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DEVELOPMENT AND VALIDATION OF A UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR RILUZOLE USING ANALYTICAL QUALITY BY DESIGN APPROACH

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ABSTRACT

An Analytic Quality by Designs (AQbD's) strategy was used to create and validate a quick, easy, economical, & environmentally friendly UV-visible spectrophotometric technique for the measurement of Riluzole. Design of Experiments (DoE) was used in the method development process utilizig Design Expert® software 13.0 to investigate the impact of important method variables on absorbance, such as solvent type and analysis speed. Ethanol was selected as the optimum solvent based on maximum absorbance and green chemistry considerations. Riluzole showed maximum absorbance (λmax) at 222 nm. The results showed that solvent type had a substantial impact on absorbance using a two-factors, two-levels factorial designs, whereas speed of analysis showed no significant influence. The ICH Q2(R1) recommendations were followed in the validation of the optimised method. With a strong correlation coefficient, the technique showed good linearity in the concentration range of 2–10 µg/ml. Precision studies showed the method's intermediate precision and repeatability with %RSD values below 2%. Recovery studies confirmed accuracy with mean recoveries between 98–102%. The method was found to be robust and rugged with minimal variation in absorbance. The results showed that the LOD and LOQ were 0.5657 µg/ml and 1.7143 µg/ml, respectively. For periodic quality control evaluation of Riluzole in pharmaceutical products, the established approach is straightforward, dependable, eco-friendly, and appropriate.

KEYWORDS: UV-Visible Spectroscopy, QbD, Method development, Validation, Design Expert 13.0, Riluzole.

INTRODUCTION

Riluzole[1] Its chemical name is 2-amino-6-(trifluoromethoxy) benzothiazole. Currently, amyotrophic lateral sclerosis (ALS) is treated using it.[2] Neurological disease that progresses motar over time. Riluzole's anti-glutamatergic action is responsible for the favorable and enhanced survival of ALS patients.[3,4]

Firstly, Quality by Designs (QbD) introduced by Juran & Godfrei (1998)[5], is mostly used in the automotive sector. The core idea of QbD is to use an optimization technique to design quality to fully understand how the quality system responds to certain variables. The Design of Experiments (DoE) its primary application of QbD in their analytical and scientific research domain.[6] The DoE significantly lowers the number of experiments required to find each factor's optimal value when compared to the conventional approach, which takes factors one at a time. By changing these variables simultaneously, this is accomplished. These are frequently the key to significant advancements.[7,8]

A methodical approach to method creation and validation is called Analytical Quality by Designs (AQbD). It focuses on predetermined goals, quality risk management(QRM), and a deep understanding of processes and controls.[9] Compared to conventional approaches, Analytical Quality by Designs (AQbD) expedites development processes by cutting down on experimentation and time.[10,11] AQbD application is more significant than other traditional methods in the development of UV-visible spectrophotometric methods.[12] Before developing a method, we must first choose the analytical targets profile (ATP) or the designated study objectives. Risk assessment studies were then used to identify the critical method variable (CMV) and critical analytical attribute (CAA).[13,14]

A few analytical techniques were developed for the measurement of Riluzole in various matrices, including the rat brain, according to the literature review[15], mouse plasma[16], in human plasma or serum[17], in human plasma using liquid extraction and the protein precipitation method[15], using LC-ESI-MS/MS in human plasma[18], utilizing HPLC in pharmaceutical dose-based formulations[19], UPLC[20] and using the spectrophotometric method[21] were reported. There is a lot of potential to improve Riluzole's pharmacokinetic

characteristics because of its low absorption and numerous side effects. Some of the negative effects of riluzole can be reduced by localizing it in brain tissue through brain-targeted administration.[22]

Application of the Quality by Designs (QbD) methodology. Trial-and-error was used in the development of earlier UV spectrophotometric techniques. This approach does not provide a systematic understanding of method variables. This method was developed by using a QbD approach, which is a scientifically proven and regulatory-approved approach. By implementing QbD, Critical Method Parameters (CMPs) such as solvent type and analysis speed. Applied Design of Experiments (DoE) to study how solvent type and analysis speed affect absorbance response. We used response surface plots and a desirability function to find the best method conditions.

Unlike previous UV methods that only assessed robustness during validation, the current method also examines robustness during the development stage. Shows minimal variation in absorbance with small changes in analytical conditions. It reduces the risk of method failure during routine quality control analysis.

Green Analytical Chemistry (GAC) Assessment most reported UV spectrophotometric methods do not assess environmental impact. The current method builds on existing literature by including Green Analytical Chemistry principles. It uses eco-friendly solvents, such as ethanol, instead of toxic organic solvents. It reduces solvent usage and energy consumption. It evaluates method greenness using green analytical metrics, such as the analytical eco-scales or the AGREE tool.

This methods also reduces chemical and operational costs compared to previous methods. The optimized method uses less solvent; There is no need for complex reagents or buffer preparation, and shorter analysis time improves lab productivity. Improved regulatory and industrial relevance, the integration of QbD and green assessment connects the method with ICH Q8, Q14 guidelines, and current regulatory expectations for managing the lifecycle of analytical methods.

Despite the availabilities of some analytical method for the analysis of Riluzole, many are complex, time-consuming, or not thoroughly validated. Regulatory authorities such as ICH (International Council for Harmonisation) mandate the development of validated analytical

methods for drug substances to support routine quality control, stability testing, and regulatory submissions.

Method development and validation are extremely crucial steps in the development of drug material. For many pharmaceutical products, the analytical methods used by scientists during the development of submissions for Investigational New Drugs (IND), New Drug Applications (NDA), and Abbreviated New Drug Applications (ANDA), especially for describing excipients and active pharmaceutical ingredients (APIs), are often not adequately developed or fully validated. To Meet Regulatory Requirements which ensures legal approval and marketability of the product. For the detection of impurities or degradation products that could impact patient safety. New methods can offer better sensitivity, specificity, speed, or cost-effectiveness.

These analytical methods help in in-process quality control (IPQC) and batch release testing. Analytical technique development and validation are crucial for meeting regulatory standards (such as those of the FDA and ICH) for product approval, manufacturing, and quality control. Previous analytical methods are critical for identifying impurities and degradation products that may impact patient safety and drug stability.

It is clear that some research departments have investigated pharmacokinetic[23,24] and formulation strategies to improve Riluzole's efficacy in animal models. Determining the plasma-concentration-time profile is necessary for evaluating the pharmacokinetic features of riluzole in animal models, and such studies require an easy-to-use, repeatable, and affordable analytical technique.[25]

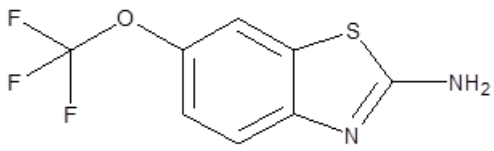

Figure 1: Riluzole 2-amino-6 (trifluoromethoxy) benzothiazole.

MATERIALS AND METHODS

Experimental

Reagents and chemicals

Riluzole is obtained from Swapnroop Drugs and Pharmaceuticals. Methanol and Ethanol of UV grade were purchased from Catalyst. Every other chemical utilized was of analytical quality.

Instrumentation

The investigation utilized UV-Visible Spectroscopy equipment (UV/VIS Shimadzu-1900i), Digital Ultrasonic Cleaner (UC1.8 Liter type). Optimization is performed using AGREE software (Analytical Chemistry). All the components were weighed on a sensitive analytical digital balance (Wensar model) with 0.1 mg accuracy.

Analytical method development and optimization

A reviews of the literature & the analyte profile served as the basis for defining the method objective. Compared to more sophisticated analytical techniques, the UV-visible spectrophotometric method was chosen for its ease of use and speed of analysis. To satisfy the Riluzole absorbance is selected as the critical analytical attribute (CAA) for the analytical target profiles (ATP).

Selection of Solvent With QbD Software

The optimal solvent for dissolving Riluzole was determined using the QbD program. To determine the maximum absorbance of riluzole with various solvents, such as ethanol and methanol, numerous tests were carried out. At 222 nm, the highest absorbance (λ max) in ethanol was determined. As a result, ethanol was chosen as Riluzole's validating solvent.

End Results Of Independent Variables on λmax

Design Experts 13 is a QbD program that we utilized to observe the effects of various factors. We chose the solvent ratio and the sonication time, as the things we wanted to change. Then we looked at how these things affected absorbance and lambda max. We were interested in the effects of solvent ratio and sonications durations on lambda max and absorbance.

Table 1: Experimental Design Matrix and Observed Absorbance Response.

	Factor 1	Factor 2	Response 1
Run	A: Solvent	B: Speed of Analysis	Absorbance

1	-1	-1	2.02
2	1	-1	1.508
3	1	1	1.492
4	1	-1	1.508
5	-1	-1	2.02
6	1	-1	1.508
7	-1	1	2.032
8	1	1	1.492
9	-1	1	2.032

Preparation of Standard Solution

Riluzole solubility tests were conducted using different solvents such as Ethanol and Methanol. It was found that the drug dissolves best in Ethanol. Therefore, stock solutions and working solutions are normally prepared using Ethanol solvent. Preparations of the stock solution involves dissolving 10 mg of Riluzole in 10ml Ethanol, producing 1000 µg/ml concentrations of the drug. Using dilution procedures, a working solution of 10µg/ml was made from the stock solution and utilized to determine UV spectra.

Determination of λ max (Selection of Wavelength)

To prepare a 10 µg/ml solution, 1 ml of the above working standard solution was added to a 10 µg/mL volumetric flask, and the necessary volume was added with ethanol. A UV spectrophotometer operating in the 200–400 nm wavelength range was then used to measure the sample, with ethanol serving as the blank. At 222 nm, the maximum absorption was noted.

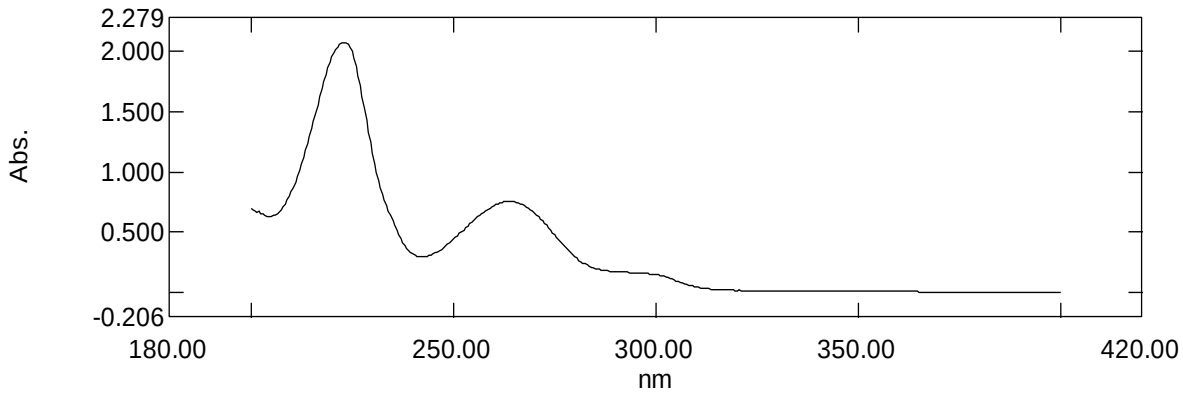

Figure 2: Absorbance maxima of Riluzole.

In our Analytical method, we started with a factorial design. In Factorial design, Two Factor Two Level (2^2) was selected. Factor one consists of solvents such as Ethanol and Methanol. Factor two consists of the Speed of Analysis, which is fast or slow. This resulted in nine

batches. Out of four batches are actual, and five batches were repeated. At the end of analysis, one batch is found Optimized, Ethanol at Slow speed with absorbance 2.020 at 222nm, we got significant results as a 3D surface plot and desirability graph.

METHOD VALIDATION

In order to achieve an analytical target profile, the selected essential parameters should be in line with the performance attributes of an analytical methods. Performance characteristics for an analytical method have been defined in the ICH guideline Q2(R1). Hence, it would be appropriate to validate a spectrophotometric analytical method based on the above-mentioned ICH guidelines, taking into account the chosen critical parameters and adopting the concept of AQbD. Therefore, the suggested approach would be validated in compliance with ICH guideline Q2 (R1). System appropriateness, linearity, precision, accuracy, specificities, LOD, and LOQ have all been examined.

Linearity

According to ICH guidelines, linearity of the analytical process demonstrates the relationship between test results and concentrations of the analyte in samples. A stock solution of ethanol in ethanol was used to generate five different concentrations of ethanol solutions (2, 4, 6, 8, and 10µg/ml) for the linearity research. The absorbances of all these solutions were determined at 222nm. The absorbance vs. ethanol concentrations were then used to plot a calibration curves. Regression analysis was used to get the correlation coefficient and percentage RSD.

Precision

As per the ICH guideline, precision is one of the measures for evaluating the degree of similarity of results achieved by using different methods or different sets of experiments for measuring the same homogenous sample. By evaluating both intra-day and inter-day precisions, the precision of the suggested analytical method was examined. Six replicates of samples at a concentration of 10 µg/ml were used for intra-day precision, and the percentage RSD was calculated. Similarly, Six repetitions of samples at a concentration of 10 µg/ml were utilized for three days in a row to ensure inter-day precision.

Accuracy

The ability of a test procedure to produce a result that is nearly identical to the analyte's real value is known as accuracy. A recovery experiment was used to verify Riluzole's accuracy. A

stock standard solution was used to spike solutions with known concentrations of drug product (10 µg/ml) at 80%, 100%, and 120%. Reanalysis of the solutions was done, where triplicates of each concentration were made.

Robustness

This procedure has been developed on the basis of data obtained at two different wavelengths (240+/-1). In case there are no fluctuations in absorbance. It shows that this technique is strong.

Ruggedness

This has been done through analyzing six samples from the same batch of Riluzole using two different analysts. It is crucial to remember that the equipment used in experiments and the persons performing the test must be distinct from one another. Both the standard deviation and the percentage RSD have to be less than 2%.

Limit of Detection (LOD) And Limit of Quantification (LOQ)

The Limit of Detection (LOD) represents the minimum amount of substance that may be detected but is not necessarily quantified. The Limit of Quantification (LOQ) is the lowest concentration that may be reliably measured. LOD and LOQ of Riluzole were estimated through residual Standard deviation is the variance of the response and slopes in accordance with the ICH's Q2B guidelines. These parameters were estimated using the calibration curve obtained during the linearity study. The equations for LOD and LOQ are (3.3 x σ)/S and (10 x σ)/S, respectively.

RESULTS AND DISCUSSIONS UV SPECTROSCOPIC METHOD VALIDATION

Linearity

First, we plotted the peak area against the analyte concentration for the calibration curve (Figure 3). Table 2 below displays the results of the linearity test.

Table 2: Linearity study for Riluzole.

Sr No.	Concentration (ppm)	Absorbance
1.	2	0.646

2.	4	1.015
3.	6	1.436
4.	8	1.842
5.	10	2.128

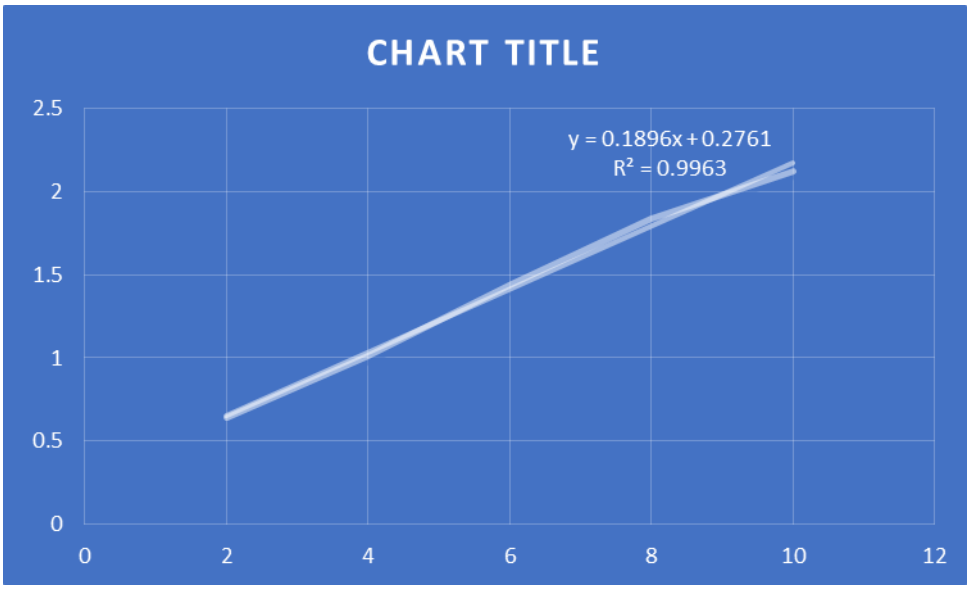

Figure 3: Standard Curve for Riluzole.

Precision

This technique was tested using a stock solution of tablets for the parameter under discussion. The intraday precision experiments involved six assays run on one single day, while interday precision tests used assays run on separate days. This is what the analysis concluded.

Table 3: Evaluation data for the Intra-day study of Riluzole.

Intraday Precision data					
Sr. No	**Conc. (µg/ml)**	**Abs**	**Mean Abs**	**SD(±)**	**%RSD**
1	2	0.548 0.555 0.567	0.556666667	0.010	1.7262
2	6	1.34 1.344 1.346	1.343333333	0.003	0.227
3	10	2.074 2.079 2.082	2.078333333	0.004	0.194

Table 4: Evaluation data for the inter-day study of Riluzole.

Sr. no.	Conc. (µg/ml)	Abs	Mean Abs	Mean	SD(±)	%RSD

1	Day 1	2	0.448	0.456666667	0.01	2.104
	Day 2		0.455			
	Day 3		0.467			
2	Day 1	6	1.34	1.343333333	0.00	0.227
	Day 2		1.344			
	Day 3		1.346			
3	Day 1	10	2.074	2.080666667	0.01	0.367
	Day 2		2.079			
	Day 3		2.089			

Accuracy

The accuracy of the analytical method gives insight into how close the reading is to the standard value. An alternative calibration method was applied in the experiment of recovery tests. The readings taken were found to be acceptable in the following way.

Table 5: Evaluation data of the accuracy study of Riluzole.

Observation Table for Accuracy								
Conc. Taken as 09 ppm (80%)			**Conc. Taken as 10 ppm (100%)**			**Conc. Taken as 11 ppm (120%)**		
Abs	**Conc found**	**% Recovery**	**Abs**	**Conc found**	**% Recovery**	**Abs**	**Conc found**	**% Recovery**
1.21	5.00	27.80	1.402	6.04	30.22	1.511	6.64	30.16
1.214	5.03	27.92	1.402	6.04	30.22	1.512	6.64	30.19
1.215	5.03	27.95	1.405	6.06	30.31	1.514	6.65	30.24
Mean Conc	5.020	27.891	Mean Conc	6.05	30.25	Mean Conc	6.64	30.19
SD of Conc	**0.0143**	**0.0797**	**SD of Conc**	**0.0094**	**0.0469**	**SD of Conc**	**0.0083**	**0.0376**
%RSD	**0.286**	**0.286**	**%RSD**	**0.155**	**0.155**	**%RSD**	**0.125**	**0.125**

Robustness

The strength of this approach was tested by varying the wavelength by ±1 nm from 220nm to 224nm and the outcome was positive.

Table 6: Evaluation data for Robustness of Riluzole.

Riluzole				
Concentration (µg/ml)	**Wavelength**	**Absorbance**	**Mean**	**% RSD**
10	220	1.967	1.971	0.268467916
10	220	1.969		
10	220	1.977		

Ruggedness

The ruggedness was determined through the testing of the Riluzole sample through several means and different analysts. The results were expressed in %RSD.

Table 7: Evaluation data for Ruggedness of Riluzole.

Riluzole			
Analyst 1		**Analyst 2**	
Concentration (µg/ml)	**Absorbance**	**Concentration (µg/ml)**	**Absorbance**
10	2.074	10	2.081
10	2.079	10	2.095
10	2.082	10	2.098
Mean	2.07833333	**Mean**	2.091333333
% RSD	0.19445639	**% RSD**	0.433874963

Limit of Detection & Limit of Quantification

The formulas LOD = 3.3 × σ/s and LOQ = 10 × σ/s were used to calculate Limits of Detection's (LOD) and Limits of Quantification's (LOQ), respectively. S is the calibration curve's slope, while σ is the drug response areas' standard deviations, which can be thought of as noise. LOD and LOQ values were acquired and confirmed to be within the constraints.

Table 8: Evaluation data for LOD & LOQ of Riluzole.

Riluzole	
LOD	0.5657 µg/ml
LOQ	1.7143 µg/ml

OPTIMIZATION FORMULATION BY THE CENTRAL COMPOSITE DESIGN METHOD BY USING DESIGN EXPERT SOFTWARE 13.

ANOVA for Linear Model.

Table 9: Response 1: Absorbance.

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**	
Model	0.6111	2	0.3056	4287.22	< 0.0001	Significant
A-Solvent	0.6057	1	0.6057	8498.98	< 0.0001	
B-Speed of Analysis	0.0000	1	0.0000	0.3306	0.5862	
Residual	0.0004	6	0.0001			
Lack of Fit	0.0004	1	0.0004			
Pure Error	0.0000	5	0.0000			
Cor Total	0.6116	8				

The constructed linear model's statistical significance and the impact of independent variables on the response (absorbance) are examined using the ANOVA table (Table 9). The model is highly significant, as evidenced by its F-value of 4287.22 and p-value of less than 0.0001. This means that the selected factors (solvent and speed of analysis) have a significant effect on absorbance, and the developed model is statistically valid.

Factor A (Solvent) shows F-value = 8498.98 and p-value = < 0.0001. This shows that the solvent has a highly significant effect on absorbance. That prove choice of solvent play a major role in their UV spectrum of the drug. Factor B (Speed of Analysis) shows F-value = 0.3306 and p-value = 0.5862. The p-values is greater than 0.05; this factor is not statistically significant. This means that the speed of analysis does not have a significant effect on absorbance within the studied range.

The residual sum of squares (0.0004) is very small, which shows that there is very little unexplained variation in the model. This confirms that the model fits the experimental data. Since the pure error is almost zero, the lack of fit is hardly important. This demonstrates that the created model is appropriate and sufficient for absorbance prediction.

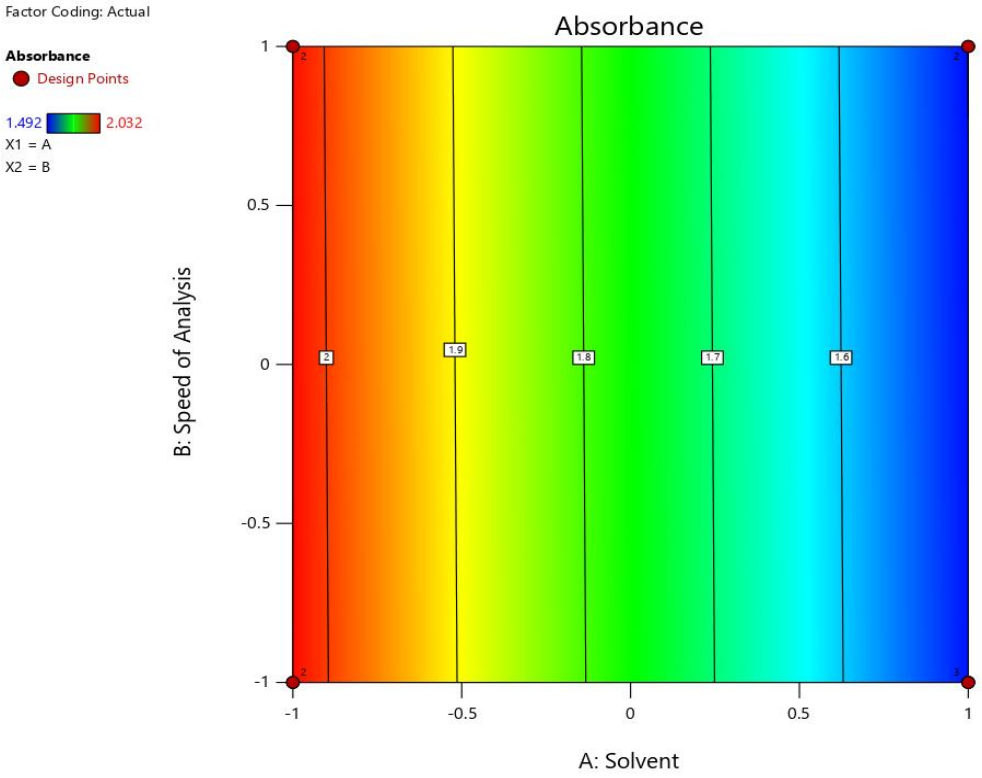

Figure 4: Effect of independent variables on dependent variables.

Above 2-D response surface (contour) plot (Fig. 4) showing solvent effect and speed of analysis on absorbance. The colour range from Blue to Red, showing intensity of absorbance, in which blue colour indicates low absorbance while red colour shows high absorbance. The colour transition shows that absorbance decreases slowly as the solvent changes from a high level to a low level. This colour transition shows that the solvent used for analysis is the most

effective factor, leading to a change in absorbance. On the other hand, contour lines are vertical and parallel, indicating minimal change in absorbance with a change in speed of analysis. This is an indication of change in the speed of analysis has no considerable effect on the result.

Parallel and vertical contour lines are a clear indication of very limited interaction between the solvent and the speed of analysis. The design points are uniformly distributed and are close to the standard predicted contours, supporting the reliability of my developed model's.

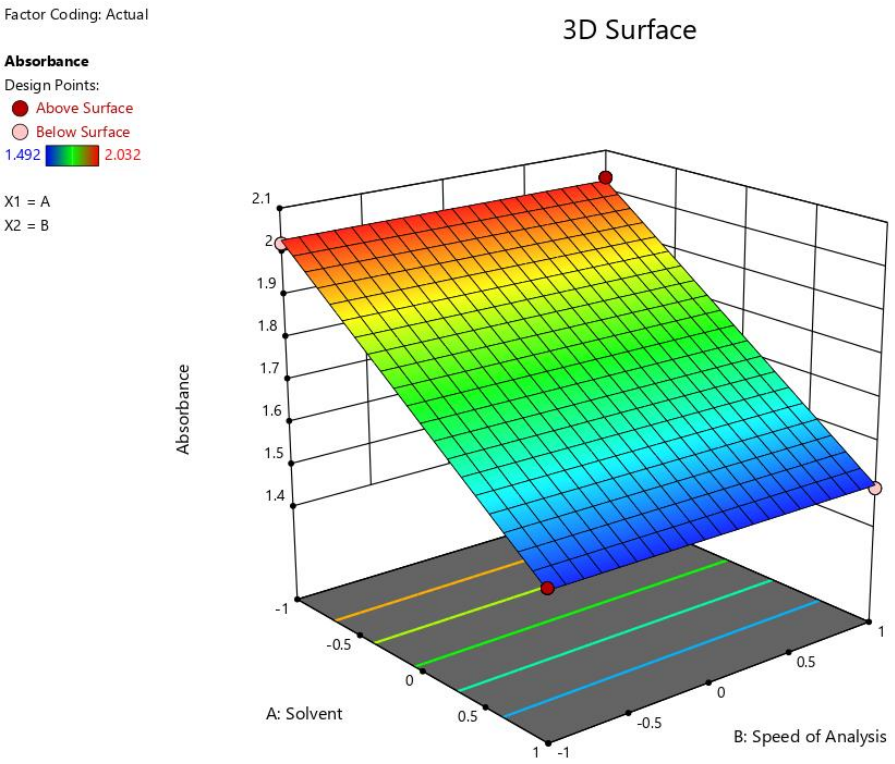

Figure 5: 3D surface plot for ANOVA results.

The 3D surface plot for ANOVA (Fig. 5) shows the effect of solvent (A) and Speed of Analysis (B) on absorbance using actual factor levels. These vertical lines show the response (absorbance). The response surface graph shows a characteristic slope along the solvent axis (A), showing a change in absorbance with a change in solvent type. Absorbance is decreasing as the solvent changes from low to high levels. The flat surface along the Speed of Analysis (B), Change in speed of analysis has a very minimal effect on absorbance in the studied range.

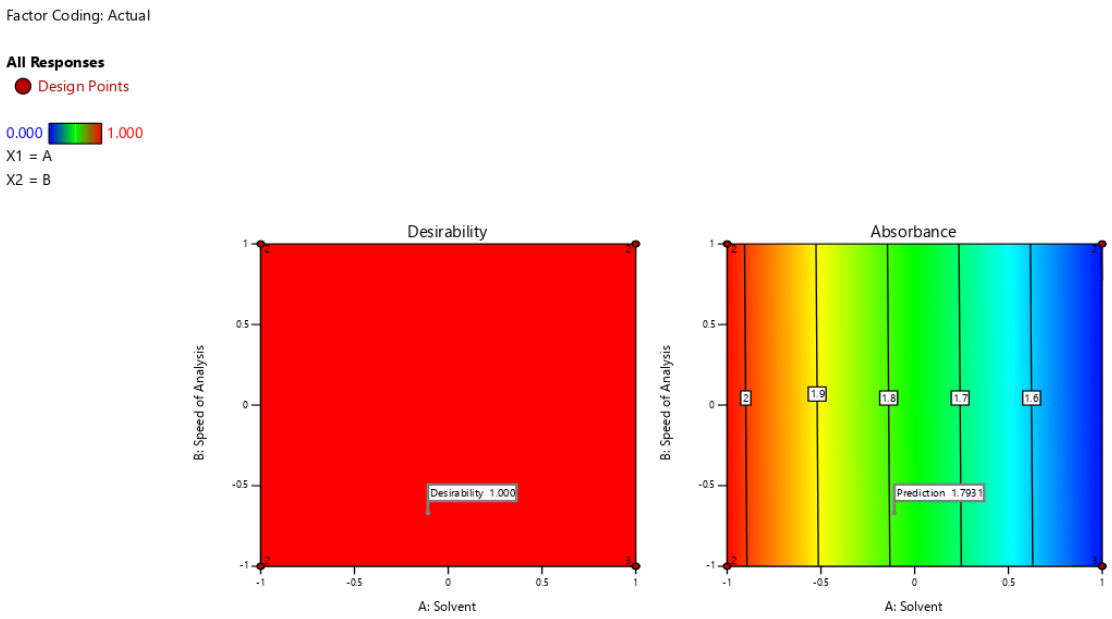

Figure 6: Plot to determine the desirability of the method.

The (Fig.6) Overlay plot of responses with the desirability function for the optimization of the analytical method by using solvent (A) and speed of analysis (B).

The desirability plot (left panel), showing a uniform red colour in the experimental study with a desirability value of 1.000, shows that the selected criteria for the analysis of absorbance are completely satisfied in the overall study range of both the solvent and speed of analysis. The results obtained are showing robust analytical values, as the method performance is comparable with the defined acceptance range.

The absorbance contour plot (right panel) indicates predicted absorbance values. A clear gradient is observed along the solvent axis (A), with absorbance decreasing from the lower level to the higher level. On the other hand, minimum variation is observed with the speed of the analysis axis (B), which confirms that analysis speed has no or a very negligible effect on absorbance. The predicted optimal absorbance value of approximately 1.79 is seen within the design space.

The consistency between the desirability and absorbance plots shows that solvent is the main controlling factor, and the speed of analysis has no significant effect on the result of analysis. Additionally, the overlay plot verifies that the analytical technique functions in the most desirable and dependable design space.

CONCLUSION

The current study successfully designed, optimized, and validated a novel UV-visible spectrophotometric technique based on Analytics Quality by Designs (AQbD) for the quantification of Riluzole. Critical method variable and their effects on analytical performance were systematically evaluated thanks to the use of Design of Experiments (DoE). The ANOVA results confirmed that solvent type is the most influential factor affecting absorbance, while speed of analysis has no statistically significant effect.

The optimized method using ethanol as a solvent at 222 nm proved to be simple, rapid, precise, accurate, robust, and rugged. Validation results complied with ICH Q2(R1) guidelines, demonstrating excellent linearity, low %RSD values for precision, high recovery for accuracy, and acceptable limits of detection and quantification.

Additionally, incorporation of Green Analytical Chemistry (GAC) principles by using ethanol as an eco-friendly solvent makes the method environmentally sustainable and cost-effective. The AQbD approach provided a better understanding of method behavior, reduced experimental trials, and ensured method reliability throughout its lifecycle.

For the purpose of estimating Riluzole in bulk and pharmaceutical dosage forms, the established UV spectrophotometric approach is therefore appropriate for routine quality control, in-process analysis, and regulatory applications.

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