CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.21936288

Available online at: http://www.iajps.com Research Article

SIMULTANEOUS ESTIMATION OF NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DAPALIFLOZIN AND VILDAGLIPTIN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Palla Sushma*[1], Dr. Rama Krishna Mungi[1]

[1]Department of Pharmaceutical Analysis, Avanthi institute of pharmaceutical science, Gunthapally, Abdullapurmet, near Ramoji film city, Ranga Reddy, Telangana-501512.

Abstract:

A systematic analytical method development approach based on Design of Experiments (DoE) was employed for the simultaneous quantification of Dapagliflozin and Vildagliptin by Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). Dapagliflozin, a sodium-glucose co-transporter 2 (SGLT2) inhibitor, and Vildagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, are widely used in combination therapy for the management of type 2 diabetes mellitus. The objective of the present study was to develop a robust, accurate, and precise RP-HPLC method using a Quality by Design (QbD) framework. Critical method parameters such as mobile phase composition, flow rate, and pH were systematically optimized using factorial design to evaluate their impact on critical quality attributes including retention time, peak area, resolution, and tailing factor. Chromatographic separation was achieved using a C18 column with an optimized mobile phase under isocratic conditions and UV detection. The developed method was validated according to ICH guidelines for specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method demonstrated excellent linearity over the selected concentration ranges for both analytes with satisfactory recovery and precision. Statistical analysis confirmed the significance of selected factors and their interactions, ensuring method robustness. The proposed DoE-based RP-HPLC method is simple, rapid, cost-effective, and suitable for routine quality control analysis of combined pharmaceutical dosage forms containing Dapagliflozin and Vildagliptin.

Keywords: *Dapagliflozin; Vildagliptin; RP-HPLC; Design of Experiments (DoE); Quality by Design (QbD); Method Development; Simultaneous Estimation; Analytical Validation; Factorial Design; Pharmaceutical Analysis.*

Corresponding author:

Palla Sushma,

Department of Pharmaceutical Analysis, Avanthi Institute of Pharmaceutical Science,Gunthapally, Abdullapurmet, Telangana.

Mobile:

Email ID: *pallasushma0@gmail.com*

QR CODE

Please cite this article in press Palla Sushma et al., Simultaneous estimation of new analytical method development and validation of Dapaliflozin and Vildagliptin by high performance liquid chromatography. Indo Am. J. P. Sci, 2026; 13(08).

INTRODUCTION:

High-performance liquid chromatography (HPLC) stands as a powerful analytical tool in modern chemistry. It excels at identifying, measuring, and separating components within liquid-dissolved samples. Widely employed in pharmacological product analysis, HPLC is prized for its precision in both quantitative and qualitative assessments, contributing significantly to advancements in analytical chemistry.[1] In high performance liquid chromatography (HPLC), a sample solution (stationary phase) is injected into a porous column. A liquid (mobile phase) is then pumped through the column at high pressure. Components in the sample exhibit different migration rates through the column due to partitioning between stationary and mobile phases. This leads to elution at distinct times, allowing separation. HPLC's precision arises from nuanced component behaviours during partitioning, offering a robust method for analysing diverse samples in fields like pharmaceuticals and analytical chemistry.[2] In high-performance liquid chromatography, a compound with lower affinity for the stationary phase travels faster and covers a longer distance, while a compound with higher affinity moves slower and covers a shorter distance.

This differential migration facilitates effective separation and analysis of sample components.[3] High performance liquid chromatography (HPLC) proves invaluable in pharmaceutical analysis, efficiently isolating and quantifying major medications, reaction impurities, synthesis intermediates, and degradants. As a preeminent analytical tool, HPLC excels in identifying, measuring, and separating diverse sample components soluble in liquid. Its precision is paramount for both quantitative and qualitative drug product analysis, playing a pivotal role in determining drug product stability. By offering a meticulous approach to characterizing pharmaceutical samples, HPLC stands as an indispensable technique in ensuring the quality and safety of medicinal formulations in the field of analytical chemistry.[4]

HPLC principle

High-performance liquid chromatography (HPLC) relies on the distribution of the analyte between a stationary phase and a mobile phase (eluent), typically within the column's packing material. The chemical structure of the analyte dictates its movement rate through the stationary phase, forming the basis for separation. This principle enables precise separation and analysis of diverse compounds, making HPLC a fundamental technique in analytical chemistry, particularly in pharmaceutical and chemical industries.

Types of HPLC

The choice of High-Performance Liquid Chromatography (HPLC) method for analysis is dictated by the employed phase system. Normal Phase HPLC, or normal phase chromatography (NP-HPLC), classifies analytes based on polarity. In NP-HPLC, a non-polar mobile phase and a polar stationary phase interact with polar analytes, retaining them. Elution time increases with rising analyte polarity due to this interaction. NPHPLC provides effective separation, revealing insights into sample composition based on polarity, making it a valuable analytical tool, particularly in characterizing compounds with different polarities in diverse fields such as chemistry, pharmaceuticals, and environmental analysis.[5,6]

Classification of HPLC can be done as

1. HPLC is classified into analytical and preparatory categories based on the scale of operation. 2. Various chromatographic techniques include size exclusion, affinity, and adsorption chromatography.
3. Chiral phase and ion exchange chromatography are categorized based on the principle of separation.
4. Isocratic and gradient separation methods distinguish chromatography based on elution technique.
5. Chromatography operates in normal and reverse phases, determined by modes of operation.[7,8]

1. Size exclusion chromatography

Size Exclusion Chromatography (SEC), also known as gel permeation or gel filtration chromatography, separates particles based on size. It is utilized to determine the quaternary and tertiary structures of amino acids and proteins. This technique is commonly employed for assessing the molecular weight of polysaccharides, providing valuable insights into their structural characteristics in various scientific and analytical applications.

2. Ion exchange chromatography

Ion-exchange chromatography relies on the retention of solute ions attracted to charged sites on the stationary phase. Ions with similar charges are repelled. This method finds application in water purification, protein ion-exchange chromatography, ligand-exchange chromatography, and high-pH anion-exchange chromatography of carbohydrates and oligosaccharides. Its versatility makes it a crucial technique in various fields, allowing selective separation and analysis based on the charged characteristics of different substances.[9,10]

EXPERIMENTAL METHODS

INSTRUMENTS USED

Table: Instruments used

S. No	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Lab man

CHEMICALS USED:

Table: chemicals used

S. No	Chemical	Brand names
1	Dapagliflozin	Forxiga
2	Vildagliptin	Galvus
3	Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC METHOD DEVELOPMENT:
TRAILS

Preparation of standard solution:
Accurately weigh and transfer 10 mg of Dapagliflozin and Vildagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 2.25ml of the above Dapagliflozin and 0.45ml of the Vildagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to 0.1% Perchloric acid: ACN in proportion (55:45) v/v respectively.

Optimization of Column:
The method was performed with various columns like C18 column, X- bridge column, Altima C18, 150mm x 4.6 mm, 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:
Accurately measured 550ml (55%) of HPLC 0.1% Perchloric acid and 450ml of Acetonitrile (45%) were mixed and degassed in a digital ultra sonicated for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION
(Optimized chromatogram):

Column : Altima C18, 150mm x 4.6 mm, 5µ
Column temperature : 35˚C
Wavelength : 210nm
Mobile phase ratio : 0.1% Perchloric acid: ACN in a 55:45 (v/v)
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 7minutes

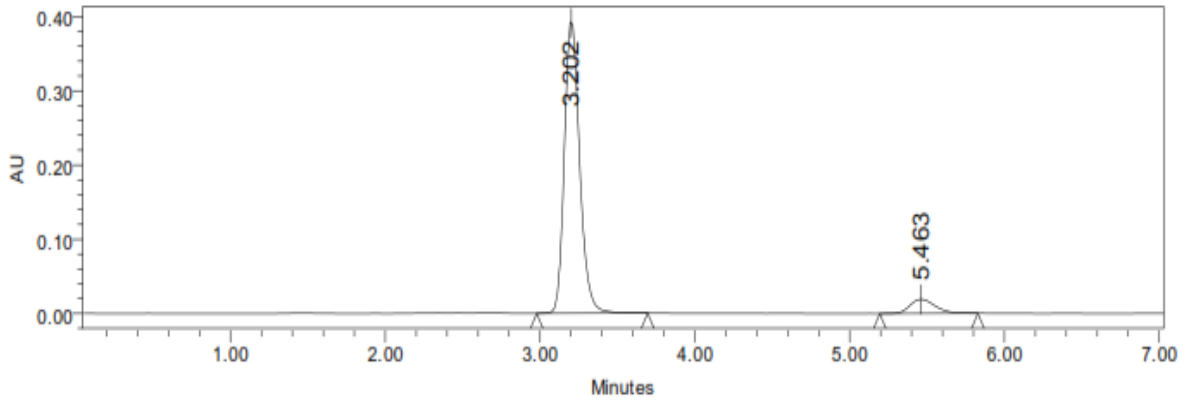


Figure: Optimized Chromatogram (Standard)

Table: Optimized Chromatogram (Standard)

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Dapagliflozin	3.202	2391746	39726	1.2	9028	
2	Vildagliptin	5.463	194627	8497	1.1	7398	7.4

Observation:
This trial shows improper separation sample peaks, baseline and show very less plate count in the chromatogram. So, it's required more trials to obtain good peaks.

From the above chromatogram it was observed that the Dapagliflozin and Vildagliptin peaks are well separated and they show proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

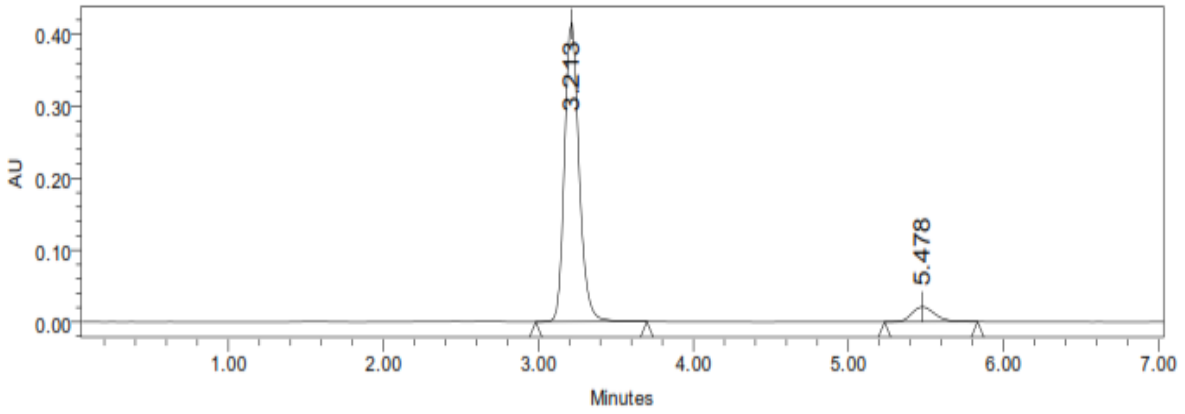


Table: Optimized Chromatogram (Sample)

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Dapagliflozin	3.213	2381649	391846	1.2	9472	
2	Vildagliptin	5.478	191057	8104	1.1	8936	7.5

Acceptance criteria:
Resolution between two drugs must be not less than 2
Theoretical plates must be not less than 2000
Tailing factor must be not less than 0.9 and not more than 2.
It was found from above data that all the system suitability parameters for developed method were within the limit.

VALIDATION
Blank:

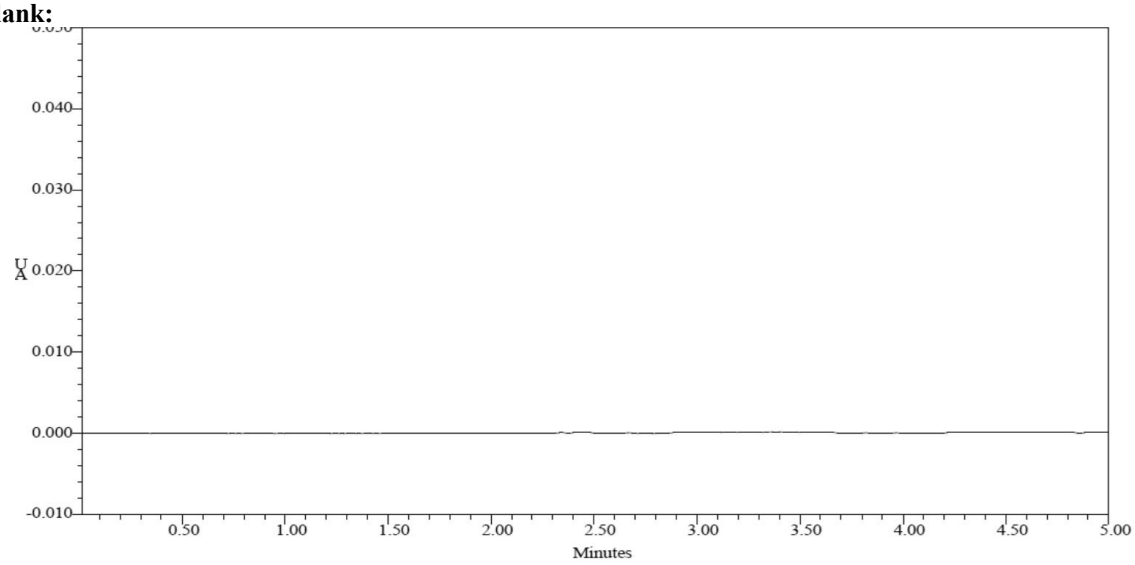


Fig: Chromatogram showing blank (mobile phase preparation)

System suitability:

Table: Results of system suitability for Dapagliflozin

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Dapagliflozin	3.200	2391746	394171	8952	1.2
2	Dapagliflozin	3.248	2391647	381946	9561	1.2
3	Dapagliflozin	3.299	2381647	391746	6572	1.2
4	Dapagliflozin	3.297	2385631	386562	6452	1.2
5	Dapagliflozin	3.297	2385635	389164	7452	1.2
Mean			2387261			
Std. Dev.			4363.771			
% RSD			0.182794			

Acceptance criteria:
%RSD of five different sample solutions should not more than 2
The %RSD obtained is within the limit, hence the method is suitable.

Table: Results of system suitability for Vildagliptin

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vildagliptin	5.413	198362	7917	5272	1.1
2	Vildagliptin	5.484	197486	7486	6291	1.1
3	Vildagliptin	5.405	198354	7859	6184	1.1
4	Vildagliptin	5.405	197352	7926	7145	1.1
5	Vildagliptin	5.409	198453	7946	6946	1.1
Mean			198001.4			
Std. Dev.			535.1774			
% RSD			0.27029			

Acceptance criteria:
%RSD of five different sample solutions should not more than 2
The %RSD obtained is within the limit, hence the method is suitable.

SPECIFICITY
The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.
Analytical method was tested for specificity to measure accurately quantitate Dapagliflozin and Vildagliptin in drug product.
Assay (Standard) :

Table: Peak results for assay standard

Dapagliflozin

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Dapagliflozin	3.211	2397162	397161	1.2	9472
2	Dapagliflozin	3.222	2394721	389173	1.2	9745
3	Dapagliflozin	3.254	2389461	391723	1.2	8917

Vildagliptin

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Vildagliptin	5.414	198462	7811	1.1	8492	7.49
2	Vildagliptin	5.453	198472	8193	1.1	8916	7.52
3	Vildagliptin	5.424	198735	7972	1.1	9372	7.44

Assay (Sample):
Table: Peak results for Assay sample
Dapagliflozin

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Dapagliflozin	3.297	2391741	381612	1.2	9472
2	Dapagliflozin	3.294	2389166	391746	1.2	8927
3	Dapagliflozin	3.295	2361731	381634	1.2	9017

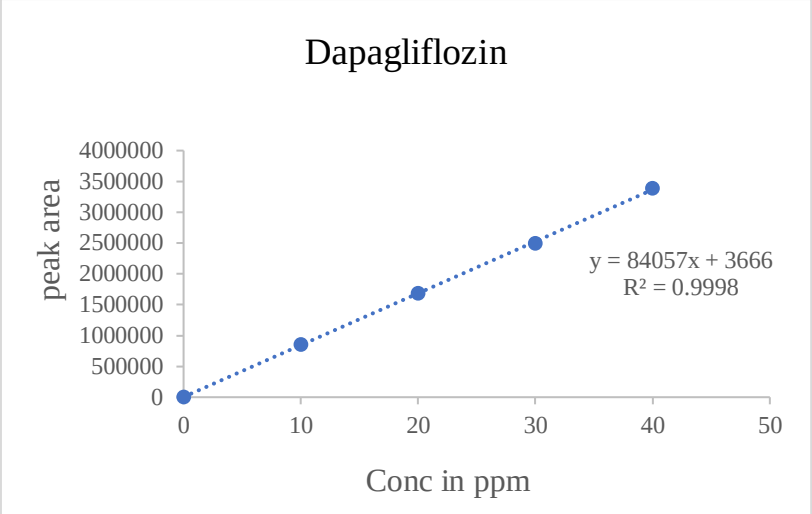
Vildagliptin

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Vildagliptin	5.435	198641	8174	1.1	9284	7.18
2	Vildagliptin	5.417	196547	8942	1.1	8974	7.44
3	Vildagliptin	5.434	194027	7294	1.1	9017	7.38

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Dapagliflozin and Vildagliptin in pharmaceutical dosage form was found to be 99.2%.
LINEARITY
CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:
Dapagliflozin

Concentration μγ/μλ	**Average Peak Area**
0	0
10	859889
20	1683641
30	2495378
40	3385089



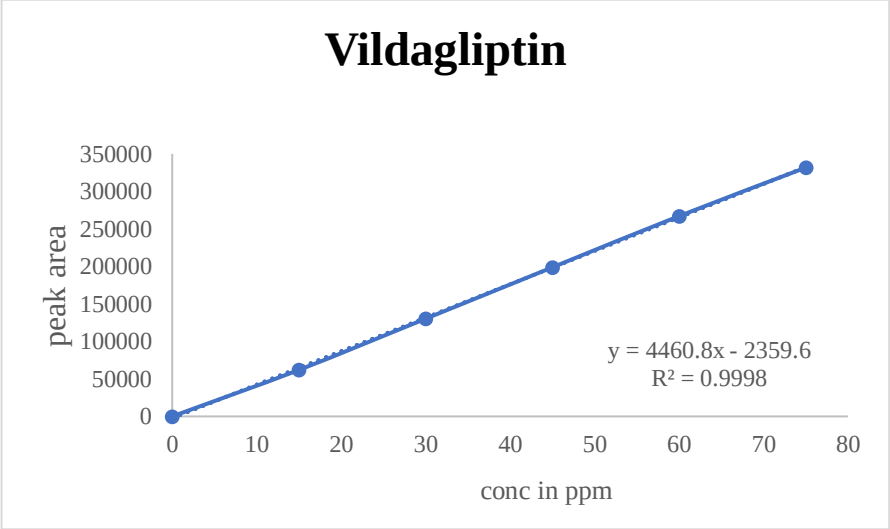
LINEARITY PLOT:
The plot of Concentration (x) versus the Average Peak Area (y) data of Dapagliflozin is a straight line.
Y = mx + c Slope (m) =11208
Intercept (c) = 3666
Correlation Coefficient (r) = 0.9998

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 48956. These values meet the validation criteria.

Vildagliptin

Concentration μγ/μλ	**Average Peak Area**
0	0
15	61953
30	130213
45	198697
60	267002
76	331658



LINEARITY PLOT:
The plot of Concentration (x) versus the Average Peak Area (y) data of Vildagliptin is a straight line.
Y = mx + c
Slope (m) = 4460.8
Intercept (c) = 2359.6
Correlation Coefficient (r) = 0.9998

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 454.8. These values meet the validation criteria.

Precision:
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY
Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table: Results of repeatability for Dapagliflozin:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Dapagliflozin	3.213	2397164	381741	8155	1.2
2	Dapagliflozin	3.253	2391741	371742	9174	1.2
3	Dapagliflozin	3.297	2371846	391746	7154	1.2
4	Dapagliflozin	3.215	2361748	391847	9917	1.2
5	Dapagliflozin	3.254	2371649	384622	9247	1.2
Mean			2378830			
Std.dev			14958			
%RSD			0.628797			

Acceptance criteria:
%RSD for sample should be NMT 2
The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table: Results of repeatability for Vildagliptin:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Vildagliptin	5.441	198464	7291	6274	1.1
2	Vildagliptin	5.442	193643	7219	6592	1.1
3	Vildagliptin	5.409	196462	7194	6028	1.1
4	Vildagliptin	5.520	194644	8174	6927	1.1
5	Vildagliptin	5.424	198464	8653	5920	1.1
Mean			196335.4			
Std.dev			2190.191			
%RSD			1.115536			

Intermediate precision:
Day 1:

Table: Results of Intermediate precision for Dapagliflozin

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USPTailing
1	Dapagliflozin	3.211	2389572	395275	9375	1.2
2	Dapagliflozin	3.211	2391847	392175	9275	1.2
3	Dapagliflozin	3.210	2319472	312947	8265	1.2
4	Dapagliflozin	3.212	2306842	310585	6254	1.2
5	Dapagliflozin	3.211	2375972	310694	9028	1.2
6	Dapagliflozin	3.297	2396746	358373	8928	1.2
Mean			2363409			
Std. Dev.			39730.83			
% RSD			1.681082			

Acceptance criteria:
%RSD of six different sample solutions should not more than 2

Table: Results of Intermediate precision for Vildagliptin

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USPPlate count	USPTailing
1	Vildagliptin	5.411	197284	7194	8264	1.2
2	Vildagliptin	5.410	197849	7294	9174	1.2
3	Vildagliptin	5.420	196572	7147	9164	1.2
4	Vildagliptin	5.423	195028	7927	9733	1.2
5	Vildagliptin	5.419	199474	8238	9194	1.2
6	Vildagliptin	5.409	197482	7638	8973	1.2
Mean			197281.5			
Std. Dev.			1466.354			
% RSD			0.74328			

Acceptance criteria:
%RSD of six different sample solutions should not more than 2

Day 2:

Table: Results of Intermediate precision Day 2 for Dapagliflozin

S. No	peak Name	RT	Area (µV*sec)	Height (µV)	USPPlate Count	USPTailing
1	Dapagliflozin	3.211	2389562	391741	9264	1.2
2	Dapagliflozin	3.233	2381654	391047	9746	1.2
3	Dapagliflozin	3.244	2381946	391748	9816	1.2
4	Dapagliflozin	3.297	2391741	391746	9917	1.2
5	Dapagliflozin	3.297	2386452	381641	9742	1.2
6	Dapagliflozin	3.202	2374763	381645	9017	1.2
Mean			2384353			
Std. Dev.			6183.339			
% RSD			0.25933			

Acceptance criteria:
%RSD of six different sample solutions should not more than 2

Table: Results of Intermediate precision Day 2 for Vildagliptin

S.No	PeakName	RT	Area (µV*sec)	Height (µV)	USPPlate Count	USPTailing
1	Vildagliptin	5.411	197486	7582	6272	1.1
2	Vildagliptin	5.410	197486	7184	6174	1.1
3	Vildagliptin	5.420	196746	7456	5184	1.1
4	Vildagliptin	5.405	195862	7814	6194	1.1
5	Vildagliptin	5.409	196582	7194	6292	1.1
6	Vildagliptin	5.463	198463	7745	6191	1.1
Mean			197104.2			
Std. Dev.			903.542			
% RSD			0.458408			

Acceptance criteria:
%RSD of six different sample solutions should not more than 2

6.3.4: ACCURACY:
Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

The accuracy results for Dapagliflozin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1217218	112.5	112.4	99.6	99.3
100%	2397141	225	225	100	
150%	3514547	337.5	332.5	98.5	

Acceptance Criteria:
The percentage recovery was found to be within the limit (98-102%).
The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

The accuracy results for Vildagliptin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	98598.67	22.5	22.4	99.9	99.6
100%	198359.7	45	45	100	
150%	291512.3	67.5	66.8	99	

Acceptance Criteria:
The percentage recovery was found to be within the limit (98-102%).
The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s
Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Dapagliflozin:
Result:
=3.3×39762/10421
=12.5µg/ml

Vildagliptin:
Result:
=3.3×5008/4365
=3.7µg/ml

Quantitation limit
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S
Were
σ = Standard deviation of the response
S = Slope of the calibration curve

Dapagliflozin:
Result:
=10×39762/10421
=38.1µg/ml

Vildagliptin:
Result:
=10×5008/4365
=11.4µg/ml

ROBUSTNESS
The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Dapagliflozin and Vildagliptin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard sample of Dapagliflozin and Vildagliptin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count.
Variation in flow

Table: Results for Robustness
Dapagliflozin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	2391746	3.202	9028	1.2
Less Flow rate of 0.9mL/min	2371831	3.639	7381	1.2
More Flow rate of 1.1mL/min	2218319	2.859	9311	1.1
Less organic phase (about 5 % decrease in organic phase)	2294821	3.460	7462	1.2
More organic phase (about 5 % Increase in organic phase)	2394811	3.022	6817	1.1

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table: Results for Robustness
Vildagliptin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.1mL/min	194627	5.463	7398	1.1
Less Flow rate of 0.9mL/min	183738	6.250	6883	1.1
More Flow rate of 0.8mL/min	198373	4.863	9917	1.2
Less organic phase (about 5 % decrease in organic phase)	178471	6.196	8372	1.1
More organic phase (about 5 % Increase in organic phase)	189462	5.010	7716	1.2

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION:

Summary

A systematic analytical method was developed for the simultaneous quantification of Dapagliflozin and Vildagliptin using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). The method development was carried out using a Design of Experiments (DoE) approach to ensure robustness, efficiency, and optimized chromatographic performance. Critical Method Parameters (CMPs) such as mobile phase composition, flow rate, pH of buffer, and column temperature were systematically evaluated using statistical experimental designs (e.g., factorial design/Box-Behnken design). Their effects on Critical Quality Attributes (CQAs) including retention time, resolution, peak symmetry, and theoretical plates were studied. Optimized chromatographic separation was achieved on a C18 column using a suitable buffer–organic solvent system, with detection performed at an appropriate wavelength using a UV detector. The developed method was validated as per ICH guidelines for parameters including specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). Results demonstrated good linearity over the selected concentration ranges for both drugs, with acceptable recovery values and low %RSD, confirming the precision and accuracy of the method. The DoE-based optimization ensured enhanced method robustness and minimized variability.

Conclusion

A robust, precise, and accurate RP-HPLC method was successfully developed for the simultaneous estimation of Dapagliflozin and Vildagliptin using a systematic Design of Experiments approach. The application of DoE enabled a comprehensive understanding of the interaction between method variables and ensured optimal chromatographic performance with minimal experimental trials. The validated method complied with ICH requirements and proved suitable for routine quality control analysis of combined pharmaceutical dosage forms containing Dapagliflozin and Vildagliptin. Furthermore, the systematic DoE strategy enhances method reliability, reproducibility, and lifecycle management, making it advantageous for regulatory and industrial applications.

ACKNOWLEDGEMENT:
The Authors are thankful to the Management and Principal, Avanthi Institute of Pharmaceutical Science, Gunthapally, Abdullapurmet, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Pharma Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

REFERENCES:

1. Rao BV, Sowjanya GN, Ajitha A, Rao Uma MV. A review on stability-indicating HPLC method development, World journal of pharmacy and pharmaceutical sciences, 2015; 4(8): 405-423.
2. Rajan HV. Development and validation of HPLC method - A Review. International Journal of current research in pharmacy, 2015; 1(2): 55-68.
3. Kumar V, Bharadwaj R, Gupta G, Kumar S. An Overview on HPLC Method Development, Optimization and Validation process for drug analysis. The Pharmaceutical and Chemical Journal, 2015; 2(2): 30-40.
4. B.V. Rao, G.N. Sowjanya1, A. Ajitha, V.U.M. Rao, Review on stability indicating hplc method development, World Journal of Pharmacy and Pharmaceutical Sciences, 2015; 4(8): 405-423.
5. Arpino, Patrick. Combined liquid chromatography mass spectrometry. Part III. Applications of thermo spray". Mass Spectrometry Reviews, 1992; 11: 3. doi:10.1002/mas.1280110103.
6. Arpino, Patrick. Combined liquid chromatography mass spectrometry. Part I. Coupling by means of a moving belt interface". Mass Spectrometry Reviews, 1989; 8: 35. doi:10.1002/mas.1280080103.
7. Gupta V, Jain AD, Gill NS, Gupta K. Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4): 17-25.
8. Sonia K, Nappinnai M. Development and validation of HPLC and UV-visible spectrophotometric method for the pharmaceutical dosage form and biological fluid –review. European Journal of Biomedical and Pharmaceutical sciences, 2016; 3(3): 382-391.
9. Sánchez MLF. Chromatographic techniques, European RTN Project, GLADNET, retrieved on, 05-09- 2013.
10. Mcpolin Oona. An Introduction to HPLC for Pharmaceutical Analysis. Mourne Training Service, 11-12.
11. United States Pharmacopoeia and National Formulary, (24th) Asian Edition, The United States Pharmacopoeia Convention Inc. U.S.A. 2126.
12. Sankar SR, Text book of Pharmaceutical Analysis. 5thEdition 2006. Rx publications, Tirunelveli, 2006; 13- 1,2.
13. M.S. Charde, A.S. Welankiwar, J. Kumar, Method development by liquid chromatography with validation, International Journal of Pharmaceutical Chemistry, 04(02): 57-61.
14. M.W. Dong, Modern Hplc for practicing scientists, John Wiley & Sons, New Jersey, 2006. 15. C.K. Kaushal, B. Srivastava, A process of method development: A chromatographic approach, J. Chem. Pharm. Res., 2010; 2(2): 519-545.