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QbD based analytical method development for simultaneous quantification of valsartan and hydrochlorothiazide in bulk and formulation

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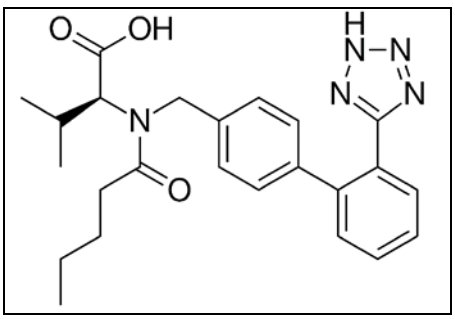
Abstract
The UV spectrophotometry analytical method was developed and validated using QbD to simultaneously assess valsartan (VAL) and hydrochlorothiazide (HCT) in both bulk and dosage form. The central composite design (CCD) approach was employed for the initial parameter selection to assess the robustness of the method. The method was found to be linear with R^2 >0.99 between the range of 2-18μg/mL for both drugs using methanol and 0.1N NaOH (55:45) as solvent. The mean percentage recovery for VAL and HCT were found to be 101.21%, 101.84%, and 100.16 %, 100.60%, respectively. The precision of the method was found within the limits (% RSD<2%). Robustness and ruggedness were found to be within limits (% RSD < 2%) for the developed methods. The LOD and LOQ were satisfactory and in the acceptable range. The QbD-developed spectrophotometric method for simultaneous VAL and HCT quantification in bulk and formulation is more precise, simple, quick, reliable, economical, and specific.

Keywords: Quality by design, method development, ICH q2 guidelines, risk assessment, UV/visible spectrophotometry

1. Introduction
Valsartan is chemically N-(1-Oxopentyl)-N- [[2-(1H tetrazol-5-yl) [1, 1-biphenyl]-4-yl] methyl]-L valine (figure 1). It is a potent, highly selective, orally active, specific angiotensin II receptor antagonist used as a hypotensive drug [1, 2]. Hydrochlorothiazide (HCT) is chemically 6-chloro-3, 4 dihydro-2H-1, 2, 4-benzothiadiazine-7sulphone-amide, 1, 1 dioxide [3]. First-line treatment with a fixed dose of VAL/HCT results in a high response rate and normalisation of blood pressure in patients with mild hypertension. This combination is supported by the fact that oral administration of VAL and HCT has been found to be more successful than the use of either drug alone in the treatment of hypertension in patients whose blood pressure cannot be adequately managed by monotherapy [4]. Figures 1 and 2 illustrate the analytes associated with the analytical method.



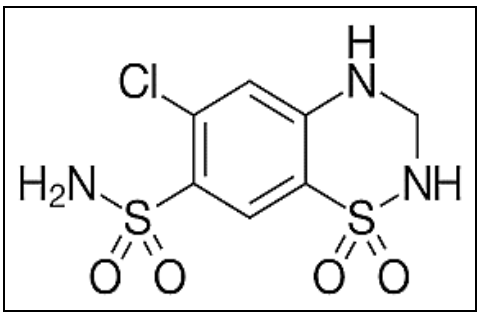


Fig 1: Structure of Valsartan **Fig 2:** Structure of Hydrochlorothiazide

After a thorough analysis of the literature, it was found that only a few analytical methods for quantifying VAL and HCT have been previously published. A liquid chromatography/tandem mass spectrometry method includes protein precipitation using acetonitrile and analytes were separated on a Zorbax SB-Aq C18 column using 10 mm ammonium acetate (60:40, v/v, pH 4.5) as mobile phase [5]. The development of a validated

stability indicating HPLC method [6] and the RP-HPLC method for the quantification of impurities in valsartan and hydrochlorothiazide in tablet dosage form [7]. There exist various techniques for the concurrent estimation of both drugs utilizing HPTLC [8, 9] and ion pair chromatography [10]. Additionally, several UV spectrophotometric methods have been documented, including the simultaneous method, the AUC method, and the first derivative method that were established [11-14]. However, the reported UV spectrophotometric method has many drawbacks, such as narrow linearity range, absence of risk assessment utility, inability to present systemic statistical optimisation, etc. Thus, efforts were made to develop an improved and new UV spectrophotometric method for quantification of VAL and HCT in formulations using the Quality-by-Design (QbD) approach. Spectrophotometric analysis offers a simpler and more cost-effective method for the simultaneous estimation of drug combinations, compared to expensive chromatographic methods such as HPLC, GC, and HPTLC which are accurate and precise, but expensive due to instrumentation, reagent, and expertise [15]. Therefore, the primary objective is to develop and validate a quick and simple approach for the synchronous quantification of VAL and HCT using a UV spectrophotometric method using the C-N-X approach and an Ishikawa fishbone diagram for risk assessment and understanding of various factors that affected method development, and central composite design (CCD) was employed to optimize certain important factors.

This research focusses on creating a Quality by Design (QbD)-driven UV spectrophotometric strategy for simultaneous estimation of VAL and HCT. QbD is a crucial technique used in product development, adhering to USFDA guidelines and industry practices. The process involves sound science, quality risk management, and process understanding, ensuring the desired outcome [16, 17]. The process of applying Quality Assurance (QA) in method development involves identifying the Analytical Target Profile and Critical Analytical Attribute, creating an Ishikawa fishbone diagram to identify six critical variables of the method through risk assessment, and optimizing selected CMVs using Design of Experiment (DoE) [18, 19]. The method control strategy should also be used in more depth for robust method development and continuous method development.

2. Materials and Methods

2.1 Materials

The pure standard samples of Valsartan and Hydrochlorothiazide were procured from Yarrow Chem Products India. The marketed preparation DIOVAAL-40H containing Valsartan (40 mg) and hydrochlorothiazide (12.5 mg) manufactured by John lee Pharmaceuticals Pvt. Ltd. was analyzed by the current method developed. Methanol and sodium hydroxide were obtained from Merck India. All chemicals utilized in this research were of analytical grade.

2.2 Instrumentation and optical characteristics

A Specord 210 plus UV/visible double beam spectrophotometer (aspect UV software) with 10mm matched quartz cuvettes matched to 10mm was used for spectral measurements, a high precision digital electronic analytical balance (Contech) was used to weigh the reagents, and an ultrasonicatior (Spectra lab) was used for the dissolution of the tablet formulation.

2.3 Selection of an appropriate solvent system for the development of analytical methods

Solubility tests were performed by dissolving the compound in various solvents such as water, methanol, ethanol, 0.1N HCl, and 0.1N NaOH. It was discovered that the pure drug VAL was more easily in methanol and 0.1N NaOH than in ethanol, water and 0.1N HCL. Pure HCT was more easily soluble in 0.1N NaOH and methanol than ethanol, water, and 0.1N HCL. Methanol and 0.1N sodium hydroxide were used as solvents for further studies.

2.4 Preparation of standard stock solution

By dissolving 100 mg of valsartan and hydrochlorothiazide separately in 100 mL of methanol and 0.1N NaOH (50:50) in a 100 mL volumetric flask while vigorously shaking, a stock standard solution of 1000 μg/mL was created. 10 mL of this stock standard solution was taken out and diluted with 100 mL of solvent to obtain a working solution of 100μg/mL.

2.5 Selection of analytical wavelengths

For the purpose of selecting analytical wavelengths, solutions of both drugs at a concentration of 10μg/mL were scanned individually within the range of 400nm to 200nm. The overlay spectra for both drugs were documented. From spectra analysis, wavelengths 250 nm (λ_{max} of VAL) and 272nm (λ_{max} of HCT) were selected for analysis of both drugs using simultaneous estimation method [20].

2.6 Preliminary trials

The scanning speed, scanning interval, and solvent proportion were tested in initial trials to determine the optimal absorbance ranges. The selected ranges were 5 nm, 10 nm & 20nm, 0.5nm, 1nm & 2nm, and 45:55, 50:50 and 55:45, providing optimal absorbance. These optimized ranges were selected for final method development and validation. Statistical validation and screening led to the selection of an optimized level.

2.7 Risk analysis-based model development

The risk assessment technique was used to identify high-risk components and develop risk processes for the quality of the final product, which were then used in the creation of experimentation research [18]. The ICH Q9 guidelines emphasis risk identification and analysis in risk assessment methodology. The Ishikawa fishbone diagram, one of the most basic tools, is useful for understanding the cause-and-effect relationship among potential method factors that may influence method performance. In this regard, a fishbone diagram was created by highlighting various variables of the method that could influence the attribute of the UV spectrophotometric method (figure 3), while the control noise experiment (C-N-X) approach performed a risk analysis [18]. In the current studies, a Cause-Effect Risk Assessment Matrix with CNX (Control-Noise-Experimentation) approach was utilized to identify high risk variables that influence analytical attributes. Critical method variables (CMVs), such as solvent variation, detection wavelength, scan speed, and sampling interval, were found to be associated with high final scores, indicating high-risk variables (figure 4). Furthermore, the CMVs were evaluated using a screening design to identify critical method parameters (CMPs), which were then subjected to response surface optimization using an appropriate experimental

design. Critical parameters were screened in Minitab using fractional factorial designs to identify high-risk variables. Certain parameters were selected as variables for the critical method following a comparison of their spectral shape, sharpness, and absorbance.

Fig 3: Typical steps of QbD involved in the development of robust analytical method

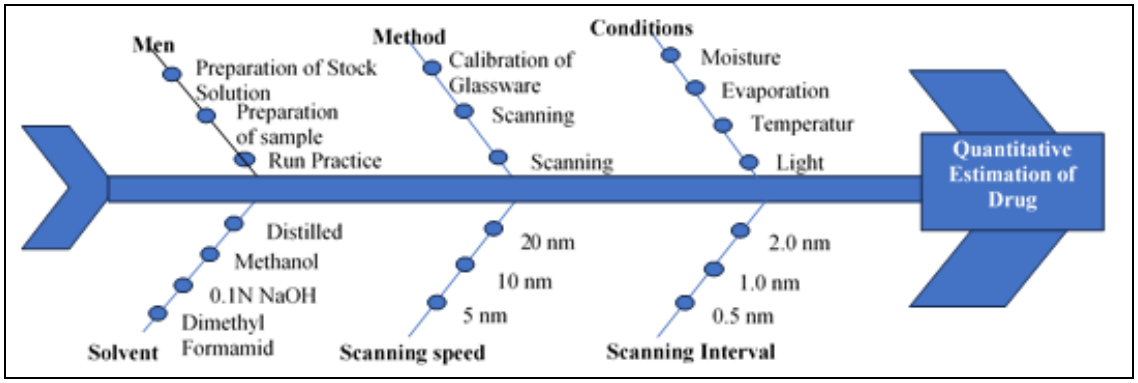


Fig 4: Ishikawa fishbone diagram for finding the probable factors that affects the method development

Prioritization studies based on prior knowledge and the Ishikawa fishbone diagram revealed that solvent type, detection wavelength, and sample were evaluated through physical observation. However, the method variables, namely scan speed (X1) and scan interval (X2), were selected by a FFD with a minimum of five runs (1 central point) using Minitab software. The variables were tested at both their highest and lowest levels. The script was then run to determine the critical method variables that influence the variable response absorbance (Y). The CMV screening was carried out by comparing the actual and predicted graphs, summarising the fit graph, and predicting the expression.

2.8 Quality-by-Design (QbD) based approach for critical method variables (CMV)

The CMV was determined using risk analysis methods and three key method variables scanning speed, scanning interval, and solvent proportion were optimized and adjusted to their optimum values [19, 21]. The study used Central Composite Design (CCD) to optimize the method variables, including scanning speed, interval, and solvent proportion. 20 runs consisting of six centered points and the same has been depicted in Table 1 were carried out for each variable, and two responses were analyzed using Design Expert software version 12. The experiments were carried out in triplicate.

2.9 Analysis of matrix design by ANOVA and model graphical presentation

The experiment data was fitted to a suitable mathematical model using multiple linear regression analysis in Design Expert software. The developed model was able to investigate both the main and interaction effects.

Table 1: Experimental design matrix obtained through central composite design.

Run	A. Scanning Speed	B. Scanning Interval	C. Solvent Proportion	Absorbance of VAL	Absorbance of HCT
1	0	1	0	0.4242	0.5479
2	1	1	-1	0.4115	0.5301
3	-1	1	-1	0.4095	0.5350
4	0	0	0	0.4288	0.5424
5	-1	0	0	0.4270	0.5585
6	1	0	0	0.4321	0.5503
7	0	0	0	0.4288	0.5424
8	0	0	1	0.4565	0.5720
9	1	-1	1	0.4488	0.5633
10	0	0	0	0.4288	0.5424
11	0	0	0	0.4288	0.5424
12	0	0	-1	0.3981	0.5161
13	0	0	0	0.4288	0.5424
14	-1	1	1	0.4392	0.5682

15	-1	-1	-1	0.4101	0.5350
16	0	-1	0	0.4270	0.5478
17	1	-1	-1	0.4091	0.5301
18	1	1	1	0.4462	0.5633
19	-1	-1	1	0.4448	0.5682
20	0	0	0	0.4288	0.5424

Coded Value	Scanning Speed	Scanning Interval	Solvent Proportion
-1	5 nm	0.5 nm	45:55
0	10 nm	1.0 nm	50:50
1	20 nm	2.0 nm	55:45

Only the coefficients of the model terms identified as significant ($p<0.05$) through ANOVA analyses were taken into account when constructing the polynomial equation and evaluating the model for aspects such as comparing the actual and predicted graphs, the fit summary and variance analysis (ANOVA), followed by examining parameters such as the coefficient of correlation (R^2), adjusted and the predicted R^2 and predicted residual sum of squares (PRESS), respectively. In order to demonstrate the relationship between the independent method variables and dependent variables or responses, the Design Expert software was used to build a suitable mathematical model in the form of a coded Eq. (1).

$$Y= X_0 + X_1 A + X_2 B + X_3 C + X_4 AB + X_5 AC + X_6 BC + X_7 A^2 + X_8 B^2 + X_9 C^2 \quad \text{Eq. (1)}$$

Where, Y= response, X_0 = intercept, X_1, X_2 and X_3 are the coefficients of the three factors A, B and C, X_4, X_5 and X_6 present the coefficients of a correlation term of factor A & B, A & C and B & C, X_7 X_8 and X_9 represent the quadratic coefficients of selected factors.

2.10 Optimization of selected variables and Design space

Other important tools such as a prediction profiler, an interaction profiler, 2-D contour plot, and a 3-D response surface profiler were used to determine the aptness of the model. The optimal solution was identified using the numerical desirability function by trading off the factors studied for the responses, which were then demarcated in the design space region. Provides optimized solutions based on goals and uses overlay plots to illustrate the relationships of variables. The design space or work space is defined as a multidimensional mix of input factors (method variables) and process parameters that have been shown to provide quality assurance. The design or working space was designed using optimization in association with response surface techniques. The design space included the variables of the operative range of the selected method, and the applied central composite design (CCD) resulting in 20 experimental runs for these variables are shown in Table 1.

2.11 Model Validation

The suitability of the developed model was evaluated using a point prediction feature, which allows optimisation of method variables. The optimized responses were compared to real experiments, validating the prediction of the model under real lab conditions [21].

2.12 Selection of solvent proportion, scanning speed, and scanning interval

On the basis of ANOVA analysis, model graphs, and optimisation study, select the optimum level of proportion of solvent, scanning speed, and scanning interval.

2.13 Preparation of standard stock solution

The stock solution of VAL and HCT (1000μg/mL) was prepared by dissolving separately using optimum level of solvent proportion to obtain working solution of 100μg/mL.

2.14 Preparation of the calibration curve

Standard dilutions of both drugs were prepared separately having a concentration of 2-18µg/mL and 2-18µg/mL for VAL and HCT, respectively. The absorbance of these standard solutions was measured at 250 and 272 nm. The calibration curves were constructed by plotting the absorbance versus concentration and subjected to least-squares linear regression analysis.

2.15 Validation of the developed method

According to the ICH Q2 (R1) guideline, the optimized UV-spectrophotometric method was validated for specificity, linearity, precision, precision, repeatability, intermediate precision, suitability of the system and robustness [22, 23]. All experiments were carried out in triplicate.

2.16 Simultaneous estimation method

Drugs (1%, 1cm) determination at selected wavelengths. A (1%, 1cm) values of drugs were calculated using the following formula (Eq. 2);

(1%, 1cm) = Absorbance / Concentration Eq. (2)

A set of two simultaneous equations were framed using these (1%, 1cm) values which are given below Eq. (3) & (4);

CVAL=$A2ay1$-$A1ay2$ / $ax2ay1$-$ax1ay2$, Eq. (3)

CHCT=$A1ax2$-$A2ax1$ / $ax2ay1$-$ax1ay2$, Eq. (4)

Where, $A1$ and $A2$ are absorbance of mixture at 250 nm and 272 nm; $ax1$ and $ax2$, (1%, 1cm) of VAL at 250 nm and 272 nm, respectively; and $ay1$ and $ay2$, A (1%, 1cm) of HCT at 250 nm and 272 nm, respectively. CVAL and CHCT are concentrations of VAL and HCT in mixture.

2.17 Drug Assay

The standard mixture of VAL and HCT was prepared by weighing 40 mg of VAL and 12.5 mg of HCT in 100 mL of volumetric flask containing methanol and 0.1 N NaOH (55:45), sonicated for 20 min; volume was adjusted to mark with the same solvent and filtered through whatmann filter

paper no. 41. The resulting solution was further diluted to get concentration 40 μg/mL of Val and 12.5 μg/mL of HCT. The prepared solution was scanned within 400 to 200 nm, and the absorbance of the sample solution at selected wavelengths was recorded against the blank. The concentrations of the two drugs in sample solutions (CVAL and CHCT) were determined, using Eq. (3) and (4).

2.18 Tablet Assay

Twenty tablets (DIOVAAL-40H containing 40 mg of Val and 12.5 mg of HCT) were weighed, average weight determined, and finely powdered. A quantity of powder equivalent to 40 mg of VAL and 12.5 mg of HCT was transferred to 100 mL volumetric flask and dissolved in mixture of methanol and 0.1 N NaOH (55:45), sonicated for 20 min; volume was adjusted to mark with the same solvent and filtered through whatmann filter paper 41. The resulting solution was further diluted to get concentration 40 μg/mL of Val and 12.5 μg/mL of HCT. The prepared solution was scanned within 400 to 200 nm and the absorbance of the sample solution at selected wavelengths was recorded against the blank. The concentrations of the two drugs in sample solutions (CVAL and CHCT) were determined, using Eq. (3) and (4).

3. Results and Discussion

3.1 Selection of solvent

Solubility is an important parameter in the development of analytical methods, especially for pharmaceuticals. It has a significant impact on solvent selection and overall method performance. For selection of a common solvent as a diluent, VAL and HCT were added to water, 0.1 N NaOH, ethanol, and methanol. VAL was freely soluble in methanol and sparingly soluble in 01 N NaOH. But HCT is not soluble in ethanol and is freely soluble in 0.1 N NaOH. However, VAL and HCT were clearly soluble in methanol and 0.1N NaOH compared to ethanol, water, and 0.1N HCL. Therefore, methanol and 0.1N sodium hydroxide (55:45) were selected as a suitable common solvent system for further studies.

3.2 Wavelengths determination

Standard VAL and HCT were scanned in the entire UV range of 400-200 nm to obtain the absorbance spectrum and overlay spectra of VAL and HCT (figure 5). The absorption maxima of VAL and HCT were found to be 250 and 272 nm respectively and same used for estimation of these drugs using simultaneous equation method.

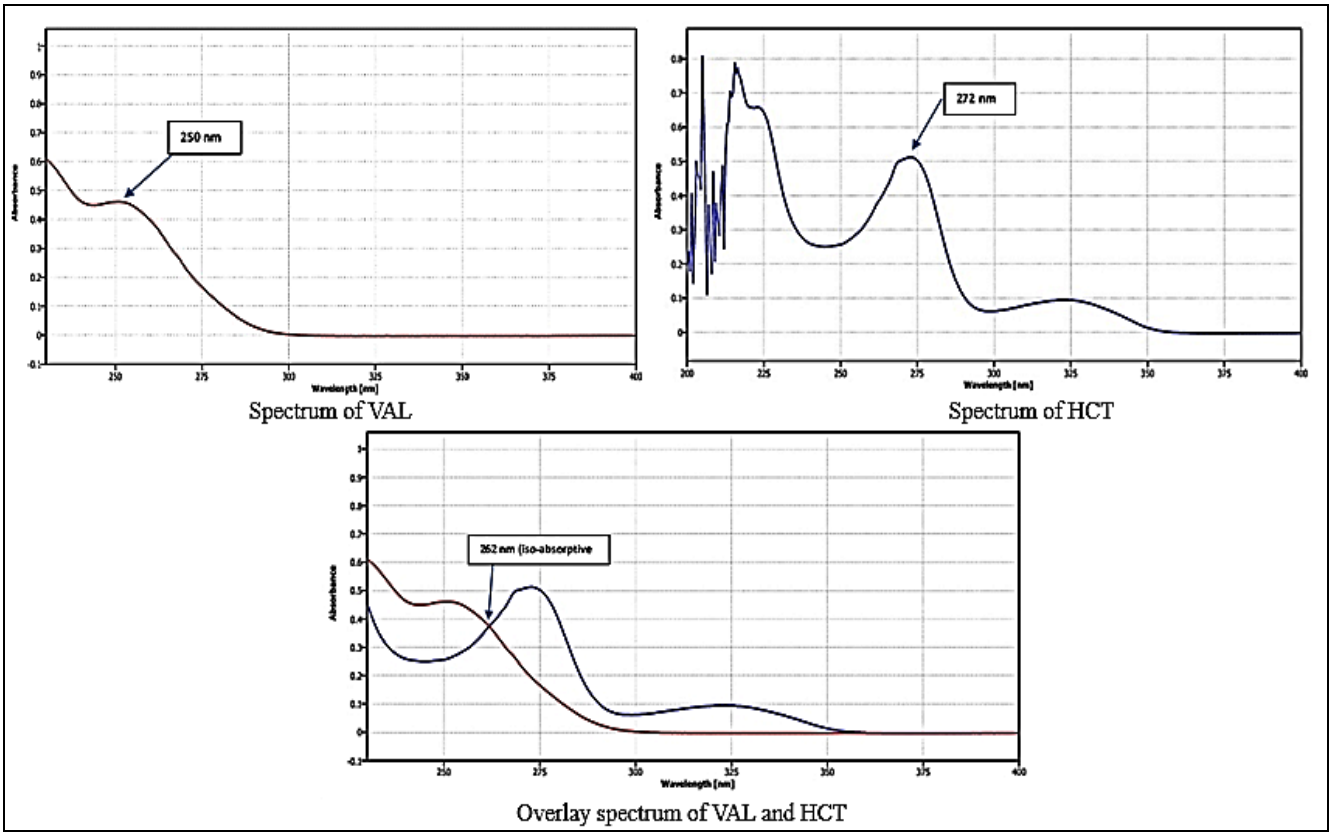


Fig 5: UV-Visible spectrum of VAL, HCT and Overlay spectrum

3.3 Risk assessment study to define ATP and CAAs

The QbD approach was used to determine the variable parameters for the final spectrophotometric conditions. A typical Ishikawa fishbone diagram was created to identify the method variables. The analytical target profile (ATP) was established for the development of analytical method utilizing the QbD approach by determining its quality attributes. The development of new UV-spectrophotometric techniques was driven by the desire to analyze drugs more quickly and easily than previous sophisticated analytical techniques. For achieving the fixed target profile, we selected the absorbance of VAL and HCT as Critical Analyte Attribute (CAA). The Ishikawa fishbone diagram (figure 4) was drawn to show the correlation among the various process variables and critical analytical attributes [24].

3.4 Risk factor analysis using the C-N-X method

The components identified using the Ishikawa fishbone diagram was subjected to a criticality analysis using the C-

N-X technique (figure 4). The total scores for various variables were calculated and prioritized using the C-E Risk Assessment matrix (Table 2) and the C-N-X approach. Physical evaluation of the method variables was performed. The variables were scored based on their impact on final quality attributes, derived from the analysis and brainstorming of the previous scientific literature.

The melting point test determined that the integrity was satisfactory. However, the variables such as scan speed, sampling, and scanning interval, required a thorough investigation to determine their impact on the robustness of the method. The FFD approach assisted in screening the CMVs based on scanning speed and scanning interval. The evaluation model, which used an actual vs. predicted plot, revealed that the adopted model was fit. The p-value (0.098), R^2 (0.989), and RMSE (0.0004) all indicated the suitability of the model. The summary of the fit revealed satisfactory values of R2 (0.997) and adjusted R2 (0.978). Based on the Pareto plot, the scanning speed (X_1) and the scanning interval (X_2) were identified as the two critical CMVs that significantly affect the response variable, absorbance (Y). A similar interpretation was noted in the prediction expression (Eq.5), which corroborated the findings of the Pareto plot.

$$\text{Absorbance (Y)} = 0.2708 + 0.00135X_1 + 0.00065X_2 \quad \text{Eq. (5)}$$

Additionally, the DoE approach was used to identify significant risk factors such as solvent proportion, scanning speed, and sampling interval, while the remaining moderate and less effective factors were kept constant [25]. Central Composite Design was applied to ensure the robustness of the method to identify optimized method conditions. The absorbances of the experimental runs obtained were evaluated at 250 nm and 272 nm as a response variable.

Table 2: C, N, X based risk assessment of various process variables and analytical attributes.

Reasons	The impact of risk on absorbance (Score)	Total Score	C-N-X	Strategy for controlling the risk
Scanning speed	10	100	X	Design of Experiment
Solvent proportion	10	100	X	Design of Experiment
Wavelength selection	4	40	C	250 nm for VAL and 272 nm for HCT
Sample Purity	4	40	N	Quality can be assessed
Sample Preparation	4	40	C	Can be controlled by following SOP.
Equilibrium of detector	3	30	C	Can be controlled by performing the qualification of equipment.
Sampling Interval	10	100	X	Design of Experiment

C: Control, N: Noise and E: Experimental Determinati0on of total score- (Risk level of the selected factors X 10), Score was given on scale of 1-10 to express Low (1), moderate (5) and high risk (10)

3.5 Analysis of the design matrix by ANOVA and optimisation of selected critical variables

CCD was used to optimize critical analytical variables, generating a design matrix consisting of 20 runs for selected independent factors using Design Expert software, and Absorbance at 250 nm and 272 nm were selected as responses or dependent variables shown in Table 1. For the optimisation of scanning speed and scanning interval, we performed analysis of 10 ppm solution at different scanning speed and scanning interval. The absorbance values of Valsartan, hydrochlorothiazide, and the iso-absorptive point show maximum absorbance at 10 nm scanning speed and 1 nm scanning interval, indicating it is the most effective for maximizing absorbance in UV-visible analysis.

As shown in Table 3, the *p-value* for the model and for the factors were found to be less than 0.05, exhibiting the significance of developed model. The F values of both responses were found to be 19.22 and 15.50 respectively, which implies that the developed model were significant [21].

Table 3: ANOVA analysis of response (absorbance at 250 nm and 272 nm).

Responses	Absorbance at 250 nm		Absorbance at 272 nm		Remarks
Source	F-value	P-value	F-value	P-value	
Model	19.22	<0.0001	15.50	<0.0001	Significant
A-Scanning speed	1.27	0.2857	2.79	0.1258	
B-Sampling Interval	0.3682	0.5575	0.00	0.9953	
C-Solvent Proportion	169.19	<0.0001	128.53	<0.0001	
AB	0.1958	0.6676	0.00	1.0000	
AC	0.5438	0.4778	0.000	1.0000	
BC	0.5438	0.4778	0.000	1.0000	
A^2	0.3366	0.5746	5.60	0.0396	
B^2	0.6180	0.4500	0.0913	0.7687	
C^2	0.0392	0.8469	0.8012	0.3918	

Coded factors were used to predict the correlation between independent and dependent variables at different levels, dividing them into low and elevated levels (-1 and +1). The equation of selected responses is shown as Eq. (6) and (7).

$$\text{Absorbance at 250 nm} = +0.4284+0.0017A-0.0009B+0.0197C+0.0007AB+0.0012AC-0.0012BC+0.0017A^2-0.0023B^2-0.0006C^2 \quad \text{Eq. (6)}$$

$$\text{Absorbance at 272 nm} = +0.5446 - 0.0028A + 0.0000B + 0.0189C + 0.0000AB + 0.0000AC + 0.0000BC + 0.0073\ A^2 + 0.0008\ B^2 - 0.0030C^2 \quad \text{Eq. (7)}$$

Where, A= Scanning Speed, B= Scanning Interval, and C= Solvent Proportion.

The interaction effect analysis was carried out employing interaction plots which explains a nonlinear pattern among all the studied factors on the response variable. To represent the correlation between variables and the response, 2D contour plot and the 3D response surface plots were constructed using Design Expert software and shown in Figures 6 and 7. With the analysis of all plots, in the 3D and 2D graphs of VAL and HCT, AB i.e. scanning speed and scanning interval show the least individual and combined effect on the absorbance. AC, i.e. scanning speed and solvent proportion, indicates that solvent proportion significantly influences the response, more than scanning speed. The BC, i.e. the scanning interval and solvent proportion, shows that the solvent proportion is the most influential factor, while the scanning interval is less effective. From this information it was concluded that among the three factors investigated, solvent proportion was the most significant factor than scanning speed & scanning interval [18]. It is crucial to choose the design space from the experimental area in order to optimize the identified critical factors (sampling interval, scanning speed, and solvent proportion) concerning the chosen response. The overlay plot illustrates the light-yellow region alongside the dark-yellow design space as depicted in figure 8.

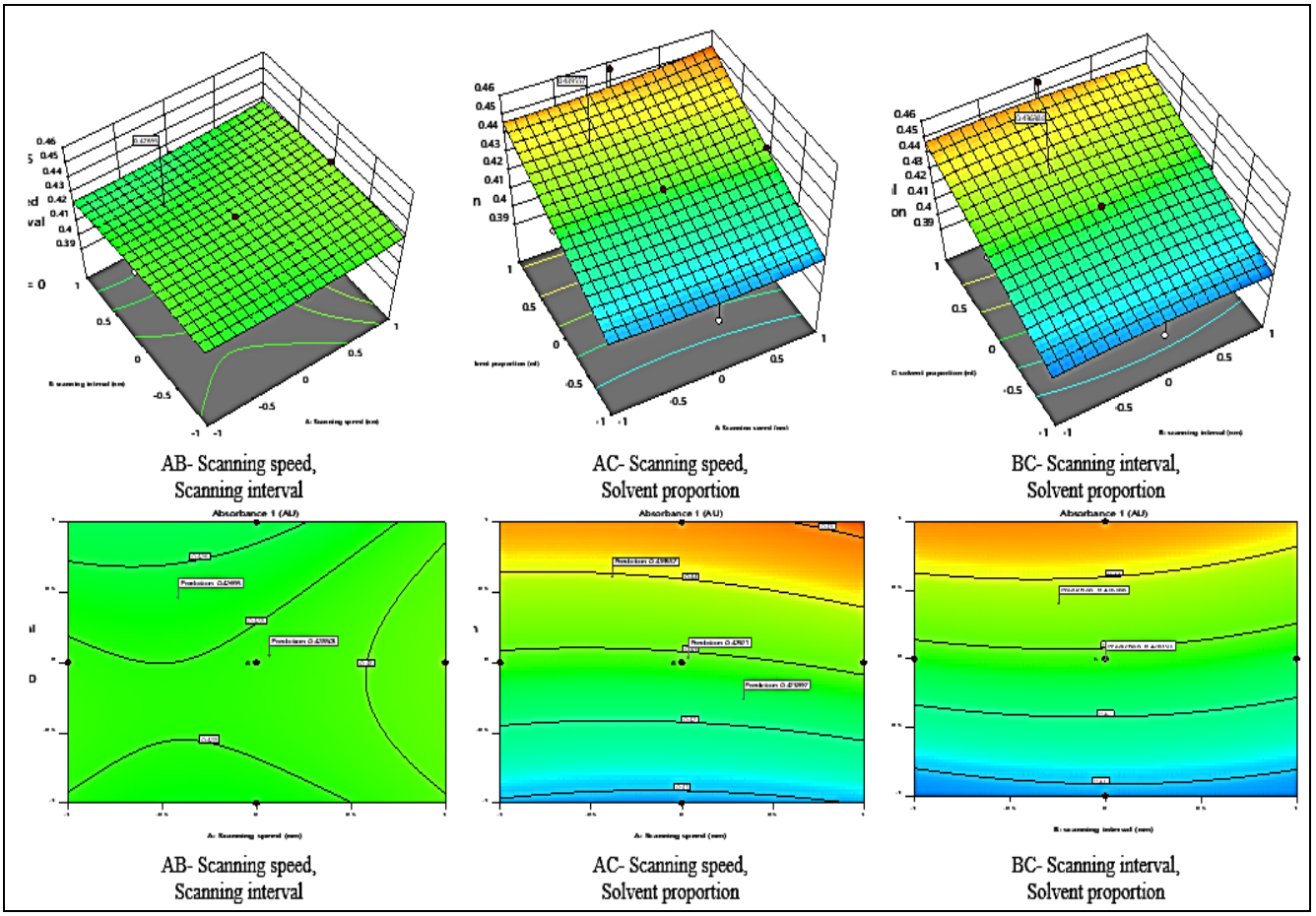


Fig 6: 3D Response surface plot and 2D contour plot of Valsartan at 250 nm

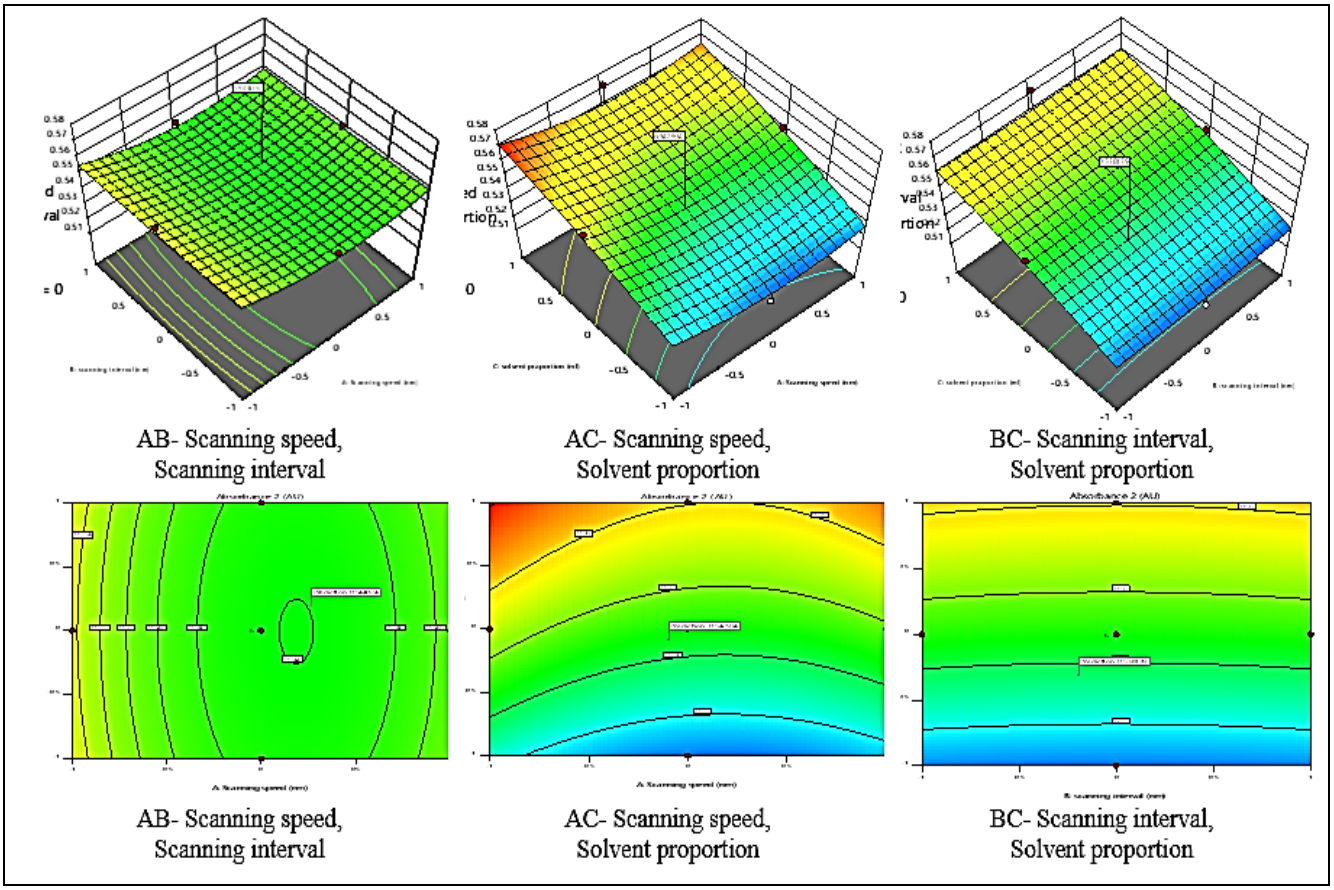


Fig 7: 3D Response surface plot and 2D contour plot of Hydrochlorothiazide at 272 nm

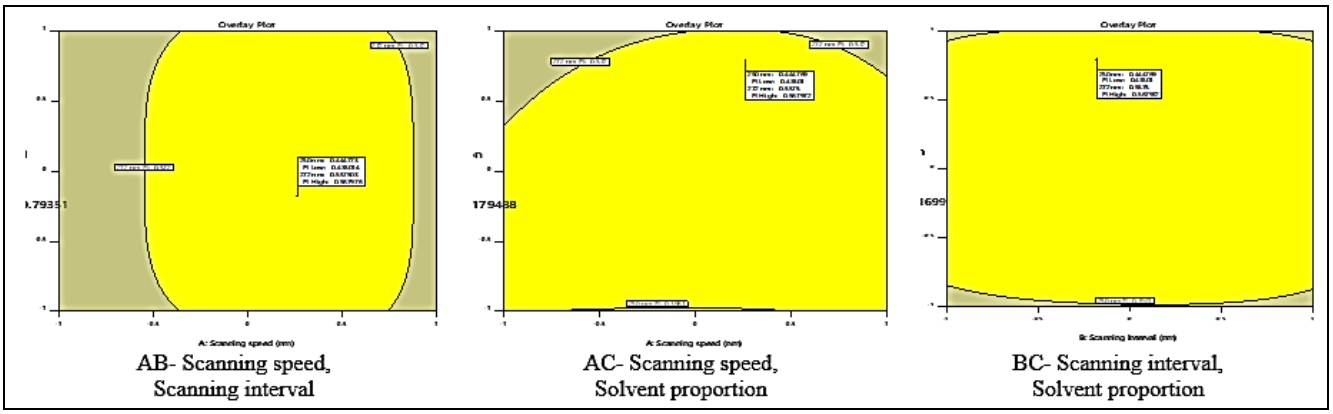


Fig 8: Overlay plot showing the design space (dark yellow) from experimental area

3.6 Statistical solution and validation of the developed experimental model

After statistical optimisation, the solutions for maximum absorbance suggested optimized variables and selected for further validation. The observed values were compared with the predicted values (Table 4). The predicted value of the developed model was found to be similar to that of the experimental value, confirming that the mathematical model can be acceptable for defining the interrelation of selected variables to obtain the desired response within the identified design space [26, 27]. After ANOVA and model analysis, the optimum level of ratio is confirmed. In this study, methanol and 0.1N sodium hydroxide (55:45) were selected as solvent.

3.7 Specificity

Specificity was established to confirm the ability of an analytical method to unequivocally measure analytes of interest in the presence of other components such as impurities, degradation products, or matrix components. It is a critical parameter that ensures that the method accurately quantifies the target substance without interference from other compounds that might be present in the sample. The absorbance, concentration, and% recovery of tablet with pure drugs, i.e., VAL and HCT were compared. The results of system specificity are shown in Table 5.

Tablet 4: Predicted and observed response for the optimized parameters.

Solution	Absorbance 1 (250nm)	Absorbance 2 (272nm)
Predicted Mean	0.4434	0.5574
Predicted Median	0.4434	0.5574
Observed	0.4288	0.5484
SD	0.00479	0.00514
SE Mean	0.00243	0.0026
95% CI low for Mean	0.438	0.5516
95% CI high for Mean	0.4488	0.5633
95% TI low for 99% Pop	0.4193	0.5316
95% TI high for 99% Pop	0.4675	0.5833

Table 5: Specificity studies of drugs and tablets.

Parameters	VAL (40ppm)	HCT (12.5ppm)
Absorbance of Tablet	1.7147	0.5355
Absorbance of Drug	1.7266	0.5655
Concentration of Tablet	39.51	11.9
Concentration of Drug	39.79	11.60
% Recovery of Tablet	98.79	95.24
% Recovery of Drug	99.49	100.84

The absorbance and concentration values of the tablet samples closely match those of the pure drug standards. Furthermore, the% recovery values for both drugs are within the acceptable range of 95-105%, indicating that there is no interference from the excipients. Consequently, this method is tailored and appropriate for the precise estimation of both drugs and in tablet formulations. The result shows that excipients with drugs, confirms the specificity of the developed method [28]. It was noted that the system-specificity parameters comply with ICH guidelines.

3.8 Linearity

A regression line was constructed by plotting drug concentrations on the X axis against absorbances on the Y axis to establish the linearity of the method. The linearity curve for VAL and HCT are represented in figures 9 and 10, respectively. The R^2 value for both wavelengths for both drug VAL and HCT was found to be 0.9949, 0.9949 and 0.992, 0.99 respectively, which was in the acceptable range of not less than 0.99 as per the ICH Q2 guidelines confirming the linearity of the developed method.

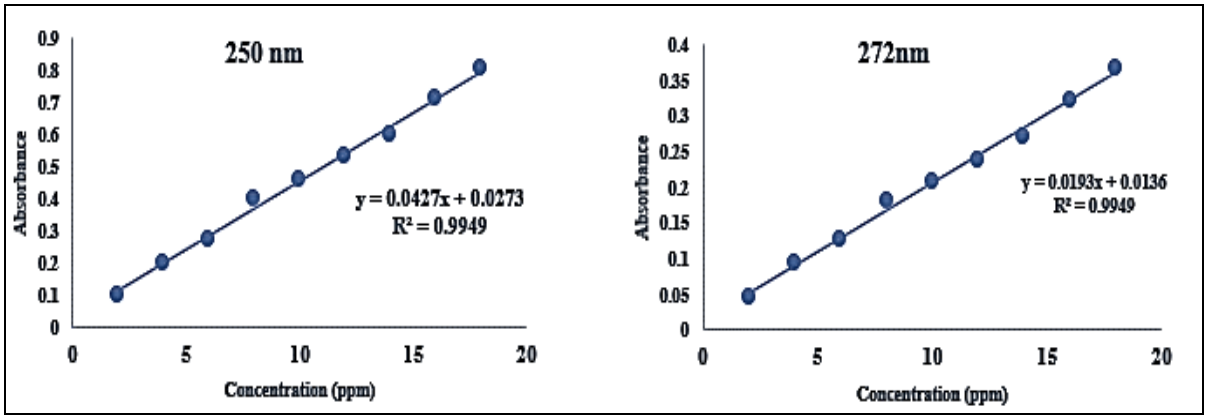


Fig 9: Linearity curve of VAL at wavelength 250 nm and 272 nm

3.9 Accuracy

Accuracy communicates the closeness of the observed arrangement between the value and the reference value. Samples with three different concentrations of recovery were prepared using the standard addition method. The mean % recovery for VAL and HCT at 250 nm and 272 nm were 101.21%, 100.16%, and 101.84% and 100.60%, respectively. The accuracy results of both drugs are shown in Table 6. The observed values were within the acceptable range of 98 -102% according to the ICH Q2 guidelines, confirming the accuracy of developed method [29].

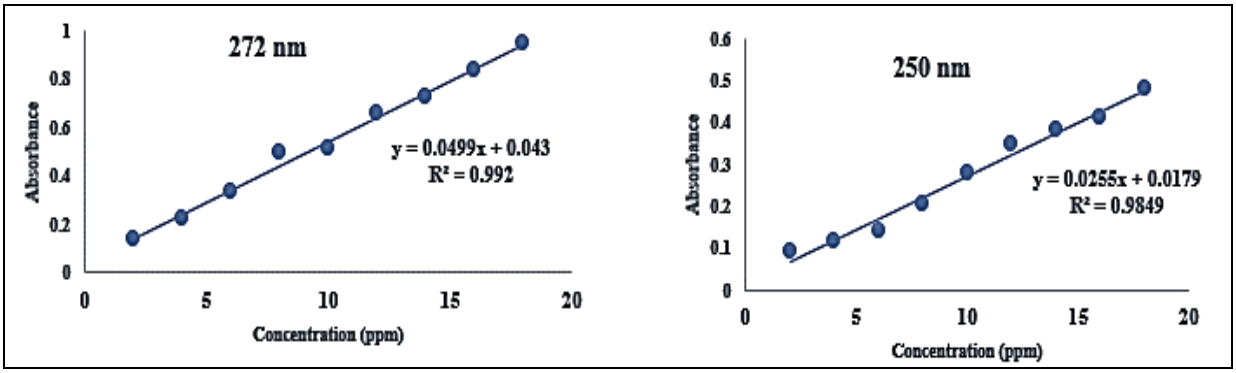


Fig 10: Linearity curve of HCT at wavelength 272 nm and 250 nm

Table 6: Recovery studies analysis at 80%, 100 % and 120% of VAL & HCT at 250nm & 272nm.

% Concentration (at specification Level)	Initial amount (□g/mL)		Conc. of std. drug added (□g/mL)		% Recovery (n=3)			
					250nm		272 nm	
	VAL	HCT	VAL	HCT	VAL	HCT	VAL	HCT
80%	10	10	8	8	101.58	100.91	101.85	100.30
100%	10	10	10	10	102.31	98.19	101.79	101.25
120%	10	10	12	12	99.73	101.37	101.89	100.25
				Mean	101.21	100.16	101.84	100.60
				SD	1.33230	1.7171	0.0504	0.568
				% RSD	1.316332	1.7143	0.0495	0.564

3.10 Intraday and Interday Precision

The intra-day and inter-day variations were determined by analysing three different solutions of VAL and HCT within the same day at an interval of 3 hours and 3 different days (Table 7). The average % RSD for intraday precision at 250 and 272 nm were found to be 1.647%, 1.8278% (VAL) and 0.5813%, 1.4543 (HCT), respectively. The average % RSD for the interday precision for VAL and HCT at 250 and 272 nm were found to be 1.7426%, 0.9024%, and 1.3829%, 1.5796 respectively. Intraday precision for both the drugs was in the acceptable range of < 2% [29, 30].

3.11 Robustness

On the basis of the observation of the absorbance of both drugs at different wavelengths, robustness was determined. When the% RSD for both VAL and HCT using the simultaneous estimation method, the robustness was determined. The results were within the acceptable range of the ICH Q2 guidelines (<2%) which validate the robustness of the developed method. The results are shown in Table 8.

3.12 Ruggedness

The ruggedness was assessed by measuring the absorbance of both drugs as evaluated by two analysts. The determination of ruggedness involved calculating the % RSD for both VAL and HCT. The results are shown in Table 9. The results were within the acceptable range of the ICH Q2 guidelines (< 2%) which validates the ruggedness of the developed method [31].

Table 7: Intraday and Interday Precision of VAL and HCT at wavelength 250 and 272 nm.

Drug	Amount taken (□g/mL)	Intraday		Interday	
		% RSD (n=3)		% RSD (n=3)	
	Wavelength	250 nm	272 nm	250 nm	272 nm
VAL	8	1.5373	1.8711	1.5447	1.7593
	10	1.7469	1.6204	1.9065	0.732
	12	1.6568	1.9919	1.7767	0.2161
Mean		1.647	1.8278	1.742633	0.902467
SD		0.105	0.189	0.183	0.785
% RSD		0.063	0.103	0.105	0.870
HCT	8	0.3794	1.496	0.4321	1.3618
	10	0.7397	0.8972	1.9528	1.8156
	12	0.625	1.9698	1.7638	1.5616
Mean		0.581	1.454	1.382	1.579
SD		0.184	0.537	0.828	0.227
% RSD		0.316	0.369	0.599	0.143

Table 8: Robustness of VAL and HCT at wavelength 250 and 272 nm.

Conc.	Valsartan						Hydrochlorothiazide					
	Abs. at 250±2 nm			Abs. at 272±2 nm			Abs. at 272±2 nm			Abs. at 250±2 nm		
	248nm	250nm	252nm	270nm	272nm	274nm	270nm	272nm	274nm	248nm	250nm	252nm
10 ppm	0.055	0.1147	0.0626	0.8282	0.1917	0.7049	0.0588	0.0879	0.0289	0.1099	0.0719	0.036
Mean	0.077			0.574			0.0585			0.072		
SD	0.0324			0.337			0.0295			0.0369		
% RSD	0.419			0.587			0.504			0.509		

Table 9: Ruggedness of VAL and HCT at wavelength 250 and 272 nm.

Wavelength	Valsartan						Hydrochlorothiazide					
	250 nm			272 nm			272 nm			250 nm		
Conc.	8	10	12	8	10	12	8	10	12	8	10	12
Analyst 1	0.1958	0.6442	0.0188	0.3675	0.1917	0.1111	0.06	0.088	0.21	0.084	0.072	0.318
Analyst 2	0.0872	0.0634	0.2242	0.0818	0.441	0.0811	0.1	0.069	0.03	0.194	0.147	0.194
Mean	0.1415	0.3538	0.1215	0.22465	0.31635	0.0961	0.08	0.0785	0.12	0.139	0.1095	0.256
SD	0.076	0.4106	0.14524	0.20202	0.176282	0.021213	0.028284	0.013435	0.127279	0.077782	0.053033	0.087681
% RSD	0.5	1.1	1.19	0.89	0.55	0.22	0.35	0.17	1.06	0.55	0.48	0.34

3.13 LOD and LOQ

Calculating the LOD and LOQ values allowed us to determine the sensitivity of the method. The LOD of the analytical method for both drugs at 250 and 272 nm was 0.0818 and 0.0530 for VAL, respectively, and the LOQ was 0.2480 and 0.1607 for HCT, respectively, indicating that the method developed for the estimation of both drugs has a high degree of sensitivity [22].

3.14 Assay of drugs in bulk and formulation

The absorptivity value, also known as the specific absorbance (1%, 1 cm), is a fundamental parameter in ultraviolet-visible spectrophotometry used to determine a substance's ability to absorb light at a particular wavelength. It is calculated using the Beer-Lambert law, which relates absorbance to concentration and path length [32]. An absorptivity value of these drugs at selected wavelengths is given in Table 10.

Table 10: Absorptivity values of drugs at selected wavelengths.

Drugs	Absorptivity Value	Absorbance maxima (nm)	
		250nm	272nm
VAL	ax1	0.0462	-
	ax2	-	0.0223
HCT	ay1	0.0339	-
	ay2	-	0.0592

By applying the developed simultaneous estimation method for drugs in bulk form, the amount found corresponds to 39.79 and 11.60 for VAL and HCT, respectively. The% label claim was found to be 99.49% and 100.84%. According to the percentage claim, the official standards the label claim for tablet as well as drug assay is 90 to 110%. Using the method developed for tablets, the amount found corresponds to 39.51 and 11.9, VAL and HCT, respectively. % label claim was found 98.77% and 95.24%. According to the percentage, the official standards show the percent label claim for tablets. Table 11 presented the results of these study findings. The results were within the acceptable range [33].

Table 11: Application of proposed developed method for analysis of drugs in bilk and tablets at 250 and 272nm.

Bulk/Tablets	Amount (mg)	Absorbance	Mean	Amt. Found	% Amount Found	SD	%RSD
Bulk drugs in mixture	VAL (40mg)	1.7258	1.7266 (A1)	39.79	99.49	0.000283	0.669
		1.7272					
		1.7269					
	HCT (12.5mg)	0.5638	0.5655 (A2)	11.6	100.84	0.0018	0.329
		0.5653					
		0.5675					
DIOVAAL-40H Tablets	VAL (40mg)	1.7143	1.7148 (A1)	39.51	98.775	0.00045	0.026
		1.7148					
		1.7152					
	HCT (12.5mg)	0.5353	0.5355 (A2)	11.9	95.24	0.0018	0.345
		0.5338					
		0.5375					

The UV spectrophotometric method was optimized considering the QbD approach to investigate this drug absorbance behavior under various scenarios [25]. This detailed analysis guarantees the safety and effectiveness of pharmaceutical products by verifying the specificity of the method and providing valuable information on the drug analysis profiles. The validation studies then made use of these idealized settings. The results of this optimisation method were good specificity, linearity, and remarkable sensitivity. Accuracy was demonstrated by the percentage recovery of all analytes that fell within the permitted limits. The precision was confirmed by the % RSD values in the intra- and inter-day precision studies, which consistently remained below 2%. The LOD and LOQ for VAL and HCT were within the acceptable range. The robustness study showed that deliberate adjustments to different wavelengths had no discernible impact on outcomes. Furthermore, the % test of the marketed formulation produced results of 98.78% for VAL and 95.24% for HCT, confirming the efficacy of the established test protocols. Uniqueness is supported by empirical evidence and offers advantages in terms of speed and cost-effectiveness [33]. It is revealed to be a cost-effective option because the entire analysis time was less than 3 minutes and the developed method was found to be both specific and selective, since the common formulation excipients found in tablet dosage form did not interfere with the estimation process. The validation parameters findings were well within the bounds set by the ICH Q2 recommendations, confirming the accuracy and applicability for use in pharmaceutical analysis. The validation of the method with reference to all of the aforementioned parameters indicated a % RSD of less than 2%, which was found to be satisfactory. The solutions remained stable throughout the study. A UV spectrophotometric method was developed to estimate both VAL and HCT in combination. The method was shown to be simple, accurate, precise, cost-effective, and specific for determining and quantifying these medicines in bulk mixtures and tablets. The present method has less time, provides better robustness, and higher accuracy. The method's results are within the prescribed limit, indicating that it is free of excipient interference.

4. Conclusion

As regulations require, the recently developed Ishikawa fishbone diagram and C-N-X approach, along with the central composite design (CCD), have undergone extensive

optimisation for comprehensive risk assessment and validation according to the ICH Q2 guidelines, demonstrating appropriate levels of sensitivity, accuracy, and precision. The established UV spectrophotometric method is simple, specific, accurate, reproducible and reliable for quantifying valsartan and hydrochlorothiazide in tablet and bulk formulations. This technique can be easily used for the routine simultaneous estimation of these particular drugs regularly in quality control laboratories.

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