International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 3 May–June 2025, pp: 1468-1480 www.ijprajournal.com ISSN: 2456-4494

Development and Validation of RP-HPLC Analytical Method for Simultaneous Estimation of Dapagliflozin and Vildagliptin in Pure and It's Pharmaceutical Dosage Forms.

[1]Dr.A.M.Tamboli, [2]Swapnil Bajirao Hole, [3]Mahesh Chakradhar Gore, [4]Vinay Shivaji Kamble
Sahyadri College Of Pharmacy Methwade

Date of Submission: 05-06-2025 Date of Acceptance: 15-06-2025

ABSTRACT:
The present study aimed to develop and validate a reverse phase high performance liquid chromatography (RP-HPLC) method for the simultaneous quantification of dapagliflozin and vildagliptin in a tablet dosage form that was simple, sensitive, accurate, and reproducible.The chromatographic measurement was performed on a Phenomenex C_8 column (150x4.6 mm, 5 um) column with an optimized mobile phase containing 0.1% Perchloric acid: ACN in ratio of 55:45.The flow rate was 0.5 ml/min,and the detecting wavelength was 214n nm.. In the concentration range Dapagliflozin and Vildagliptin were of 8 to 12µg/ml and 80 to 120µg/ml respectively showed a linear response of the suggested approach. The correlation coefficients (r^2values) for Dapagliflozin and Vildagliptinwere 0.999 and 0.999, respectively, and the retentiontimes of Dapagliflozin and Vildagliptinwas found to be 2.31 ± 0.5 min and 1.49 ± 0.5 min respectively. The developed chromatographictechnique was validated for specificity, linearity, precision, accuracy, LOD, and LOQ using ICH Q2(R1) criteria. Theanalysis results have been validated in accordance to ICH guidelines.

I. INTRODUCTION:

Vildagliptin and dapagliflozin is a combination of two antidiabetic medications. Dapagliflozin increases the excretion of glucose in the urine and decreases blood glucose levels [1]. Vildagliptin reduces the amount of glucose produced by the liver by raising insulin levels and decreasing glucagon levels, a hormone that raises blood glucose levels [2].

A lack of insulin synthesis or a loss of tissue sensitivity to insulin are the two main causes of diabetes, a disorder marked by faulty protein, carbohydrate, fat, and metabolism. Patients with diabetes frequently experience fatigue, excessive thirst, frequent urination, and weight loss. Type 2 Diabetes Melitus (T2DM) affects a large number of the population. It is typified by insufficient secretion and synthesis of insulin due to insulin resistance. T2DM is an illness that worsens over time. As people age, the incidence and prevalence of type 2 diabetes increase. Eighty to ninety percent of all DM cases are DM2. Insulin resistance is associated with intra-abdominal obesity, which is seen in the majority of persons with Type 2 diabetes. This is the most common kind of diabetes mellitus and is closely associated with advanced age, obesity, a lack of activity, and a family history of diabetes. Alpha glucoside inhibitors, dipeptidyl peptidase 4 inhibitors, and SGLT2 inhibitors are offered to treat type 2 diabetes. [3-5]

Vildagliptin (VIL) and Dapagliflogin (DAP) can be estimated using a variety of techniques, including UPLC [6], RP-HPLC [7–12], UPLC, and LC-MS/MS [13–15], both in combination with metformin and saxagliptin and in individual dosage forms. However, there was no single technique for the simultaneous measurement and stability-indicating test of VIL and DAP using RP HPLC. In the present situation, the regulatory body additionally advises the company to use advanced HPLC equipment for both quantitative and qualitative estimation. Thus, the use of a sensitive, established, and validated method for the simultaneous measurement of dapagliflozin and vildagliptin by liquid chromatography in pharmaceutical dosage forms is advantageous.

II. MATERIALS AND METHODS:

Chemicals and reagents:

Reagents and chemicals For the duration of our investigation, Dalton Pharma Chem, of Vadodara, Gujarat, India, kindly provided us with a gift sample of Dapagliflozin and Vildagliptin as our reference standard. All additional solvents, chemicals, and excipients (specificity) utilized in this study were supplied by LobaChemiePvt. Ltd., Mumbai, India.

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Instrument:

The HPLC system consisted of Agilent connected with PDA detector

Chromatographic condition:

Phenomenex C_8 column (150x4.6 mm, 5 um) column, at 0.5 ml/min flow rate, detection wavelength is 214 nm, mobile phase containing 0.1% Perchloric acid: ACN in ratio of 55:45.

Different mobile phases like 0.1% Perchloric acid: ACN were used with different ratio and analyzed for best resolution of peaks in chromatogram.

Chromatographic Conditions:

a. Oven Temp: 30ºC
b. Flow rate: 1 ml/min.

1. Preparation of 0.1% Perchloric acid: In 1000 ml HPLC water, 1 ml of Perchloric acid was added and mixed well and filtered through 0.45-micron membrane filter and sonicated to degas for 10 minutes.

a. Runtime: 5 minutes
b. Injection Volume: 10 µl
c. Wavelength: 214 nm
d. Diluents: 0.1% Perchloric acid: Acetonitrile (50: 50, % v/v)
e. Column: PhenomenexKinetex XB-C_8 (150 x 4.6 mm, 5µ)

2. Standard Preparation:

a. Vildagliptin Standard Stock solution-I (SSS-I):

i. Prepare a Standard Stock Solution (SSS-I) of by adding 10mg of Vildagliptin in 10 ml volumetric flask & add 5 ml diluents, sonicate for 5 minutes and make the volume to 10 ml with diluent. (Conc. of Vildagliptin in SSS-I = 1000 µg/ml).

b. Dapagliflozin Standard Stock solution-II (SSS-II)

i. Prepare a Standard Stock Solution (SSS-II) of by adding 10mg of Dapagliflozin in 100 ml volumetric flask & add 50 ml diluent, sonicate for 5 minutes and make the volume to 100 ml with diluent. (Conc. of Dapagliflozin in SSS-II = 100 µg/ml).

c. Then add 1 ml of SSS-I and 1 ml of SSS-II in 10 ml volumetric flask and add 5 ml diluents and vortex and make up the volume with diluent. (Conc. of Vildagliptin = 100 µg/ml and Conc. of Dapagliflozin = 10 µg/ml)

3. Preparation of Drug Product sample solution:

The drug product sample solution was prepared by taking 10 tablets and crushing them using mortar and pestle and powder equivalent to 10 mg of Vildagliptin and 1 mg of Dapagliflozin weighed accurately in 10 ml volumetric flask and 5-7 diluent was added to it and sonicated for 5 minutes and made up to the mark with diluent.

4. Selection of Wavelength:

The sample was scanned from 190-400 nm with DAD detector. The Wavelength selected for analysis chosen was 214 nm on the basis of isobestic point.

5. Method Validation:

a. Specificity & Assay:

i. Individual sample of Blank, Vildagliptin working standard (100 µg/ml), Dapagliflozin working standard (10 µg/ml), Mixture working standard and Drug product of was prepared and peak was for identified from Retention Time.

ii. % Assay was calculated as follows:

$$\% \text{ Assay} = \frac{\text{Samplearea}}{\text{Standardarea}} \times 100$$

b. Repeatability & System Suitability:

i. A single working standard was prepared as described in section 2 and 6 injections were made from same solution and checked for system suitability.

ii. System suitability parameters are as below:

1. Retention Time,
2. Theoretical plates,
3. Asymmetry (Tailing factor),
4. Resolution.

c. Linearity & Range:

i. 5 samples of varying concentrations ranging from 80-120% were prepared.

ii. The concentrations are given below

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Table:1 Concentration for linearity Study of For HPLC

% Level	Vildagliptin Conc. (µg/ml)	Dapagliflozin Conc. (µg/ml)
80	80	8
90	90	9
100	100	10
110	110	11
120	120	12

iii. The sample preparations are given as below;
iv. X ml of Vildagliptin and Y ml of Dapagliflozin standard solution was added to 10 ml diluent to make up the concentrations given above:

X ml of SSS-I	X ml of SSS-II	Diluted to
0.8	0.8	10 ml
0.9	0.9	10 ml
1.0	1.0	10 ml
1.1	1.1	10 ml
1.2	1.2	10 ml

d. Accuracy:

i. Samples were prepared of 80%, 100% and 120% concentration by spiking the same amount of concentration given in table for Linearity.
ii. Samples were injected in triplicate to calculate % RSD.
iii. % Recovery was also calculated.

e. LOD/ LOQ:

i. Was calculated by using ANOVA technique.
ii. Formula:
iii.

$$\text{LOD} = \frac{3.3 \times \text{Std. Error of Intercept}}{\text{Coefficients of X Variable 1}}$$

$$\text{LOQ} = \frac{10 \times \text{Std. Error of Intercept}}{\text{Coefficients of X Variable 1}}$$

f. Robustness:

i. The Robustness was performed by changing the column temperature and Wavelength by ± 2˚C and ± 2 nm.
ii. Each Sample was injected and % RSD of peak area was calculated at each condition.

Table 2:Column Oven Temperature Robustness Study.

Condition	Increased	Normal	Decreased
Column Oven Temperature	32˚C	30˚C	28˚C
Wavelength	216 nm	214 nm	212 nm

g. Intra & Inter-day Precision:

i. Single mixture working standard and drug product was prepared and injected twice in a day at different time intervals to evaluate intra-day precision.
ii. Same mixture working standard was analysed on second day to evaluate the inter-day precision.
iii. % RSD of peak was calculated at each interval and stability of solutions were estimated.

III. RESULTS AND DISCUSSION:

i) Selection of analytical wavelength:

The sample was scanned from 200-400 nm with PDA detector. The Wavelength selected for analysis chosen was 214 nm on basis of appropriate intensity of Vildagliptin.

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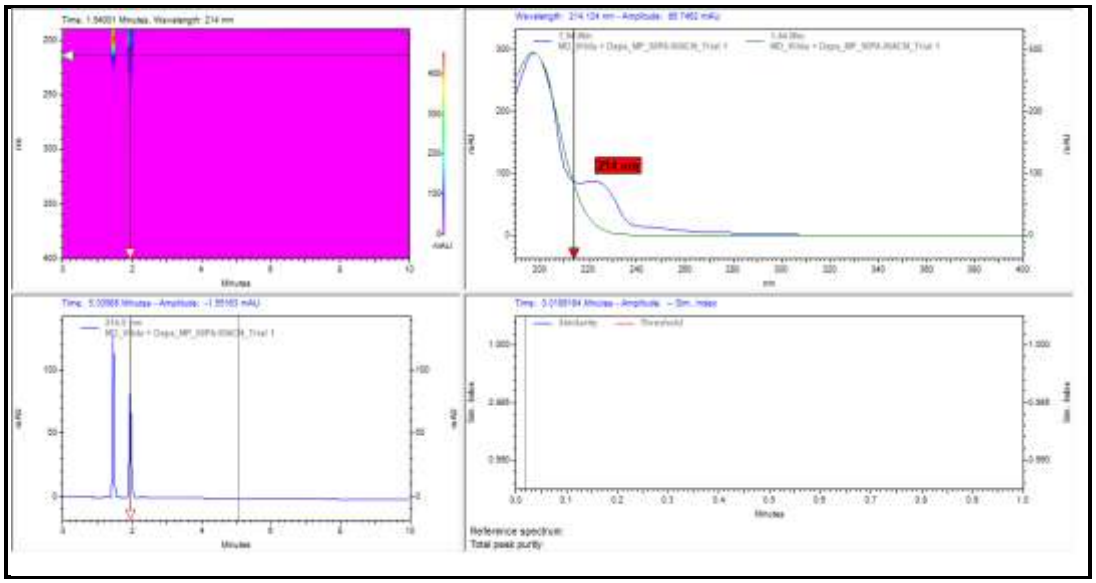

Figure 1: Spectrum of Dapagliflozin and Vildagliptinbetween 200-400nm in mobile phase.

Dapagliflozin RT 2.31 min and Vildagliptin RT 1.49 min show the maximum absorbance at 214nm. Hence, HPLC analysis was carried out at 214 nm. **(Figure. 1)**

ii) Optimization of Chromatographic Conditions for Dapagliflozin and Vildagliptin:

The column was saturated with the mobile phase. Standard solution of Dapagliflozin and Vildagliptin was injected to get the chromatogram. The retention times for the two drugs were found to be:

Drug name:
Retention time.
Dapagliflozin
2.31 ± 0.5 min
Vildagliptin
1.49 ± 0.5 min

Chromatogram of Dapagliflozin and Vildagliptinshown in Fig. 7.9. The separation of two drugs was confirmed from the Figure 1.

Final Method: Phenomenex C_8 column (150x4.6 mm, 5 um) column, at 0.5 ml/min flow rate, detection wavelength is 214 nm, mobile phase containing 0.1% Perchloric acid: ACN in ratio of 55:45.Different mobile phases like 0.1% Perchloric acid: ACN were used with different ratio and analyzed for best resolution of peaks in chromatogram.

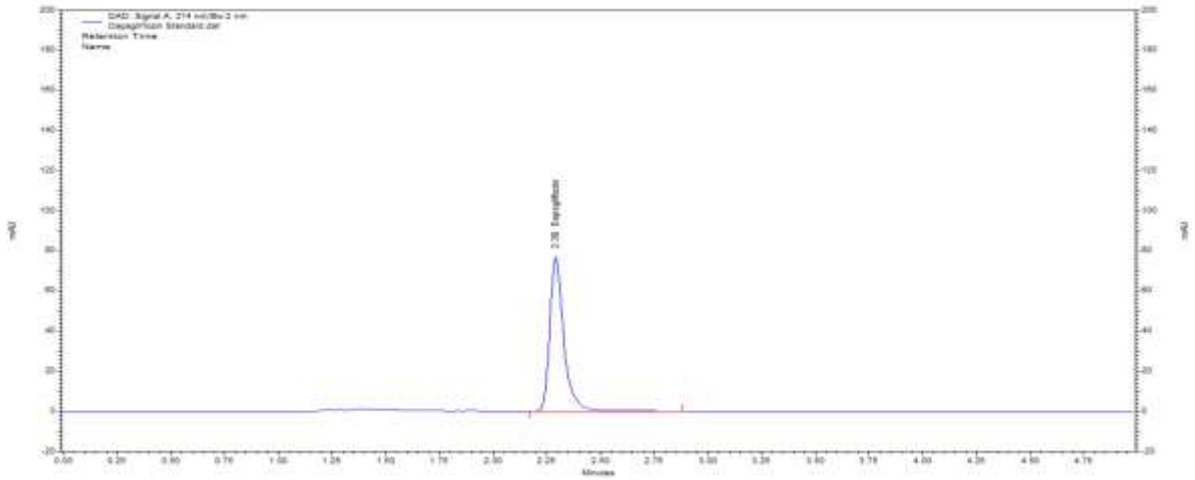

Figure 2: Chromatogram of Standard Dapagliflozin.

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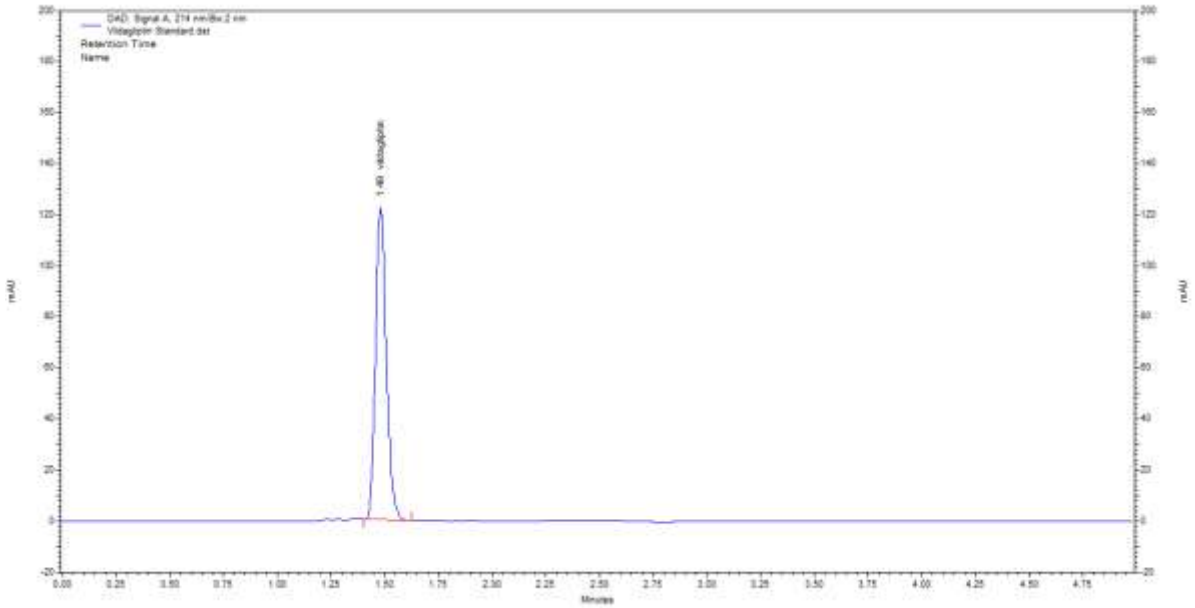

Figure 3: Chromatogram of Standard Vildagliptin.

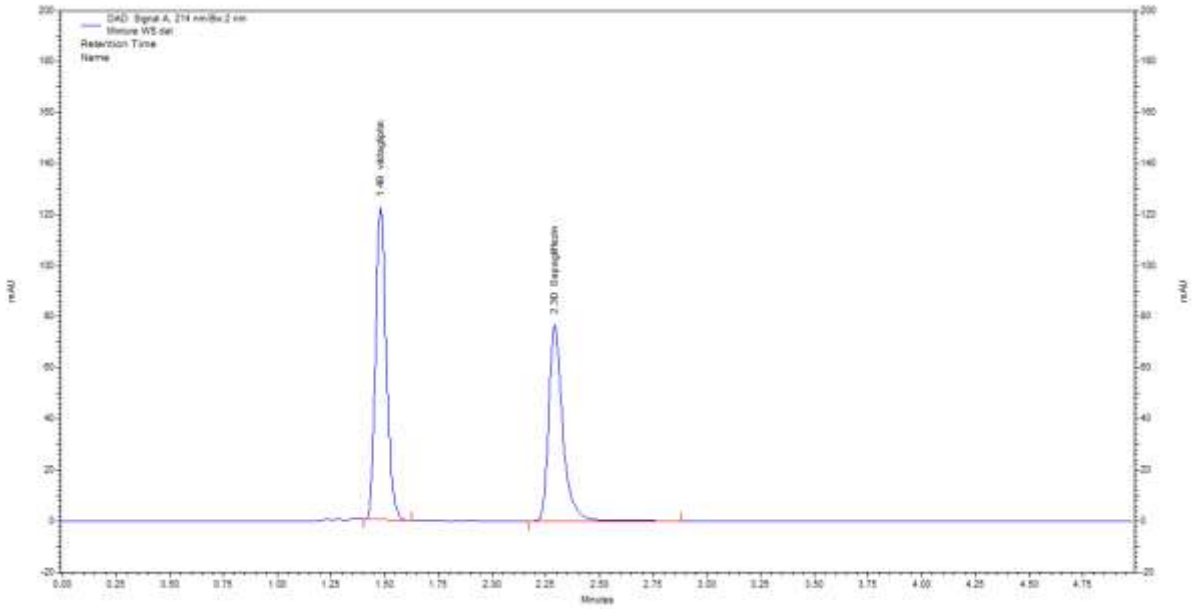

Figure 4: Chromatogram of Standard Mixture of Dapagliflozin and Vildagliptin in optimized chromatographic conditions.

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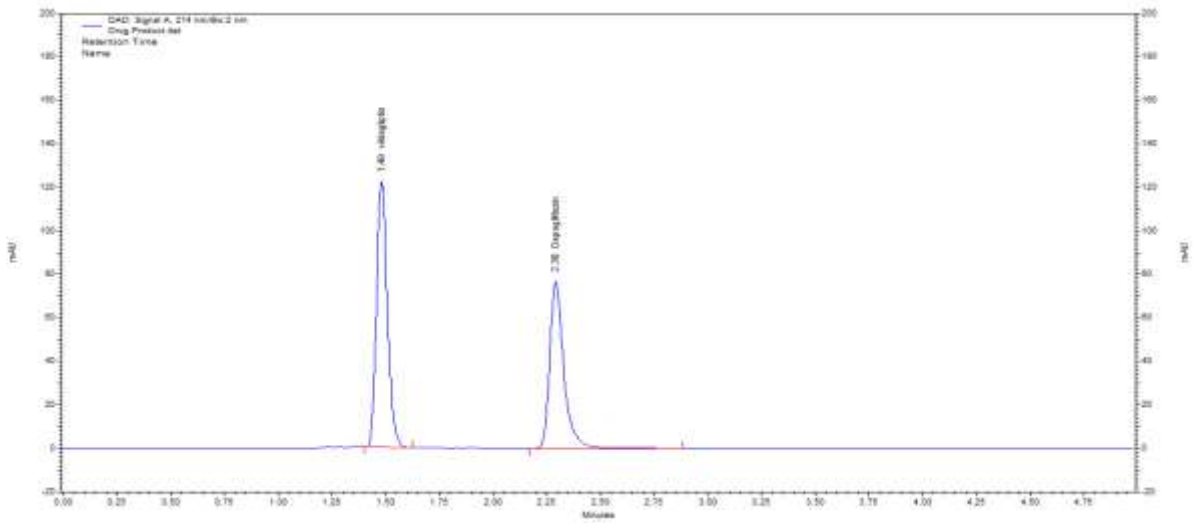

Figure 5: Chromatogram of Sample of Dapagliflozin and Vildagliptin in optimized chromatographic conditions.

Table 3: Details of chromatogram of standard mixture containing Dapagliflozin and Vildagliptin.

Sr. No	Name of drug	RT (min)	Plates	Tailing factor
1.	Dapagliflozin	2.30	6706	1.25
2.	Vildagliptin.	1.49	4165	1.13

iii) Analysis of tablet formulation:-

Table 4 Analysis of marketed formulation.

Sample ID	Dapagliflozin			Vildagliptin		
	RT	Area	% Assay	RT	Area	% Assay
VDG WS	-	-	-	1.49	882545	-
DAPA WS	2.30	729457	-	-	-	-
MIX WS	2.30	725375	-	1.49	893166	-
Drug Product	2.30	720587	**99.34**	1.49	890544	**99.71**

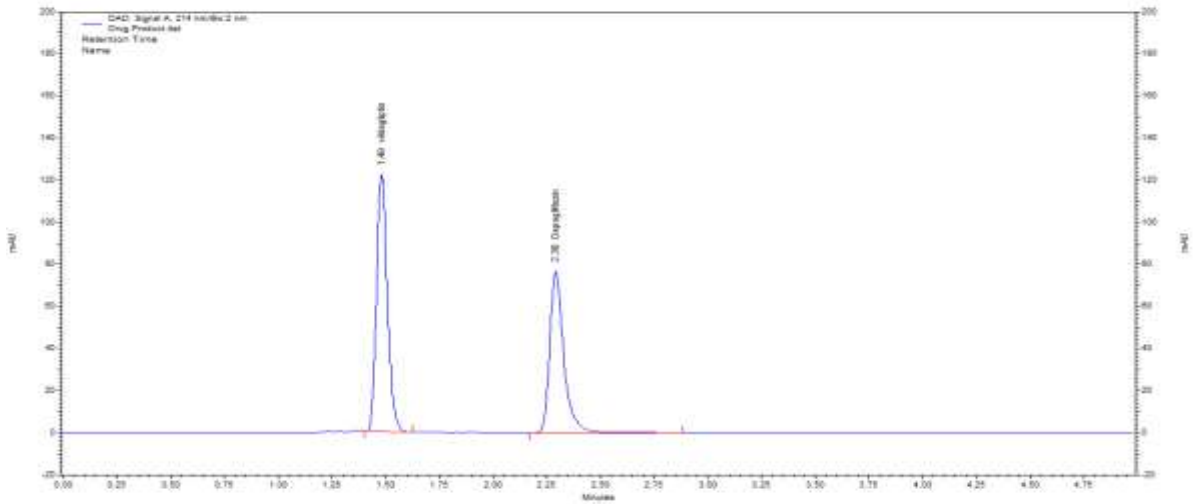

Figure 6 Chromatogram of Dapagliflozin and Vildagliptin in tablet formulation.

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Amount of drug present in the marketed formulation was calculated using RP-HPLC. Amount of Dapagliflozin and Vildagliptinwas found to be 99.34 &99.71% respectively. This method can be employed for routine analysis of Dapagliflozin and Vildagliptin. The result of assay of marketed formulation are given in Table 4.The separation was achieved by PhenomenexKinetex XB-C18 (150 x 4.6 mm, 5µm) column, at 0.5 ml/min flow rate, mobile phase containing 0.1% Perchloric acid: ACN in ratio of 55:45. The detection was carried out at 214 nm. The retention time of Dapagliflozin and Vildagliptinwas found to be 2.31 ± 0.5 min and 1.49 ± 0.5 min respectively.After establishing the chromatographic conditions, analysis of tablet formulation was done. The results are given in (Table 4 & Figure 6)

7.3) VALIDATION OF RP-HPLC METHODE :[16-19]

A. Linearity: Different concentration of solution prepared for Linearity of both Dapagliflozin and Vildagliptin are shown in (Table 5 and Table 6) calibration curves are shown in Figure 7&8 respectively.

Table 5 Linearity dilutions for Dapagliflozin.

Dapagliflozin		
% Level	**Conc. (µg/ml)**	**Area**
80	8	579586
90	9	652265
100	10	725375
110	11	794368
120	12	864607

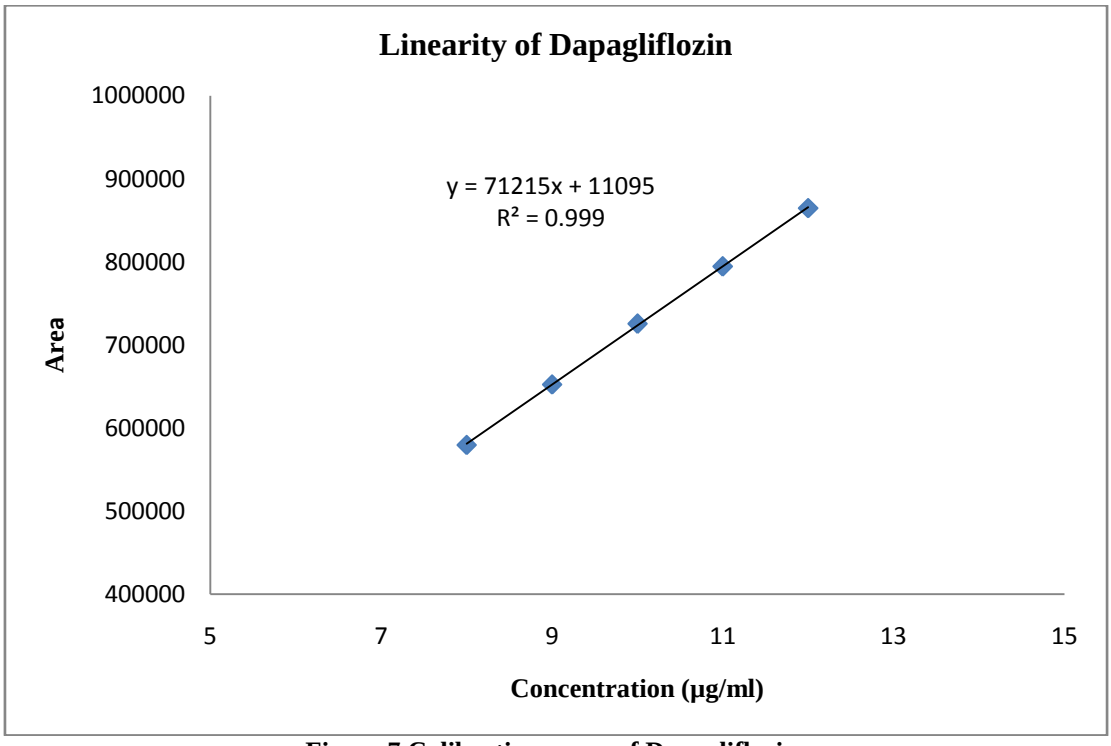

Figure 7 Calibration curve of Dapagliflozin.

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Table 6 Linearity dilutions for Vildagliptin.

Vildagliptin.		
% Level	**Conc. (µg/ml)**	**Area**
80	80	715492
90	90	801635
100	100	893166
110	110	975103
120	120	1069035

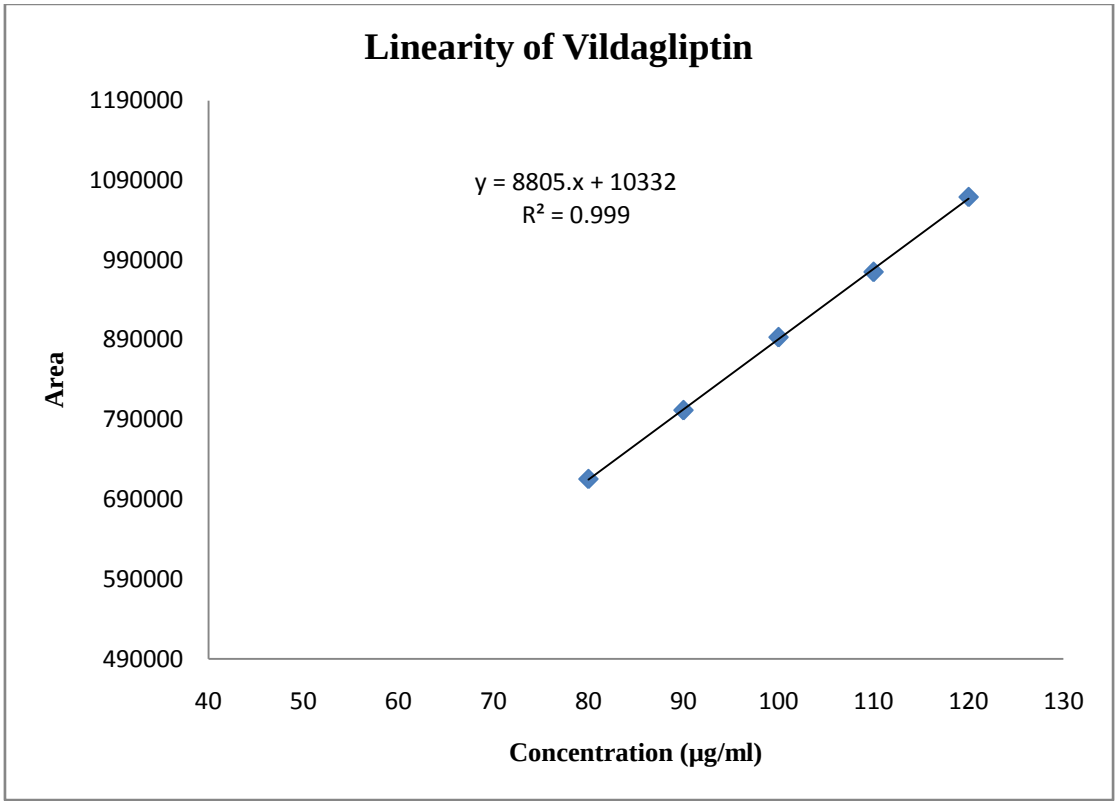

Figure 8 Calibration curve of Vildagliptin.

According to ICH guideline linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of an analyte Linearity was studied by plotting a graph of area v/s concentration. A series standard solution of Dapagliflozin and Vildagliptinwere prepared in the concentration range of 8 to 12 µg/ml and 80 µg/ml to 120 µg/ml respectively with linearity range 80-120% for both the drug and is shown in Table 5 and 6. The regression coefficient (r^2) of Dapagliflozin was found to be 0.999 & for Vildagliptin regression coefficient (r^2) was found to be 0.999. The equation of regression line for Dapagliflozinwas found to be y=71215x+11095 for Vildagliptin was found to be y=8805.5x+10332 Linearity graph of Dapagliflozin and Vildagliptinshown in figure 7 & 8 respectively.

B. Precision:

The Precision study of Dapagliflozin and Vildagliptinare shown Table 7 respectively.

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Table 7 Precision of Dapagliflozin and Vildagliptin.

Dapagliflozin				
Condition	**Sample ID**	**RT**	**Area**	**% Assay**
Morning	WS	2.30	725375	-
	DP	2.30	720587	99.34
Evening	WS	2.30	717548	-
	DP	2.30	712305	99.27
% RSD				**0.05**
Day 2	WS	2.30	712014	-
	DP	2.30	705514	99.09
% RSD				**0.13**

Vildagliptin				
Condition	**Sample ID**	**RT**	**Area**	**% Assay**
Morning	WS	1.49	893166	-
	DP	1.49	890544	99.71
Evening	WS	1.49	892104	-
	DP	1.49	888814	99.63
% RSD				**0.05**
Day 2	WS	1.49	890954	-
	DP	1.49	885142	99.35
% RSD				**0.19**

The Precision of test results is ensured by intraday and interday precision. Dapagliflozin and Vildagliptin both had % RSD values less than 2. Results are shown in **Table 7.**

C. Accuracy: The accuracy study of Dapagliflozin and Vildagliptin are shown in Table 8 and 9respectively.

Table 8.Accuracy Study of Dapagliflozin.

Sample ID	**Reps**	**Spiked Conc. (µg/ml)**	**Area**	**Amount Recovered (µg/ml)**	**% Recovery**	**AVG**	**STDEV**	**% RSD**
80%	Rep 1	7.99	579586	8.00	100.16	100.03	0.125218	0.13
	Rep 2	7.99	578142	7.98	99.91			
	Rep 3	7.99	578758	7.99	100.02			
100%	Rep 1	9.99	725375	10.02	100.28	99.99	0.272337	0.27
	Rep 2	9.99	721474	9.96	99.74			
	Rep 3	9.99	722947	9.98	99.95			
120%	Rep 1	11.99	864607	11.94	99.61	99.58	0.103388	0.10
	Rep 2	11.99	865120	11.95	99.67			
	Rep 3	11.99	863374	11.92	99.47			

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Table 9.Accuracy study of Vildagliptin.

Sample ID	Reps	Spiked Conc. (µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	AVG	STDEV	% RSD
80%	Rep 1	79.92	715492	80.03	100.14	100.05	0.08944	0.09
	Rep 2	79.92	714214	79.89	99.96			
	Rep 3	79.92	714865	79.96	100.05			
100%	Rep 1	99.90	893166	99.91	100.01	100.02	0.083585	0.08
	Rep 2	99.90	892574	99.84	99.94			
	Rep 3	99.90	894057	100.01	100.11			
120%	Rep 1	119.88	1069035	119.58	99.75	99.65	0.133078	0.13
	Rep 2	119.88	1068541	119.52	99.70			
	Rep 3	119.88	1066355	119.28	99.50			

The method's accuracy defines how close the method's results are to the true value. The results of the accuracy testing revealed that the technique is accurate within acceptable ranges. When the % RSD for Dapagliflozin and Vildagliptin is calculated, all of the results are within acceptable bounds. A maximum RSD of 2.0% indicated acceptable accuracy within the range. The results are shown in Table 8 and 9.According to the Accuracy research, the percent recovery of Dapagliflozin99.47- 100.28 % and Vildagliptin is 99.50-100.14 % both of which are within the ICH standards.

D. Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ of Dapagliflozin and Vildagliptin are shown in Table 10.

Table 10. The LOD and LOQ of Dapagliflozin and Vildagliptin.

Sr.No	Name of drug	LOD (µg/ml)	LOQ(µg/ml)
1.	**Dapagliflozin**	**0.23**	**0.70**
2.	**Vildagliptin**	**3.53**	**10.68**

Sensitivity of the method was determined with respect to limit of detection (LOD) and the quantification limit of an individual analytical procedure is the lowest amount of an analyte in a sample which can be quantitatively determined with the suitable precision and accuracy.

LOD and LOQ value for Dapagliflozin was found to be 0.23 µg/ml and 0.70 µg/ml, LOD and LOQ value for Vildagliptin was found to be 3.53 µg/ml and 10.68 µg/ml respectively. Results are shown in Table 10.

E. System suitability:

System suitability data of Dapagliflozin and Vildagliptingiven in below Table 11.

Table 11 System suitability parameter of Dapagliflozin.

Dapagliflozin					
Sample ID	**Area**	**RT**	**TP**	**Asymmetry**	**Resolution**
100% Rep 1	725375	2.30	6750	1.48	7.92
100% Rep 2	721474	2.30	6925	1.46	7.92
100% Rep 3	722947	2.30	6631	1.53	7.92
100% Rep 4	725724	2.30	6458	1.47	7.92
100% Rep 5	722178	2.30	6347	1.51	7.92

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100% Rep 6	722257	2.30	6578	1.49	7.92
AVG	723326	2.30			
STDEV	1787.905	4.86			
% RSD	0.25	0.00			

Table 12 System suitability parameter of Vildagliptin.

Vildagliptin					
Sample ID	**Area**	**RT**	**TP**	**Asymmetry**	**Resolution**
100% Rep 1	893166	1.49	4229	1.15	0.00
100% Rep 2	892574	1.49	4178	1.13	0.00
100% Rep 3	894057	1.49	4322	1.12	0.00
100% Rep 4	893575	1.49	4314	1.17	0.00
100% Rep 5	891946	1.49	4029	1.16	0.00
100% Rep 6	893354	1.49	4241	1.14	0.00
AVG	893112	1.49			
STDEV	750.7162	0			
% RSD	0.08	0.00			

The system, method, and column performance were validated by testing system suitability features. Six times, a standard solution of Dapagliflozin and Vildagliptinwas injected into the system, and the system's suitable features were evaluated. Results are shown in Table 11 and 12.

F. Robustness: Robustness data of Dapagliflozin and Vildagliptingiven in below

Table 13 Robustness parameter of Dapagliflozin.

Variation in Column temperature (Dapagliflozin)							
Condition	**Sample ID**	**RT**	**Area**	**% Assay**	**Average**	**STDEV**	**% RSD**
28˚C	WS	2.30	723788	-	99.28	0.056488	0.06
	DP	2.30	718374	99.25			
30˚C	WS	2.30	725375	-			
	DP	2.30	720587	99.34			
32˚C	WS	2.30	723874	-			
	DP	2.30	718333	99.23			

Variation in wavelength (Dapagliflozin)							
Condition	**Sample ID**	**RT**	**Area**	**% Assay**	**Average**	**STDEV**	**% RSD**
212 nm	WS	2.30	731474	-	99.30	0.08784	0.09
	DP	2.30	726787	99.36			
214 nm	WS	2.30	725375	-			
	DP	2.30	720587	99.34			
216 nm	WS	2.30	721024	-			
	DP	2.30	715244	99.20			

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Table 14 Robustness parameter of Vildagliptin.

Variation in Column temperature (Vildagliptin)							
Condition	**Sample ID**	**RT**	**Area**	**% Assay**	**Average**	**STDEV**	**% RSD**
28˚C	WS	1.49	892237	-	99.68	0.028565	0.03
	DP	1.49	889125	99.65			
30˚C	WS	1.49	893166	-			
	DP	1.49	890544	99.71			
32˚C	WS	1.49	891725	-			
	DP	1.49	888974	99.69			

Variation in wavelength (Vildagliptin)							
Condition	**Sample ID**	**RT**	**Area**	**% Assay**	**Average**	**STDEV**	**% RSD**
212 nm	WS	1.49	902224	-	99.66	0.059086	0.06
	DP	1.49	898545	99.59			
214 nm	WS	1.49	893166	-			
	DP	1.49	890544	99.71			
216 nm	WS	1.49	880440	-			
	DP	1.49	877584	99.68			

Robustness was investigated using various deliberate alterations in chromatographic settings, such as changes in column Condition like 28˚C, 30˚C and 32˚C. RSD was shown to be less than 2% in the Dapagliflozin and Vildagliptinrobustness studies. As a result, it is strong and adheres to ICH criteria. Results are shown in Table 13 and 14.

IV. CONCLUSION:

The created high-performance liquid chromatographic method was also tested for accuracy and precision, and it was found to be useful and effective for Dapagliflozin and Vildagliptin quality control. Furthermore, the 5-minute analytical run time and reduced solvent usage result in a low-cost, ecologically friendly chromatographic approach. The suggested methodology for detecting Dapagliflozin and Vildagliptin is rapid, selective, and only requires simple sample preparation.The major advantage of this technique is that it is less timeconsuming and also eco-friendly because of its low consumption of organic solvents as compared to otheranalytical techniques.

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