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Analytical Quality by Design (AQbD) Driven Development of a Robust Stability-Indicating RP-HPLC Method for the Precision Estimation of Dapagliflozin and Vildagliptin in Combined Fixed-Dose Tablets

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ABSTRACT: A simple, accurate, precise, and stability-indicating RP-HPLC method was developed and validated for the simultaneous estimation of Dapagliflozin (DAPA) and Vildagliptin (VILDA) in a combined tablet dosage form using a Quality by Design approach. Method development was carried out systematically by identifying important analytical parameters and optimizing them using the Design of Experiments. Chromatographic separation was achieved using an Inertsil ODS-3 C18 column with phosphate buffer (pH 4.5) and acetonitrile as the mobile phase at a flow rate of 1.0 mL/min. The detection was performed at 210 nm. Vildagliptin and Dapagliflozin were eluted at approximately 2.4 min and 7.7 min, respectively, with good peak shapes and satisfactory resolutions. The developed method was validated according to the ICH guidelines for parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection, and limit of quantification. The method showed good linearity for both drugs, with correlation coefficient values greater than 0.999. The accuracy results were within acceptable limits, and the percentage RSD for precision studies was less than 2%, indicating good reproducibility of the method. Forced degradation studies under acidic, alkaline, oxidative, thermal, photolytic, and humid conditions showed that the method could effectively separate the drugs from their degradation products, proving its stability-indicating nature. Therefore, the proposed RP-HPLC method is simple, reliable, cost-effective, and suitable for routine quality control analysis and stability studies of combined pharmaceutical dosage forms of Dapagliflozin and Vildagliptin.

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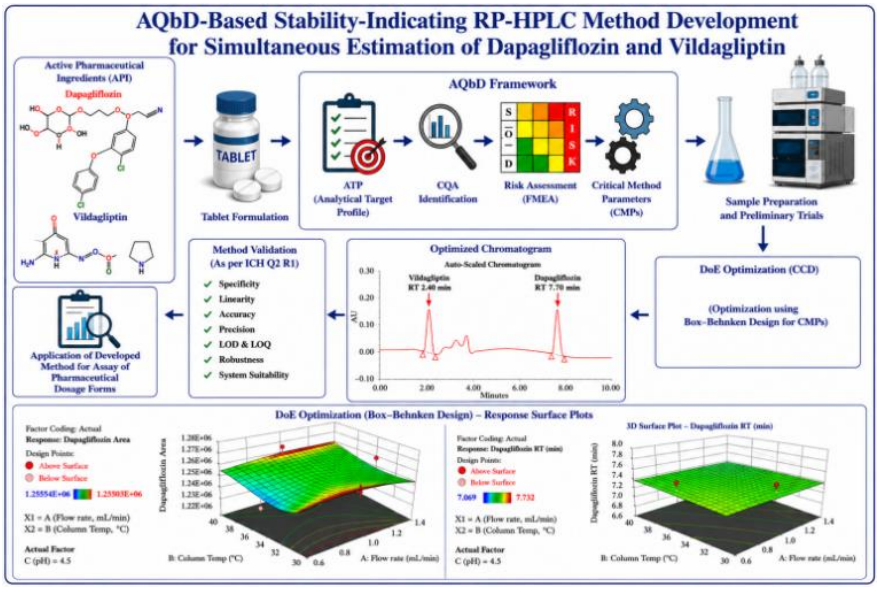
Graphical Abstract:



1. INTRODUCTION:

Chemically, DAPAGLIFLOZIN is a (2S,3R,4R,5S,6R)-2-[4-chloro-3-((4-ethoxyphenyl) methyl, phenyl)-6-(hydroxymethyl) oxane-3,4,5-triol. It is a crystalline solid with a colour ranging from white to off-white and a molecular weight of 408.9 g/mol with the formula C21H25ClO6. It is soluble in a variety of organic solvents. Slightly soluble in DMSO, it is invisible or insoluble in n-heptane but with no trouble soluble in methanol, water, as well as dichloromethane. It is also inadequately soluble in aqueous buffers. It has a melting point of 74–78°C. In addition to treating type 2 diabetes, dapagliflozin is used to treat heart attacks and chronic renal disease in adults. It is a newly invented, potent, reversible, competitive, highly selective, and orally active inhibitor of sodium glucose transport proteins (SGLT2), which are responsible for reabsorbing glucose into the kidney and lowering plasma glucose levels by excreting blood glucose through urine. As a result, patients with T2DM experience improvements in glycaemic control. The direct and insulin-independent renal clearance of glucose is a key component of DAPA's mechanism of action. [1–5]

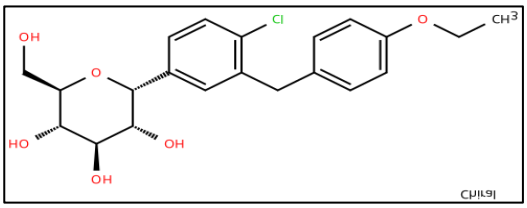


Figure 1: Chemical Structure of Dapagliflozin

VILDAGLIPTIN has the chemical formula C17H25N3O2 and a molecular weight of 303.4 g/mol. It is also known as (S)-1-(2-(3-Hydroxyadamantan-1-ylamino) acetyl) pyrrolidine-2-carbonitrile. It has a melting point between 148 and 152 °C and is white crystalline/slightly yellowish in nature. Methanol, water, and DMSO freely dissolved it. [6–8]

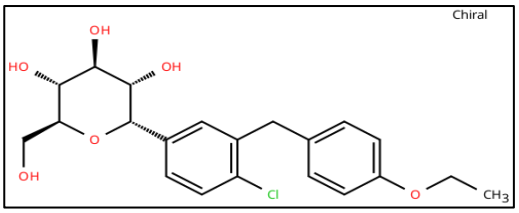


Figure 2: Chemical Structure of Vildagliptin

VILDAGLIPTIN is an orally active antihyperglycemic agent belonging to the class of "Islet Enhancer". It has a high affinity for the inhibition of DPP-4 (dipeptidyl peptidase-4) which acts on the incretin system. An incretin hormone called GLP-1 blocks glucagon secretion, promotes insulin production, and is swiftly removed by DPP-4, which increases GLP-1 levels and lowers glucose levels. VILDA reduces hyper-glycemia in T2DM patients by increasing pancreatic islet function. In this study, we developed a stability-indicating liquid chromatographic system for the simultaneous estimation of DAPA and VILDA in bulk and pharmaceutical formulations to satisfy the standards of the International Conference on Harmonization (ICH). VILDA and DAPA in bulk samples and pharmaceutical formulations can be properly analysed using the described method. [9–12]

2. MATERIALS AND METHODS:

2.1 Materials:

The Dapagliflozin working standard was generously provided by Torrent Pharmaceuticals (Ahmadabad, Gujarat, India), and the Vildagliptin working standard was sourced from Sigma-Aldrich. The commercially available formulation, comprising 5 mg of Dapagliflozin and 100 mg of Vildagliptin, was acquired from a local pharmacy. Acetonitrile and methanol of high-performance liquid chromatography (HPLC) grade were procured from Merck Chemicals (India). Potassium dihydrogen phosphate, orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide used in this study were of analytical reagent grade. HPLC-grade water was prepared using a Milli-Q water purification system.

2.2 Instrumentation and Chromatographic Infrastructure :

Chromatographic analysis was performed using a Waters Alliance HPLC system equipped with a UV/PDA detector, autosampler, and quaternary pump. Separation was achieved using an Intersil ODS-3 C18 column (150 mm × 4.6 mm, 5 µm particle size). Data acquisition, integration, and processing were performed using Empower software. UV spectral analysis was performed using a Shimadzu UV-1900 UV-visible spectrophotometer. A calibrated analytical balance (Mettler Toledo), ultrasonic bath (Citizen), pH meter (Mettler Toledo), centrifuge (REMI), photostability chamber (Thermolab), hot air oven (Memmert), and stability chamber were used during the study.

2.3 Optimized Chromatographic Conditions :

The mobile phase consisted of a phosphate buffer (pH 4.5 adjusted with orthophosphoric acid) and acetonitrile. Chromatographic separation was performed at a flow rate of 1.0 mL/min with detection at 210 nm. The column temperature was maintained at 35°C ± 2°C, and the injection volume was 20 µL. Methanol: water (50:50, v/v) was used as the diluents.

2.4 Preparation of Standard Stock Solutions:

To prepare a Dapagliflozin stock solution at a concentration of 100 µg/mL, precisely weigh 10.0 mg of the Dapagliflozin working standard and transfer it to a 100 mL volumetric flask. Subsequently, approximately 70 mL of diluent was added, the mixture was sonicated for 10 min, allowed to cool to room temperature, and the volume was adjusted to 100 mL with additional diluent. Vildagliptin Stock Solution (1000 µg/mL): Precisely weigh 100.0 mg of the vildagliptin reference standard and transfer it to a 100 mL volumetric flask. Approximately 70 mL of diluent was added, sonicated for 10 min, allowed to cool to room temperature, and adjusted to volume with diluent. Mixed Working Standard Solution (DAP: 10 µg/mL; VIL: 100 µg/mL): Transfer 5.0 mL of DAP stock and 5.0 mL of VIL stock solution into a 50 mL volumetric flask and dilute to volume with diluents. This represents 100% of the label-claimed concentration and is used as the working standard.

2.5 Preparation of Sample Solution (Tablet Formulation) :

Twenty tablets of Dapagliflozin and Vildagliptin were weighed accurately, and the average tablet weight was determined. The tablets were finely powdered in a clean, dry mortar and pestle. An accurately weighed quantity of tablet powder equivalent to 10 mg of Dapagliflozin and 100 mg of Vildagliptin was transferred into a 100 mL volumetric flask. Then, ~70 mL of diluent was added and sonicated for 30 min. The mixture was cooled to room temperature and diluted to volume with the diluent. The samples were centrifuged at 4000 rpm for 10 min. The supernatant was filtered through a 0.45 µm PVDF filter, and the first 5 mL of filtrate was discarded. The suitably diluted aliquots were injected into the HPLC system. [18–20]

2.6 QbD-Based Method Optimization Workflow

Quality by Design (QbD)-based method development was performed using the Design-Expert software version 12.0.0. Risk assessment and experimental models were applied to evaluate the effects of critical method parameters on chromatographic responses and to establish the analytical design space. Preliminary experimental trials were performed to identify the critical method parameters affecting chromatographic separation. Based on the initial screening studies, flow rate (A), column temperature (B), and mobile phase pH (C) were identified as the critical method parameters and selected as the independent variables for optimization using the Design of Experiments (DoE) approach. The selected variables were evaluated at three levels to study their influence on the chromatographic responses, namely Dapagliflozin peak area, Dapagliflozin retention time, Dapagliflozin theoretical plates, Dapagliflozin tailing factor, Vildagliptin peak area, Vildagliptin retention time, Vildagliptin theoretical plates, and Vildagliptin tailing factor.

3. RESULTS AND DISCUSSION:

3.1 AQbD Method Design Factor Screening

Table 1: Variables Selected in Box-Behnken Design

Factors (Independent Variables)	Symbol	Lower Level (-1)	Intermediate Level (0)	Higher Level (+1)
Flow Rate (mL/min)	A	0.9	1.0	1.1
Column Temperature (°C)	B	30	35	40
Mobile Phase pH	C	4.3	4.5	4.7

Table 2: 2A Chromatographic Responses and the Experimental Design Matrix Using Box-Behnken Design for Dapagliflozin

Std	Run	Flow Rate (mL/min)	Column Temp (°C)	Mobile Phase pH	Dapagliflozin Area (AU)	Dapagliflozin RT (min)	Dapagliflozin Plates	Dapagliflozin Tailing
14	1	1	35	4.7	1.23552×10^6	7.721	19309	1.2
4	2	1.1	40	4.3	1.24787×10^6	7.015	19031	1.2
10	3	1.1	35	4.5	1.24909×10^6	7.043	19403	1.1
7	4	0.9	40	4.7	1.23975×10^6	7.709	19094	1.2
13	5	1	35	4.3	1.25093×10^6	7.057	19422	1.1
8	6	1.1	40	4.7	1.24093×10^6	7.721	19542	1.1
1	7	0.9	30	4.3	1.25763×10^6	7.722	19532	1.1

12	8	1	40	4.5	1.25902 ×10⁶	7.321	19320	1.2
2	9	1.1	30	4.3	1.24432 ×10⁶	7.732	19742	1.1
15	10	1	35	4.5	1.24314 ×10⁶	7.344	19594	1.1
3	11	0.9	40	4.3	1.25092 ×10⁶	7.003	19555	1.1
9	12	0.9	35	4.5	1.23665 ×10⁶	7.721	19511	1.2
6	13	1.1	30	4.7	1.24445 ×10⁶	7.022	19511	1.1
11	14	1	30	4.5	1.25873 ×10⁶	7.703	19799	1.1
5	15	0.9	30	4.7	1.25094 ×10⁶	7.021	19410	1.1

Table 2: 2B Chromatographic Responses and the Experimental Design Matrix Using Box-Behnken Design for Vildagliptin

Std	Run	Flow Rate (mL/min)	Column Temp (°C)	Mobile Phase pH	Vildagliptin Area (AU)	Vildagliptin RT (min)	Vildagliptin Plates	Vildagliptin Tailing
14	1	1	35	4.7	3.56054 ×10⁶	2.571	4658	1.3
4	2	1.1	40	4.3	3.50888 ×10⁶	2.187	5095	1.2
10	3	1.1	35	4.5	3.50245 ×10⁶	2.154	5098	1.2
7	4	0.9	40	4.7	3.50754 ×10⁶	2.166	5294	1.2
13	5	1	35	4.3	3.50466 ×10⁶	2.159	5340	1
8	6	1.1	40	4.7	3.50993 ×10⁶	2.169	5166	1.1
1	7	0.9	30	4.3	3.55984 ×10⁶	2.544	4999	1.1
12	8	1	40	4.5	3.54905 ×10⁶	2.354	5167	1.2
2	9	1.1	30	4.3	3.55098 ×10⁶	2.51	5102	1.1
15	10	1	35	4.5	3.54456 ×10⁶	2.365	5169	1.1

3	11	0.9	40	4.3	3.5583 ×10⁶	2.497	5110	1.1
9	12	0.9	35	4.5	3.56094 ×10⁶	2.537	5987	1.1
6	13	1.1	30	4.7	3.50886 ×10⁶	2.187	5095	1.2
11	14	1	30	4.5	3.56632 ×10⁶	2.577	5077	1.1
5	15	0.9	30	4.7	3.50855 ×10⁶	2.154	5056	1.3

Table 3: ANOVA Data Summary for Response Surface Models

Statistical Parameter	RT of DAPA	RT of VILDA	Area of DAPA	Area of VILDA	Plates of DAPA	Plates of VILDA	Tailing of VILDA
Adjusted R^2	0.4877	0.0813	0.3171	0.0457	0.2105	0.0000	0.3243
Predicted R^2	-0.1861	-0.3007	-1.0226	-0.3268	-0.3878	-0.1480	-0.7264
R-Squared (R^2)	0.7073	0.2782	0.7561	0.2502	0.3797	0.0000	0.6139
% C.V.	3.23	7.36	0.5014	0.7290	0.9496	5.30	5.94
Suggested Model	2FI	Linear	Quadratic	Linear	Linear	Mean	2FI
F-value (Model)	3.22	1.41	1.72	1.22	2.24	N/A	2.12
P-value (Model)	0.0648	0.2912	0.2849	0.3472	0.1402	N/A	0.1606

Final Polynomial Equations in Terms of Coded Factors:

- **Dapagliflozin Peak Area** = +1.247E+06 - 923.10 A - 1758.10 B - 4007.70 C + 2241.00 AB + 1381.50 AC - 1444.25 BC - 5418.72 A^2 + 10584.28 B^2 - 5065.72 C^2
- **Dapagliflozin Retention Time** = +7.39 - 0.0643 A - 0.0431 B + 0.0665 C + 0.0016 AB - 0.0011 AC + 0.3529 BC
- **Dapagliflozin Theoretical Plates** = +19451.67 + 12.70 A - 145.20 B - 41.60 C
- **Vildagliptin Peak Area** = +3.534E+06 - 11407.40 A - 6117.30 B - 8723.80 C

The chromatographic method was optimized using a Box Benthehen Design under the Analytical Quality by Design approach. A total of 15 experimental runs were evaluated using software tools to study the effects of these factors on the responses. The relationship between the variables and responses was described using a second-order polynomial model.

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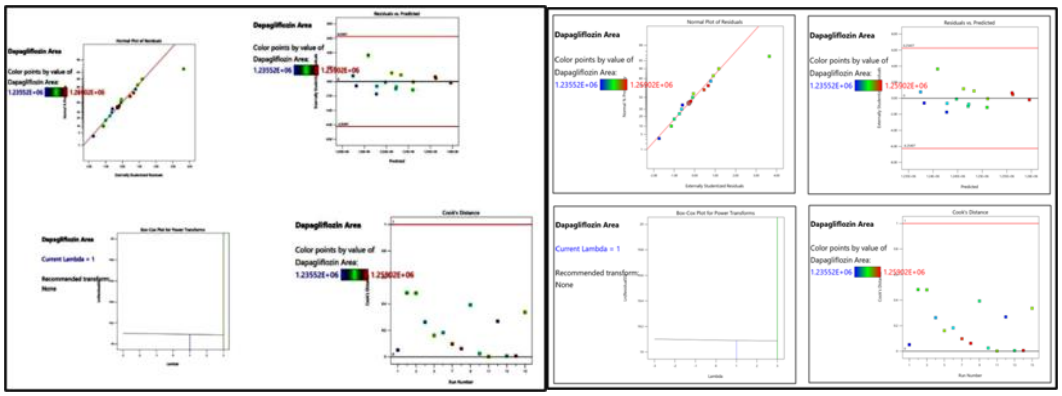

Figure 3A **Figure 3B**

Figure 3: Combined statistical diagnostic plots (Normal plot, Residuals vs. Predicted, Box-Cox, and Cook's distance for the Theoretical plates of Dapagliflozin 3A & Vildagliptin 3B

Normal Plot of Residuals [DAP / VIL]: Points line up tightly along the linear diagonal, verifying that experimental errors satisfy normal distribution assumptions.

Residuals vs. Predicted Plot [DAP / VIL]: Data points are randomly distributed above and below the zero line within standard limits boundary line (± 3.82), proving homoscedasticity.

Box-Cox Plot [DAP / VIL]: The current Lambda line (= 1.0) stays safely inside the 95% confidence interval boundary, confirming that the raw data requires no mathematical transformations.

Cook's Distance Plot [DAP / VIL]: All 15 operational matrix runs sit well below the critical value limit line (1.0), proving the complete absence of any influential outlier values in the design space loop.Figure 3: Multi-Panel Response Surface Diagnostic Plots for Dapagliflozin Area

AQbD Method Design Factor Screening (Perturbation Analysis)

The individual effects of the independent variable parameters—Flow rate (A), Column Temperature (B), and Mobile Phase pH (C)—on the critical analytical response metric (Peak Area) of the drugs Dapagliflozin and Vildagliptin were systematically evaluated using a comparative perturbation tracking plot. This visualization demonstrates how the response area of the drug changes as each factor moves away from its selected reference midpoint setting (A = 1.0 mL/min, B = 35°C, C = 4.5) while holding all other parameters completely constant.

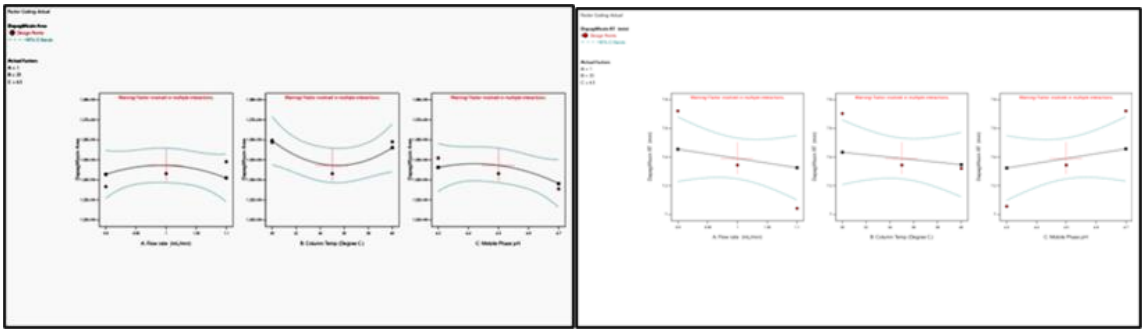

Figure 4A **Figure 4B**

Figure 4: Perturbation plots for critical method parameters on the peak area of Dapagliflozin (4A) & Vildagliptin (4B)
AQbD Method Design Factor Screening (Interaction Contour Analysis)

To comprehensively understand the synergistic interaction between the critical independent variables, a two-dimensional (2D) contour plot was analyzed. This graph maps out the joint effects of Factor A (Flow rate) and Factor B (Column Temperature) on the critical quality attribute (Peak Area) of the drugs Dapagliflozin and Vildagliptin, while keeping the third factor, Mobile

Phase pH, constant at its intermediate midpoint setting of 4.5.

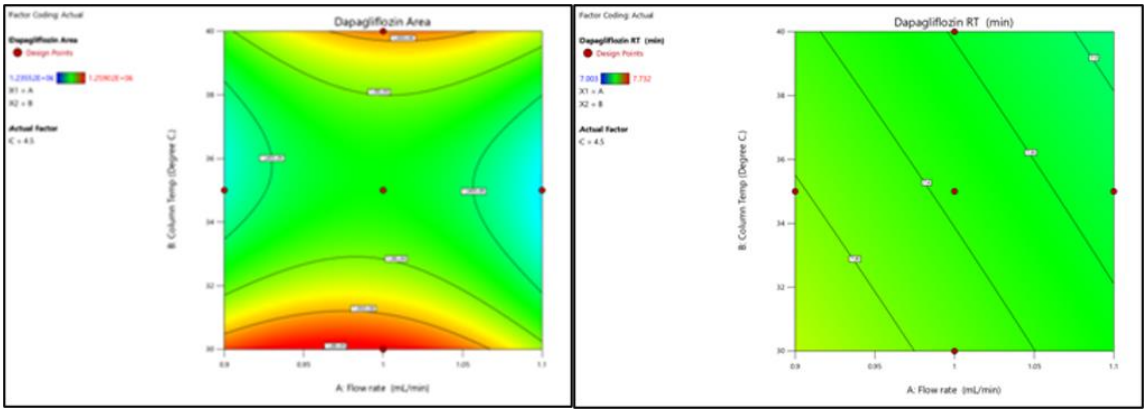

Figure 5A **Figure 5B**

Figure 5A & 5B: 2D Contour plot showing the interaction of flow rate and column temperature on Dapagliflozin (5A), Vildagliptin (5B) peak area.

AQbD Method Design Factor Screening (3D Surface Analysis)

To visualize the simultaneous interaction of the critical independent variables, a three-dimensional (3D) response surface plot was analyzed. This plot demonstrates the combined effects of flow rate (Factor A) and column temperature (Factor B) on the critical response parameter (Peak Area) of the drug Dapagliflozin, and Vildagliptin with mobile phase pH held at 4.5. The highest peak response area for Dapagliflozin (>1.259 $\times 10^6$, red-colored zone) is located at the lowest temperature boundary 30 -31 ^{0}Ccombined with intermediate mid-level flow rates (0.95 to 1.05 mL/min). The highest peak response area for Vildagliptin (>3.566 $\times 10^6$, corresponding to the red-colored zone) is located at the lowest temperature boundary 30 -31 ^{0}C combined with intermediate mid-level flow rates (0.95 to 1.05 mL/min)

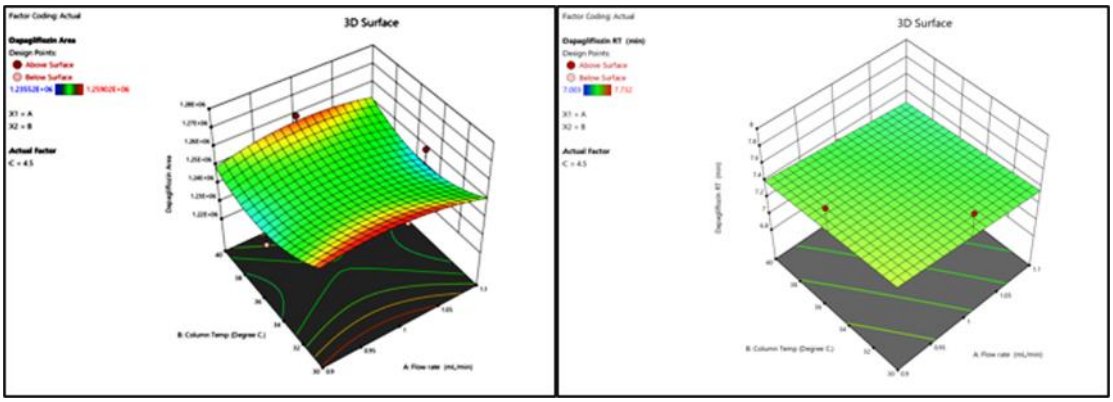

Figure 6A **Figure 6B**

Figure 6A & 6B: 3D Response surface plot showing the interactive effect of flow rate and column temperature on Dapagliflozin peak area (6A), Vildagliptin peak area(6B)

3.2 System Suitability

System suitability tests are performed prior to and during analytical runs to verify that the chromatographic system is functioning correctly. Five replicate injections of the mixed working standard solution were made and the following parameters evaluated as per USP and ICH Q14 guidelines:

Table 4: System Suitability Parameters and Operational Observations

System Suitability Parameter	Acceptance Criterion	Observed Value (n=5)	Compliant Status
Retention Time — DAP (min)	~7.5 ± 0.5	7.45	Yes
Retention Time — VIL (min)	~2.0 ± 0.5	2.06	Yes
Resolution (Rs) between DAP and VIL	≥ 2.0	19.8	Yes
Tailing Factor — DAP	≤ 2.0	1.1	Yes
Tailing Factor — VIL	≤ 2.0	1.2	Yes
Theoretical Plates — DAP	≥ 2000	14979	Yes
Theoretical Plates — VIL	≥ 2000	5587	Yes
% RSD of Peak Area — DAP (n=5)	NMT 2.0%	0.6	Yes
% RSD of Peak Area — VIL (n=5)	NMT 2.0%	0.2	Yes

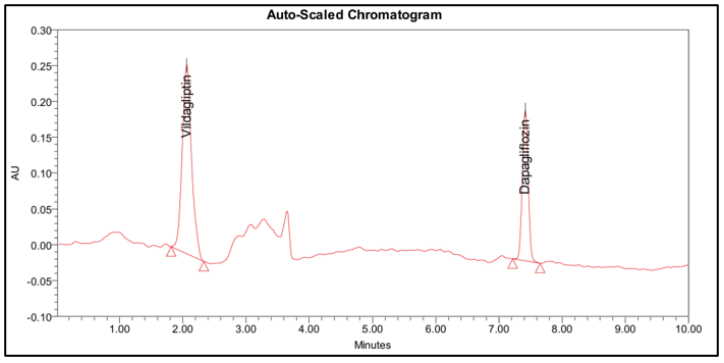

Figure 7: Auto-scaled RP-HPLC chromatogram showing resolution of Vildagliptin and Dapagliflozin.

3.3 Method Validation

The analytical method was validated as per ICH Q2 (R2) and ICH Q14 guidelines. Validation parameters included: Specificity, Linearity and Range, Accuracy, Precision, Robustness, and Solution Stability.

- **Linearity and Range:** Linearity was evaluated by preparing calibration solutions at concentration levels spanning 50-150% of nominal concentration. Dapagliflozin Linearity Range: 2.5 – 7.5 µg/mL with an R^2 value of 0.9999. Vildagliptin Linearity Range: 50 - 150 µg/mL with an R^2 value of 1.0000.

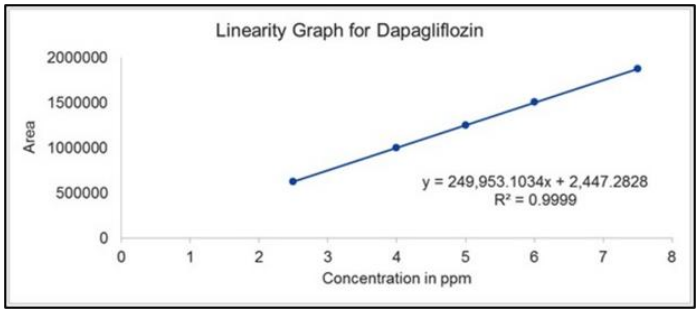

Figure 8: Linearity Calibration Plot for Dapagliflozin Standard Response

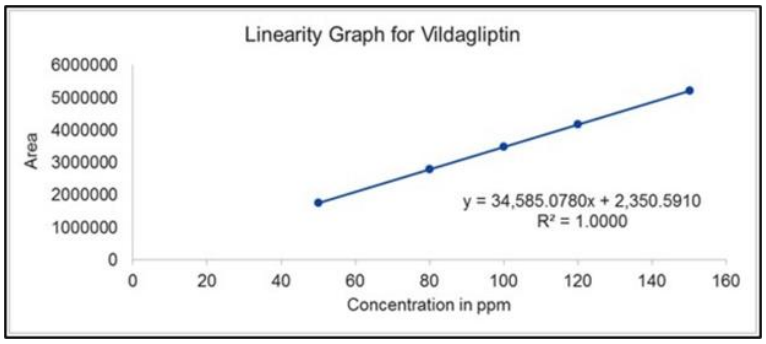

Figure 9: Linearity Calibration Plot for Vildagliptin Standard Response

- **Precision:** System precision was verified via five replicate injections. Method precision was evaluated by preparing and injecting six sample solutions of the same batch, returning percentage RSD values well under the 2.0% requirement.

- **Robustness:** Evaluated by deliberately varying critical conditions around the optimised settings; variations generated negligible effects on systemic performance parameters.

3.4 Forced Degradation Studies (Stress Testing)

Forced degradation studies were performed as per ICH Q1A (R2) guidelines to establish the stability-indicating nature of the developed method and to identify potential degradation products. Individual drug solutions were prepared at a concentration of 5 µg/mL for DAP and 100 µg/mL for VIL in their respective stress media. Peak purity was assessed using the PDA detector by comparing spectral purity angle with spectral purity threshold. The method cleanly resolved all degradation products from both analyte peaks, confirming its stability-indicating capability.

Table 5: Forced Degradation and Stress Analysis Data for Dapagliflozin (DAP)

Forced Degradation Stress Pathway	Peak Area	Weight (mg)	% Assay	% Degradation	Purity Angle	Purity Threshold
Sample preparation - Untreated	1256672	509.5	100.4	N/A	0.278	0.694
Sample preparation - Acid degradation	1230941	508.8	98.5	1.9	0.222	0.674
Sample preparation - Base degradation	1227643	509.5	98.1	2.3	0.325	0.666
Sample preparation - Oxidation degradation	1185532	509.2	94.8	5.6	0.227	0.732
Sample preparation - Heat degradation	1230943	509.7	98.3	2.1	0.276	0.777
Sample preparation - Humidity degradation	1229874	509.3	98.3	2.1	0.314	0.609
Sample preparation - Photolytic degradation	1221097	509.2	97.6	2.8	0.256	0.693

Table 6: Forced Degradation and Stress Analysis Data for Vildagliptin (VIL)

Forced Degradation Stress Pathway	Peak Area	Weight (mg)	% Assay	% Degradation	Purity Angle	Purity Threshold
Sample preparation - Untreated	3489843	509.5	100.7	N/A	0.571	1.123
Sample preparation - Acid degradation	3324249	508.8	96.1	4.6	0.499	1.208
Sample preparation - Base degradation	3345547	509.5	96.6	4.1	0.508	1.132
Sample preparation - Oxidation degradation	3021234	509.2	87.3	13.4	0.558	1.274
Sample preparation - Heat degradation	3429975	509.7	99.0	1.7	0.497	1.304
Sample preparation - Humidity degradation	3437776	509.3	99.3	1.4	0.583	1.254
Sample preparation - Photolytic degradation	3427898	509.2	99.0	1.7	0.521	1.324

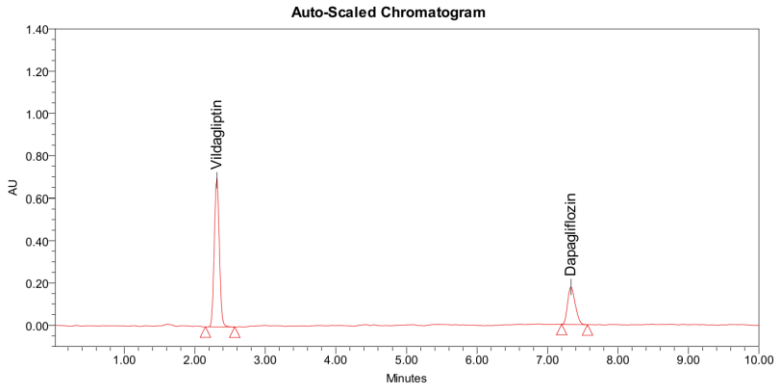

Figure 10: Acid degradation sample

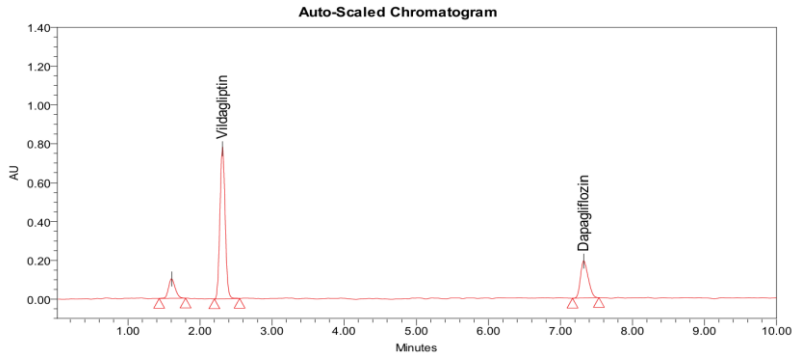

Figure 11: Base degradation sample

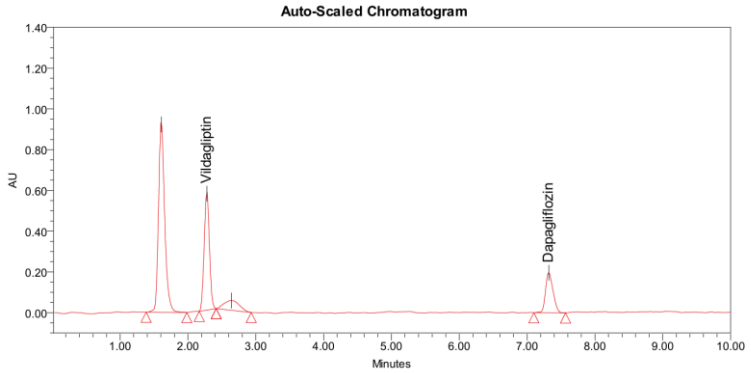

Figure 12: Oxidation degradation sample

3.5 Method Optimization and Design Space:

An overlay plot analysis was performed using Design-Expert to establish the method's design space. The yellow-highlighted zone confirms that variations in flow rate and column temperature within acceptable limits do not adversely affect chromatographic performance, verifying the method's overall robustness.

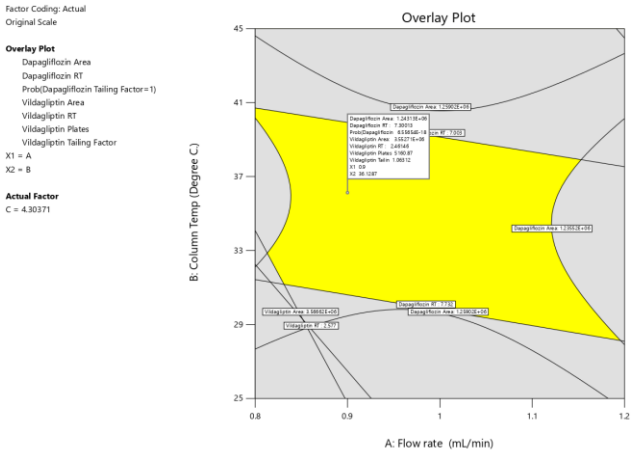

Figure 13: Overlay Contour Plot Representing the Method Operable Design Region (MODR) for Dapagliflozin and Vildagliptin

4. CONCLUSION

The present work describes a simple, rapid, precise, accurate, and stability-indicating RP-HPLC method developed for the simultaneous estimation of Dapagliflozin and Vildagliptin in a combined tablet dosage form using a QbD approach. The application of QbD and statistical tools enabled the systematic optimization of chromatographic conditions and provided a better understanding of the effect of critical method parameters on the critical quality attributes of the method. The developed method showed satisfactory peak resolution, good peak symmetry, and excellent linearity for both analytes within the selected concentration range. Validation studies proved that the method is specific, precise, accurate, and robust according to the ICH guidelines. Forced degradation studies revealed that the developed method was capable of separating degradation products from the analyte peaks under acidic, alkaline, oxidative, thermal, photolytic, and humidity stress conditions without any operational interference, confirming the stability-indicating nature of the method. Hence, it can be effectively applied for routine quality control analysis and stability studies in the pharmaceutical industry.

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6. CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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