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Analytical Method Development and Validation of Alogliptin and Dapagliflozin in Tablet Dosage Form by RP HPLC

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Abstract: *The development and validation of a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous quantification of Alogliptin and Dapagliflozin in tablet dosage forms are presented in this study. Methanol was selected as the optimal solvent due to its superior solubility for both drugs. The wavelength for detection was set at 286 nm, as determined by the overlay PDA-spectrum, ensuring accurate quantification. The method was optimized using an HPLC C18 column (150 mm × 4.6 mm, 2.5 µm particle size) with a mobile phase comprising acetonitrile and 0.05% ortho phosphoric acid (80:20 % v/v), adjusted to pH 6.5 with 0.1% triethylamine. The chromatographic conditions were fine-tuned to achieve excellent resolution and symmetrical peak shapes for both drugs, with retention times of 5.690 ± 0.02 min for Alogliptin and 3.044 ± 0.022 min for Dapagliflozin. The method was validated as per ICH guidelines, demonstrating linearity, accuracy, precision, sensitivity, robustness, and specificity. Linearity was confirmed for Alogliptin (5–25 µg/mL) and Dapagliflozin (40–200 µg/mL) with correlation coefficients (r^2) of 0.9999 and 0.9996, respectively. The method exhibited satisfactory accuracy with recovery rates within 99-101% and precision with %RSD less than 2%. Sensitivity analysis revealed limits of detection (LOD) and quantification (LOQ) appropriate for routine analysis. The method was successfully applied to the analysis of both bulk samples and marketed tablet formulations, proving its utility for quality control and routine analytical purposes in the pharmaceutical industry.*

Keywords: RP-HPLC, Alogliptin, Dapagliflozin, analytical method, validation, tablet dosage, linearity, accuracy.

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a prevalent chronic metabolic disorder characterized by persistent hyperglycemia resulting from insulin resistance and impaired insulin secretion.[1] Effective management of T2DM requires a multifaceted approach that targets various pathways involved in glucose metabolism. In recent years, combination therapy has become a cornerstone in diabetes management, with medications that act through different mechanisms to achieve better glycemic control. Among these therapies, Alogliptin and Dapagliflozin have emerged as significant players due to their distinct but complementary mechanisms of action.[2,3]

Alogliptin is a potent and selective inhibitor of dipeptidyl peptidase-4 (DPP-4), an enzyme responsible for the degradation of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). By inhibiting DPP-4, Alogliptin prolongs the action of these hormones, which enhances insulin secretion in response to meals and suppresses glucagon release, thus contributing to improved blood glucose levels. This mechanism helps in reducing postprandial glucose spikes and overall glycemic variability, which are crucial in the long-term management of diabetes.[4,5]

Dapagliflozin, on the other hand, functions as a sodium-glucose co-transporter 2 (SGLT2) inhibitor. SGLT2 is a protein located in the renal proximal tubules responsible for reabsorbing glucose from the urine back into the bloodstream. By inhibiting this transporter, Dapagliflozin promotes glucose excretion through the urine, thereby lowering blood glucose levels independently of insulin. This not only aids in glycemic control but also has beneficial effects on body weight and blood pressure, making it a valuable addition to diabetes therapy.[6,7]

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The combination of Alogliptin and Dapagliflozin represents a strategic approach to diabetes management, leveraging the strengths of both medications. Alogliptin addresses the insulin secretion and glucagon suppression pathways, while Dapagliflozin provides a glucose-lowering effect through renal excretion. This dual-action approach enhances the overall efficacy of treatment, providing more comprehensive glycemic control than either drug alone.[8,9]

To ensure the efficacy and safety of these combination therapies, accurate and reliable quantification of Alogliptin and Dapagliflozin in tablet dosage forms is essential. High-Performance Liquid Chromatography (HPLC) is a sophisticated analytical technique that offers the precision and sensitivity required for this task. HPLC allows for the simultaneous estimation of both drugs, facilitating quality control processes and ensuring that each tablet meets the required specifications for active ingredient content.[10] This not only ensures the therapeutic effectiveness of the medication but also supports regulatory compliance, safeguarding patient health and optimizing treatment outcomes. The development and validation of an HPLC method for this purpose are thus critical for maintaining high standards in pharmaceutical manufacturing and quality assurance.[10]

II. MATERIALS AND METHOD

The selection of the appropriate solvent was critical for ensuring the solubility of Alogliptin and Dapagliflozin, with methanol identified as the optimal solvent for these drugs. For wavelength selection, the overlay PDA spectrum revealed that 286 nm provided the highest absorption peak intensities for both drugs, thus this wavelength was chosen for quantification. The preparation of the stock standard solution involved dissolving 5 mg of Alogliptin and 40 mg of Dapagliflozin in a 100 ml volumetric flask with mobile phase to achieve final concentrations of 50 µg/ml and 400 µg/ml, respectively. Working standard solutions were then prepared by diluting 1.0 ml of this stock solution into 10 ml volumetric flasks to obtain concentrations of 5 µg/ml and 40 µg/ml. Reversed-phase HPLC was selected as the chromatographic technique to ensure the development of a stability-indicating assay method. An HPLC C18 column (150 mm × 4.6 mm, i.d., 2.5 µm particle size) was chosen for its efficacy in separating the analytes. The solvent system, comprising 80:20 % v/v Acetonitrile and 0.05 % Orthophosphoric Acid adjusted to pH 6.5 with 0.1% triethylamine, was prepared, sonicated for 20 minutes, and filtered through a 0.2 µm Ultipor® N66® Nylon 6, 6 membrane filter paper to ensure purity and optimal performance of the analytical method.[11,12]

Optimization of a solvent system

The view to resolve and quantify the Alogliptin and Dapagliflozin from the tablet matrix or degradation product is mostly meticulous based upon several variables comprised of Alogliptin and Dapagliflozin s polarities.

The solubility of drugs into specific and combination of solvents and also on reported data on the literature. Hence, selecting a solvent system for resolution and identification of Alogliptin and Dapagliflozin was done on various proportions of solvent systems. Primarily, acetonitrile (100 %) was tested. However, the low resolution and splitting of the peak were noticed, and early elution's of Alogliptin and Dapagliflozin peaks were noticed. [13]

Therefore, the efforts were made to experience various proportions of acetonitrile: 0.05% Ortho Phosphoric Acid/0.1 % Ortho Phosphoric Acid extending from 90:10 % *v/v* to 20:80 % *v/v* and methanol: 0.05% Ortho Phosphoric Acid/0.1 % Ortho Phosphoric Acid were employed, and it was noticed that reducing the proportion of Acetonitrile/methanol in the solvent leads to a longer retention time, although solvent interference was also observed.[14]

Thus, the proportion of acetonitrile: 0.05% Ortho Phosphoric Acid (80:20 % *v/v*) pH 6.5 adjusted with 0.1% triethylamine was found to be better in all respect, except interference from the solvent system. Accordingly, the samples were prepared using the solvent system and pH adjusted with 0.1% triethylamine to eliminate the interference from the solvent system. [15]

Finally, the solvent system comprises a proportion of acetonitrile: 0.05% Ortho Phosphoric Acid (80:20 % *v/v*) pH 6.5 adjusted with 0.1% triethylamine demonstrated good symmetrical peaks shape and excellent resolution of both eluents was also appropriate due to acceptable system suitability tests. [16]

The total analysis time for quantification of Alogliptin and Dapagliflozin was below 6 min. The R*t* of Alogliptin and Dapagliflozin was 5.690 ± 0.02 min and 3.044 ± 0.022 min, respectively. [17]

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Validation: [18,19]

Linearity Studies for Alogliptin

Linearity studies were conducted to establish the relationship between the concentration of Alogliptin and its peak area in HPLC analysis. The study involved preparing solutions of Alogliptin at various concentrations and recording their peak areas to determine the linearity of the method.

Linearity Studies for Dapagliflozin

Similar to Alogliptin, linearity studies for Dapagliflozin were performed to assess the correlation between its concentration and peak area. Solutions of Dapagliflozin at different concentrations were prepared, and their peak areas were recorded to establish the method's linearity.

Analysis of Alogliptin and Dapagliflozin in Bulk Samples

Bulk samples of Alogliptin and Dapagliflozin were accurately weighed, dissolved in methanol, and diluted to prepare solutions for HPLC analysis. The analysis involved determining the amount of Alogliptin and Dapagliflozin in these samples by measuring the peak areas and calculating their respective concentrations.

Analysis of Marketed Formulation

Marketed tablet formulations containing Alogliptin and Dapagliflozin were analyzed to determine their content. The tablets were ground, and the powder was dissolved in methanol. The resulting solutions were analyzed by HPLC to quantify the Alogliptin and Dapagliflozin content in the tablets.

Validation

The RP-HPLC method was validated according to ICH guidelines, covering accuracy, precision, sensitivity, robustness, and specificity.

Accuracy

Accuracy was assessed by determining the recovery of Alogliptin and Dapagliflozin at various concentration levels (80%, 100%, and 120%) and comparing the measured values with the expected values.

Precision

Precision studies included intra-day, inter-day, and repeatability tests to evaluate the consistency of the method. The results helped determine the method's reliability and reproducibility.

Sensitivity

Sensitivity was evaluated by calculating the limit of detection (LOD) and limit of quantification (LOQ) for both Alogliptin and Dapagliflozin.

Robustness

The robustness of the method was tested by varying chromatographic conditions such as the % proportion of acetonitrile, wavelength, and flow rate. The method's performance under these variations was assessed to ensure its reliability.

Specificity

Specificity was tested to ensure that the HPLC method accurately measures Alogliptin and Dapagliflozin without interference from excipients or other substances in the formulations.

The RP-HPLC method for Alogliptin and Dapagliflozin demonstrated satisfactory performance in terms of linearity, accuracy, precision, sensitivity, robustness, and specificity. This method is suitable for the analysis of Alogliptin and Dapagliflozin in bulk and tablet formulations, making it a valuable tool for quality control and routine analysis.[18,19]

III. RESULTS AND DISCUSSION

Chromatographic conditions

An HPLC system of Agilent (1100) Gradient System pump and with UV VWD detector was used working via Chemstation10.1software. The separation was carried on Fortis column with C18 packaging and 4.6 x100 mm dimensions, 2.5µm particle size. The mobile phase consists of acetonitrile: 0.05 % ortho phosphoric acid pH 6.5 with 0.1% triethylamine in the ratio of 80:20 with a flow rate of 1mL/min. Before the execution of chromatographic analysis, the solvent system was filtered through a 0.2 µm membrane (Ultipor N_{66} Nylon 6, 6) and sonication of it for 20 min. The wavelength selected for the determination of Alogliptin and Dapagliflozin was 286.0nm. The total analysis

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time for quantification of Alogliptin and Dapagliflozin was below 6 min. The average retention times for Alogliptin and Dapagliflozin were 5.690 ± 0.02 min and 3.044 ± 0.022 min, respectively.

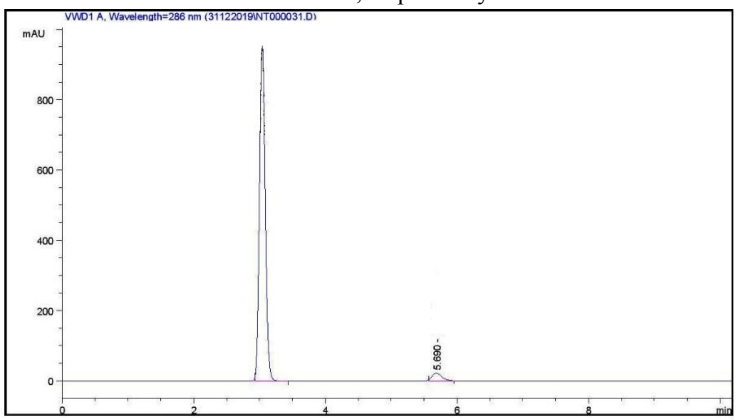

Figure 1: Optimized chromatogram for Alogliptin and Dapagliflozin

System suitability test

The system suitability was assessed using 5µg/mL and 40µg/mL concentrations of Alogliptin and Dapagliflozin (six determinations). The RSD values of peak area and retention time for Alogliptin and Dapagliflozin are within 2% indicating the suitability of the system. Both analytes i.e. Alogliptin and Dapagliflozin were continuously well resolved and retained at 5.7 and 3.0 min with RSD % less than 2 percent depicting strong reproducibility of the duplicate injections used on the integral LC system according to USP. In all chromatographic cycles, theoretical plate number still crossed over 2000 maintaining strong column efficacy across the entire separation process of investigation. The tailing factor and the number of USP plates were both found to be within reasonable limits.

Table 1: System suitability test

Parameters	Estimates for Alogliptin HCl	Estimates for Dapagliflozin
Retention time (Rt) (min)	5.701 ± 0.04	3.045 ± 0.022
Theoretical Plates	5035.56 ± 0.11	2545.14 ± 0.23
Tailing factor	1.09 ± 0.08	1.26 ± 0.09
Resolution	6.59	

Calibration curve

The calibration curve for Alogliptin and Dapagliflozin were assessed using the five working solutions. It was prepared using the precise aliquots (1 – 5 mL from combined stock standard solution) were accurately moved into the 10 mL series of a calibrated flask, and the volume was diluted to the mark of a calibrated flask with a solvent system to get the 5 – 25 µg/mL and 40 – 200 µg/mL concentrations of Alogliptin and Dapagliflozin, respectively. By making use of 100 µL Hamilton Syringe (Muttenz, Switzerland), a constant proportion of 20 µL solution (for each determination) was introduced into the HPLC system; repeated multiple times (five) for every single determination. The calibration curves of peak area against the µg/mL concentrations for Alogliptin and Dapagliflozin were plotted and analysed using the equation of linear regression in order to develop a relationship as a calibration curve. The determination coefficient (r^2 0.9999 and 0.9996) of the calibration curve obtained from the line indicates the excellent connection between the peak area and the Alogliptin and Dapagliflozin concentrations.

Table 2: Linearity studies for Alogliptin

Sr. No	Concentration of Alogliptin [µg/mL]	Peak Area [n=5]	Average%RSD
1	5	79.97	0.18
2	10	161.76	
3	15	246.87	

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4	20	327.38	
5	25	407.46	

Table 3: Linearity studies for Dapagliflozin

Sr. No	Concentration of Dapagliflozin [µg/mL]	Peak Area [n=5]	% RSD
1	40	2148.59	0.32
2	80	4153.98	
3	120	6292.13	
4	160	8393.11	
5	200	10272.85	

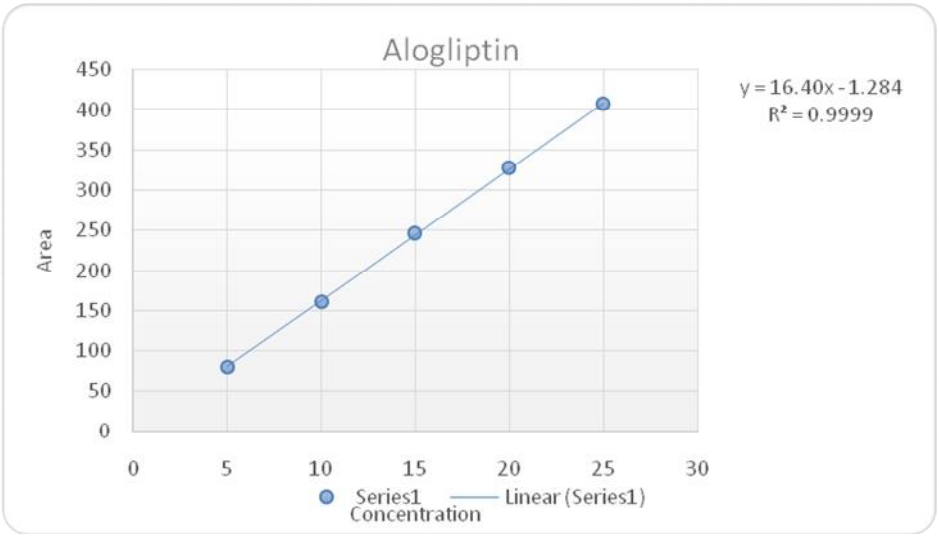

Figure 2: Calibration curve for Alogliptin

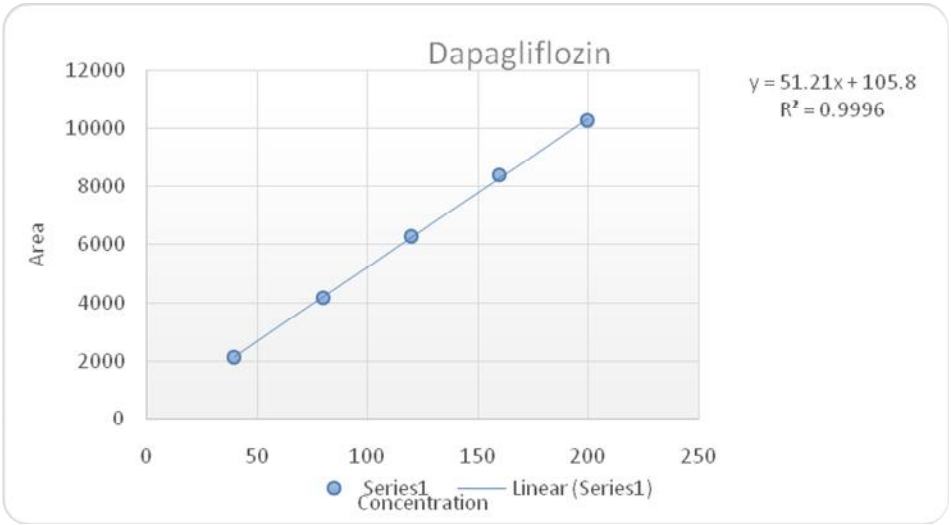

Figure 3: Calibration curve for Dapagliflozin

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Analysis of Alogliptin and Dapagliflozin in bulk samples

Alogliptin and Dapagliflozin, precise quantities were accurately weighed and transferred in 100 mL of the calibrated flask; solubilized in methanol, and the volume was diluted to the mark of a calibrated flask with same to have 50 µg/mL and 400 µg/mL concentrations of Alogliptin and Dapagliflozin. The suitable volumes of this were diluted with a solvent system to get the final concentrations of 20 µg/mL and 160 µg/mL of Alogliptin and Dapagliflozin that was analysed according to the procedure of chromatographic conditions; the peak areas of both analytes were estimated.

Table 4: Analysis of Alogliptin and Dapagliflozin in bulk material

Drugs	Amount taken [µg/mL]	Amount found [µg/mL] ± SD	% Amount found	% RSD [n=6]
Alogliptin HCl	20.0	19.94 ± 0.06	99.70 ± 0.45	0.01
Dapagliflozin	160.0	161.48 ± 0.03	100.92 ± 0.26	0.01

Analysis of marketed formulation

The assay of Alogliptin and Dapagliflozin in the marketed pharmaceutical formulation was performed for two different pharmaceutical matrices. To estimate the in tablet matrix, twenty tablets label claim Alogliptin - 5 mg and Dapagliflozin - 40 mg) were evaluated to estimate the average weight of the tablets and then ground and mixed through pestle and mortar. A portion of tablet powder corresponds to a weight of one tablet was precisely solubilized into 50 mL of methanol and sonicated for 15 min to obtained the complete dissolution of Alogliptin and Dapagliflozin and before made the volume to mark with same to achieved the concentrations of 50 µg/mL of Alogliptin and 400 µg/mL of Dapagliflozin; were filtered through a 0.45 µm membrane. The suitable volume of this was diluted with methanol to get the final concentrations of 20 µg/mL and 160 µg/mL of Alogliptin and Dapagliflozin that was analysed according to the procedure of chromatographic conditions; the peak area was estimated for selected peak.

Table 5: Analysis of Alogliptin in tablet formulation

Drug	Amount taken [µg/mL]	Amount found [µg/mL]	% Amount found
Alogliptin HCl	20	19.89	99.45
	20	19.99	99.95
	20	19.99	99.95
	20	19.96	99.80
	20	19.94	99.70
	20	19.87	99.35
	Mean ± SD	19.94 ± 0.12	99.70± 0.30
	% RSD	0.26	0.21

Table 6: Analysis of Dapagliflozin in tablet formulation

Drug	Amount taken [µg/mL]	Amount found [µg/mL]	% Amount found
Dapagliflozin	160	161.40	100.87
	160	161.90	101.18
	160	162.20	101.37
	160	160.95	100.59
	160	161.10	100.68
	160	159.50	99.68
	Mean ± SD	161.17 ± 0.015	100.72± 0.20
	% RSD	0.31	0.20

Validation

The design RP-HPLC method for Alogliptin and Dapagliflozin was explored by ICH recommendation.

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Accuracy

The accuracy of the designed RP-HPLC method for Alogliptin and Dapagliflozin was addressed in the context of % recovery and accomplished at three distinct levels, i.e., 80%, 100%, and 120%. The % recovery was exercised by adding a fixed amount of Alogliptin and Dapagliflozin standard to pre-analysed tablet solution (Alogliptin – 5 µg/mL and Dapagliflozin - 40 µg/mL) resulting solution was ultimately addressed using the established method.

The % recovery of the planned method was determined through the formula; Recovery (%) = A-B/C×100; where, A- total concentration of Alogliptin and Dapagliflozin; B- initial concentration of Alogliptin and Dapagliflozin and C- concentration added of Alogliptin and Dapagliflozin. The results of the % recovery of the planned method are given in **Table 3.5.8** and **3.5.9** for Alogliptin and Dapagliflozin respectively.

Table 7: Investigation of accuracy study for Alogliptin

Initial amount [µg/mL]	**Amount added** [µg/mL]	**Amount found** [µg/mL]	**% Recovery**	**% RSD**
		Alogliptin HCl	**Alogliptin HCl**	**Alogliptin HCl**
Level of recovery study 80 %				
5	4	8.99	99.83	0.99
5	4	9.04	101.24	
Mean ± SD = 100.54±0.04				
Level of recovery study 100 %				
5	5	5.06	101.27	0.40
5	5	5.01	100.70	
Mean ± SD = 100.99 ± 0.04				
Level of recovery study 120 %				
5	6	5.98	99.75	0.47
5	6	6.02	100.45	
Mean ± SD = 99.54 ± 0.03				

Table 8: Investigation of accuracy study for Dapagliflozin

Initial amount [µg/mL]	**Amount added** [µg/mL]	**Amount found** [µg/mL]	**% Recovery**	**% RSD**
		Dapagliflozin	**Dapagliflozin**	**Dapagliflozin**
Level of recovery study 80 %				
40	32	71.99	99.97	0.38
40	32	71.81	99.47	
Mean ± SD 99.70 ± 0.34				
Level of recovery study 100 %				
40	40	79.90	99.76	0.34
40	40	79.71	99.28	
Mean ± SD 99.52 ± 0.30				
Level of recovery study 120 %				
40	48	87.75	99.48	0.09
40	48	87.80	99.60	
Mean ± SD 99.54 ± 0.17				

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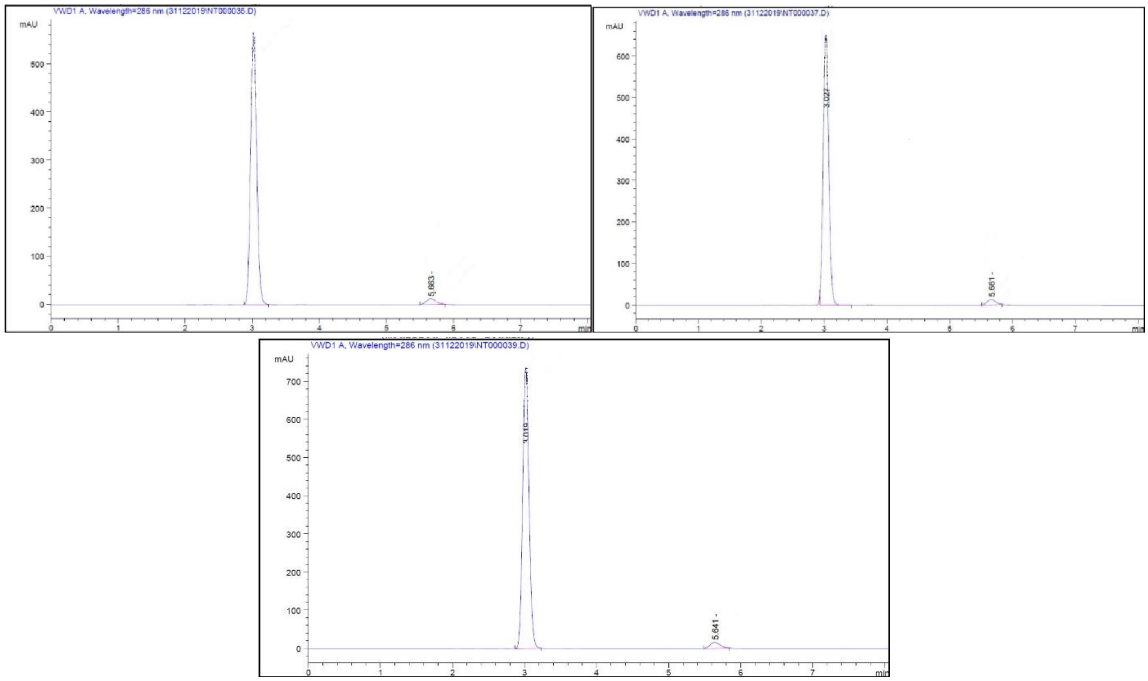

Figure 4: Accuracy of Alogliptinand Dapagliflozin (80%,100% and 120%)

Precision

The precision analysis of the method for Alogliptin and Dapagliflozin was investigated for intra, inter-day and repeatability and are expressed as % RSD. The three distinct concentrations 10, 15, and 20 µg/mL of Alogliptin and 80, 120, and 160 µg/mL of Dapagliflozin were assayed at different time on same day for intra-day precision and continuous for three successive days as per the ICH guidelines. Additionally, repeatability variability assessed using six determinations of 15 µg/mL of Alogliptin and 120 µg/mL of Dapagliflozin concentrations.

Table 9: Precision study for Alogliptin and Dapagliflozin

Intra-day Precision			Inter-day Precision		
Concentrations	**% Amount Found**	**% RSD**	**Concentration**	**% Amount Found**	**% RSD**
For Alogliptin HCl					
10	101.46	0.23	10	100.40	0.59
15	98.85	1.48	15	98.93	0.41
20	99.60	1.44	20	100.45	0.77
For Dapagliflozin					
80	99.13	0.23	80	99.14	0.24
120	100.65	0.02	120	100.62	0.03
160	101.69	0.05	160	101.11	0.06

Sensitivity

The sensitivity of the designed RP-HPLC method (LOD and LOQ) were calculated using standard deviation (N) of outcomes of the Alogliptin and Dapagliflozin (n=3) and calibration curve slope (B). The formulae exploited were LOD = 3.3 × N/B and LOQ = 10 × N/B. The planned method recorded LOD and LOQ values of 0.66 µg/mL and 1.84 µg/mL for Alogliptin and 2.11 µg/mL and 6.40 µg/mL for Dapagliflozin, respectively.

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Table 10: A Sensitivity study for Alogliptin and Dapagliflozin

Name of drug	LOD	LOQ
Dapagliflozin	2.11	6.40
Alogliptin HCl	0.66	1.84

Robustness

Robustness analysis of the designed RP-HPLC method was carried out by attempting to make significant changes in % proportion of acetonitrile and buffer in a solvent system, the wavelength, and flow rate. The influence of each of the independent variables was determined for the peak areas of both analytes. The selected independent variables for this analysis were varied as proportion of acetonitrile: buffer as (79:21 and 81:19), the absorption wavelength to 285 – 287 nm, and flow rate (0.9 – 1.1 mL/min). It was recognized that selected independent variables did not positively influence the analysis of both analytes. Analysis has been addressed with 20 µg/mL of Alogliptin and 160 µg/mL of Dapagliflozin concentrations.

Table 11: Robustness studies for Alogliptin

ChromatographicConditions	Mean Peak Area ± SD	% RSD
Proportion of Acetonitrile: buffer		
79 : 21	304.20 ±2.72	0.90
81 : 19	323.16 ±6.13	1.90
Wavelength change		
285 nm	335.00 ±2.11	0.63
287 nm	326.46 ±1.64	0.50
Flow rate change		
0.9 mL/min	396.73 ±3.94	0.99
1.1 mL/min	320.46 ±5.27	1.64

Table 12: Robustness studies for Dapagliflozin

Chromatographic Conditions	Mean Peak Area ± SD	% RSD
Proportion of Acetonitrile: buffer		
79 : 21	8372.90 ±24.72	0.30
81 : 19	8122.17 ±15.98	0.20
Wavelength change		
285 nm	8111.70 ±21.96	0.27
287 nm	8044.71 ±70.59	0.88
Flow rate change		
0.9 mL/min	9134.71 ±17.41	0.19
1.1 mL/min	7531.67 ±111.45	1.48

Specificity and selectivity

Specificity is the process for experimentally determining the interest of the analyte in the context of components that can also be supposed to present in the sample matrix; thus, selectivity is the process for qualitatively defining the interest of analyte in the context of components likely to be present in the sample matrix. The proposed method is quite well selective and specific. It was noticed that there was no other specific intervention was recorded around the R*t* of Alogliptin and Dapagliflozin; neither the baseline exhibits a substantial unavoidable noise.

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IV. CONCLUSION

In conclusion, the developed RP-HPLC method for the simultaneous estimation of Alogliptin and Dapagliflozin in tablet dosage forms is a highly effective analytical tool, characterized by its robustness, accuracy, and precision. The method demonstrates excellent linearity, ensuring reliable quantification across a range of concentrations, and is sensitive enough to detect low levels of both drugs, which is crucial for quality control. Its precision is reflected in consistent and reproducible results, making it well-suited for routine analysis in pharmaceutical quality control. Overall, this method ensures that the content of Alogliptin and Dapagliflozin in tablet formulations meets the required specifications, supporting the effective management of Type 2 diabetes mellitus.

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