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Research Article

Analytical Method Development and Validation of Dapagliflozin and Vildagliptin using High Performance Liquid Chromatography

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ABSTRACT:
A simple, Accurate, precise method was developed for the simultaneous estimation of the Dapagliflozin and Vildagliptin in Tablet dosage form. Chromatogram was run through Agilent 250 x 4.6 mm, 5µ. Mobile phase containing Buffer 0.1% OPA: Acetonitrile taken in the ratio 45:55 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was Potassium dihydrogen orthophosphate buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 260.0 nm. Retention time of Vildagliptin and Dapagliflozin were found to be 2.290 min and 2.896 min. %RSD of the Dapagliflozin and Vildagliptin were and found to be 0.5 and 0.4 respectively. %Recovery was obtained as 99.05% and 99.28% for Dapagliflozin and Vildagliptin respectively. LOD, LOQ values obtained from regression equations of Dapagliflozin and Vildagliptin were 0.01, 0.03 and 0.33, 1.0 respectively. Regression equation of Dapagliflozin is y = 32384x + 806.79, and y = 28076x + 6435.3 of Vildagliptin.
Key Words: Vildagliptin, Dapagliflozin, Rp Hplc, Validation.

INTRODUCTION

Dapagliflozin and Vildagliptin represent two important classes of oral medications used to manage type 2 diabetes mellitus (T2DM). Both drugs are essential components of modern pharmacotherapy for diabetes due to their efficacy in controlling hyperglycemia and their additional benefits in reducing diabetes-related complications. Dapagliflozin, a sodium-glucose co-transporter 2 (SGLT2) inhibitor, works by inhibiting the SGLT2 protein in the proximal renal tubules. This leads to increased urinary glucose excretion, thereby lowering blood glucose levels. In addition to glycemic control, Dapagliflozin has demonstrated significant benefits in reducing cardiovascular risk, as well as slowing the progression of chronic kidney disease (CKD), making it an ideal choice for patients with coexisting heart failure or CKD [1-4]. Dapagliflozin weight-reducing effects further enhance its appeal in patients with T2DM who are often overweight or obese [5].On the other hand, Vildagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor that works by preventing the breakdown of incretin hormones such as glucagon-like peptide-1 (GLP-1). These hormones stimulate insulin release from the pancreas in response to meals, while also suppressing glucagon secretion. By prolonging the action of incretins, Vildagliptin helps to regulate blood glucose levels in a glucose-dependent manner, which minimizes the risk of hypoglycemia [6-8]. Vildagliptin is often used in combination with other antidiabetic drugs to enhance glycemic control and has been found to be effective in both monotherapy and combination therapy, particularly with metformin [9, 10]. Dapagliflozin is chemically written as (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-6-(hydroxymethyl)oxane-3,4,5-triol[11] Vildagliptin written as (2S)-1-{2-[(3-hydroxyadamantan-1-yl)amino]acetyl}pyrrolidine-2-carbonitrile [12].

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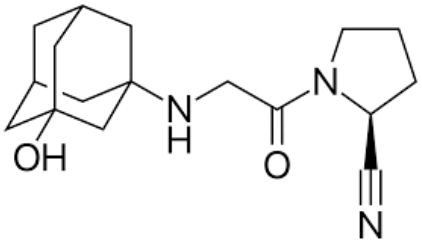

Figure 1: structure of Vildagliptin

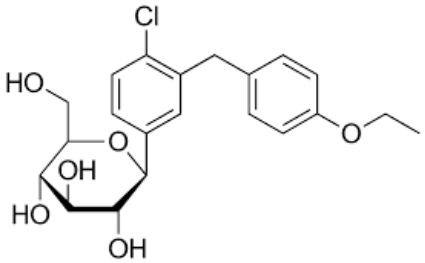

Figure 2: Structure of Dapagliflozin

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently few documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Vildagliptin, Dapagliflozin, and their medicinal dose form using RP-HPLC [13 - 17] must be validated and developed as per ICH guidelines

Materials and Methods: Spectrum pharma Research Solution provided with Vildagliptin and Dapagliflozin pure drugs (API) gift samples and Combination Vildagliptin and Dapagliflozin tablets (**Vildaily - DZ**). The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: In order to fulfill ICH standards, we need to design and test an HPLC technique that can detect Dapagliflozin and Vildagliptin in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	Acetonitrile and 0.1% OPA (55:45 v/v)
Flow rate	1 ml/min
Column	Agilent C18 (4.6 x 250mm, 5µm)
Detector wave length	260 nm
Column temperature	30°C
Injection volume	10 mL
Run time	4.0 min
Buffer	Ortho Phosphoric Acid

Buffer Preparation: 0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

API Preparation:

Preparation of Standard stock solutions: Accurately weighed 2.5mg of Dapagliflozin, 25mg of Vildagliptin and transferred to 50ml and 50ml individual volumetric flasks and 3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (50µg/ml of Dapagliflozin and 500µg/ml Vildagliptin)**.** 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (50µg/ml Vildagliptin of and 5µg/ml of Dapagliflozin)

Formulation Preparation:

Preparation of Sample stock solutions: 10 tablets were taken each tablet weigh and calculate the mean of total 10 minutes then equivalent to average weight of 1 tablet (10mg and 100mg) of dosage form was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for r the volume was made up with diluent

and filtered by HPLC filters (100µg/ml of Dapagliflozin and 1000µg/ml Vildagliptin): 0.5 ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (5µg/ml of Dapagliflozin and 50µg/ml Vildagliptin)

System suitability parameters: Vildagliptin (100 ppm) and Dapagliflozin (10 ppm) standard solutions were prepared, injected six times, and metrics such as peak tailing, resolution, and USP plate count were measured in order to evaluate the system suitability parameters. The region of six standard injection results should have an RSD of no more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. Therefore, this method was said to be specific.

Linearity: To test the drug's linearity, serial dilutions from 25% to 150% were prepared. A graph was used to demonstrate the link between peak area response and medication concentration. It was found to be linear at the indicated drug concentration. Dilution were as follows.

1. 25 µg/mL: Take 0.25 mL of stock solution and dilute to 10 mL
2. 50 µg/mL: Take 0.5 mL of stock solution and dilute to 10 mL
3. 75 µg/mL: Take 0.75 mL of stock solution and dilute to 10 mL
4. 100 µg/mL: Take 1.0 mL of stock solution and dilute to 10 mL
5. 125 µg/mL: Take 1.25 mL of stock solution and dilute to 10 mL
6. 150 µg/mL: Take 1.5 mL of stock solution and dilute to 10 mL

Accuracy: Accuracy was performed in triplicate for various concentrations equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. Dilution were as follows.

1. 50 µg/mL: Take 0.1 mL of stock solution and dilute to 10 mL
2. 100 µg/mL: Take 0.2 mL of stock solution and dilute to 10 mL
3. 150 µg/mL: Take 0.3 mL of stock solution and dilute to 10 mL

Sensitivity

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. Based on the response's standard deviation and calibration curve's slope, the LOD and LOQ can be estimated.

Assay

The assay and % purity were calculated for brand Vildaily - DZ with label claim Dapagliflozin 10g and Vildagliptin 100mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form.

Degradation studies:

Oxidation: To 1 ml of stock solution of Dapagliflozin and Vildagliptin, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 50µg/ml & 5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock s solution Dapagliflozin and Vildagliptin, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 50µg/ml & 5µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 ml of stock solution Dapagliflozin and Vildagliptin, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 50µg/ml & 5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 50µg/ml & 5µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 100µg/ml Dapagliflozin & 1000µg/ml Vildagliptin solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 50µg/ml & 5µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 50µg/ml & 5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Table 2: System suitability results

S.no	Vildagliptin			Dapagliflozin			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.286	3441	1.42	2.890	4970	1.32	3.6
2	2.291	3502	1.42	2.900	5152	1.32	3.6
3	2.299	3496	1.41	2.908	4328	1.33	3.7
4	2.306	3480	1.42	2.910	4698	1.32	3.7
5	2.307	3549	1.43	2.916	5203	1.33	3.7
6	2.310	3576	1.42	2.917	5630	1.33	3.7

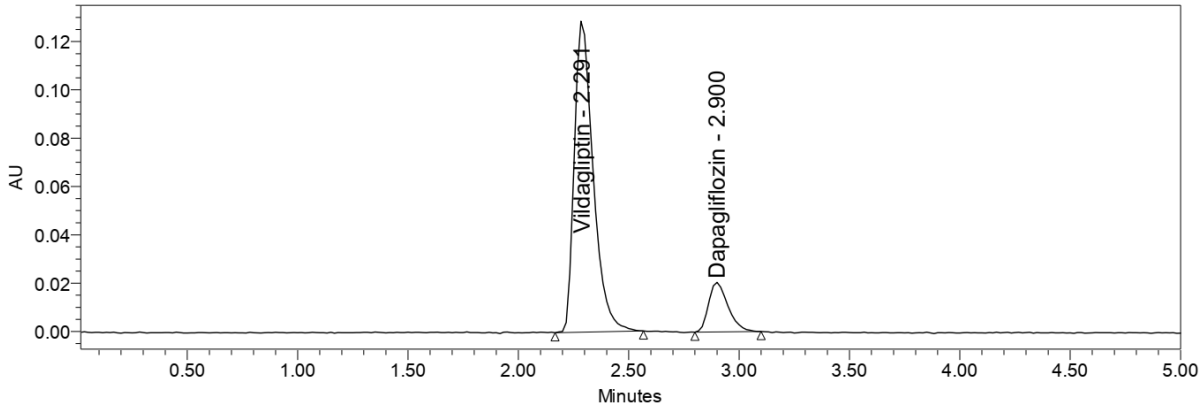

Figure 3: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time	Area	Plate count	Tailing	Resolution
Vildagliptin	2.290	770368	3470.3	1.4	
Dapagliflozin	2.896	131114	4694.	1.4	3.7

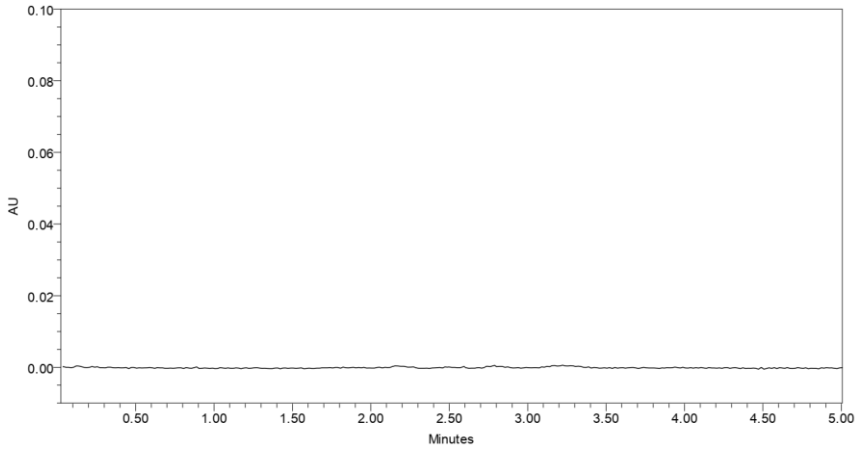

Figure.4 Specificity of Vildagliptin and Dapagliflozin

Linearity:

Calibration data is given in table 4 and regression data in table 5 and calibration curve in figure 5, 6

Table 4: Calibration data of Vildagliptin and Dapagliflozin

Vildagliptin		Dapagliflozin	
Conc (µg/mL)	**Peak area**	**Conc(µg/mL)**	**Peak area**
0	0	0	0
12.5	353719	1.25	40981
25	711545	2.5	82353
37.5	1067707	3.75	123832
50	1412802	5	161782
62.5	1774985	6.25	204378
75	2094294	7.5	242408

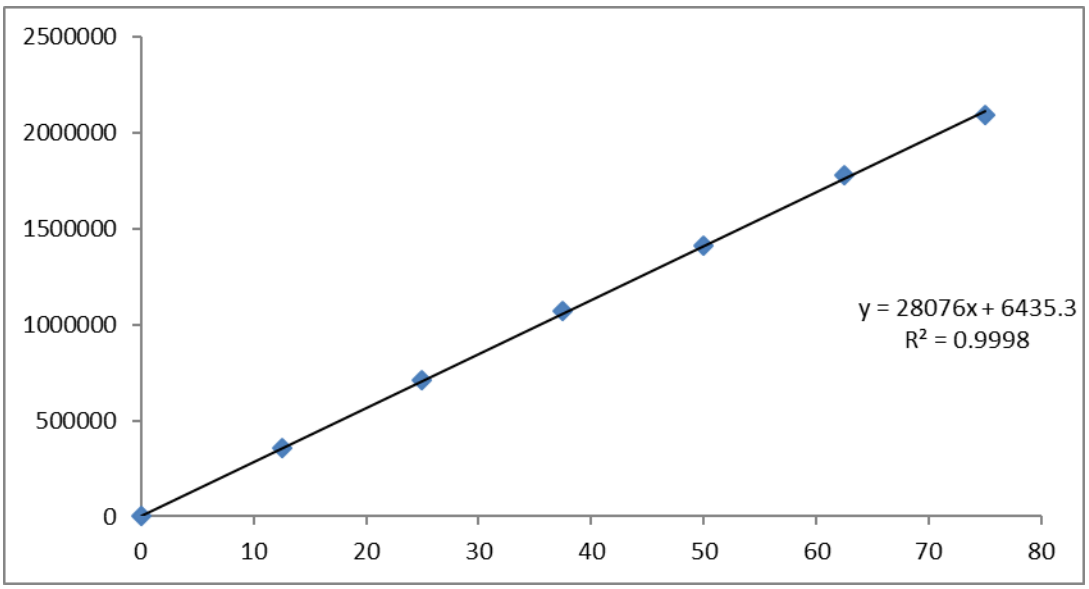

Figure 5 Calibration curve of Vildagliptin

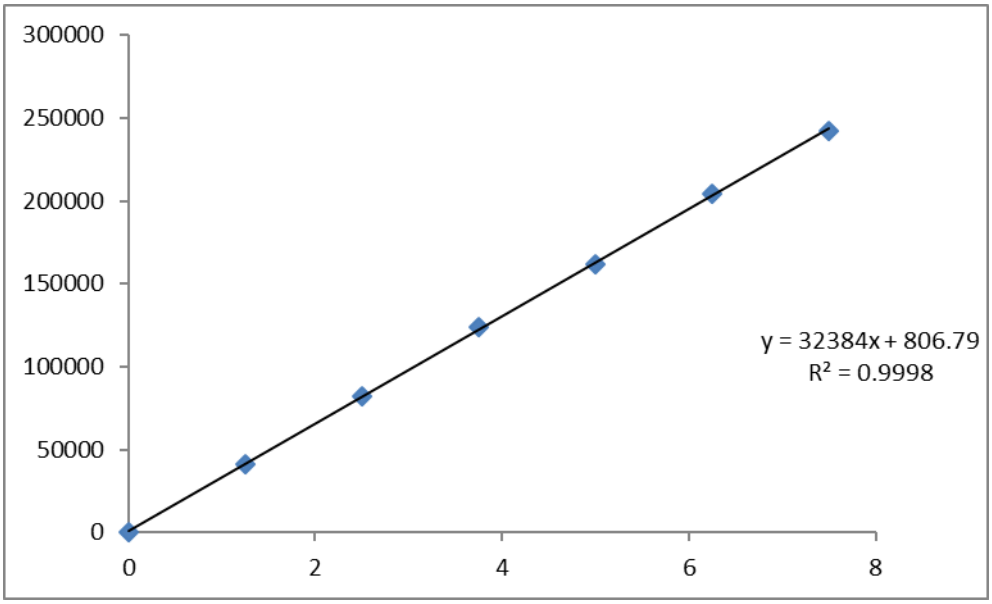

Figure 6 Calibration curve of Dapagliflozin

Table 5: regression data

Parameter	Vildagliptin	Dapagliflozin
Conc range (µg/mL)	12.5 – 75 µg/ml	1.25 – 7.5 µg/ml
Regression Equation	y = 28076x + 6435.3	y = 32384x + 806.79
Co-relation	0.999	0.999

Accuracy:

Recovery data shown in table 6

Table 6: recovery data of Vildagliptin and Dapagliflozin

	Vildagliptin			Dapagliflozin		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	25	24.939	99.75	2.5	2.490	99.61
		24.921	99.68		2.481	99.22
		24.913	99.65		2.477	99.07
100%	50	49.706	99.41	5.0	4.976	99.52
		49.745	99.49		4.971	99.41
		49.852	99.70		4.952	99.04
150%	75	74.620	99.49	7.5	7.484	99.78
		74.677	99.57		7.491	99.88
		74.636	99.52		7.485	99.80
% recovery	**99.59**			**99.48**		

System precision was performed and the data was shown in table 7

Table 7: System precision of Vildagliptin and Dapagliflozin

S. No	Area of Vildagliptin	Area of Dapagliflozin
1.	1427311	163171
2.	1436362	164577
3.	1439396	164533
4.	1420529	165018
5.	1423834	164401
6.	1429765	164876
Mean	1429533	164429
S.D	7245.1	657.7
%RSD	0.5	0.4

The % RSD for the peak areas of Vildagliptin and Dapagliflozin obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Vildagliptin and Dapagliflozin and shown in table 8.

Table 8: method Precision

S. No	Area of Vildagliptin	Area of Dapagliflozin
1.	1424756	162340
2.	1419658	163244
3.	1421313	163214
4.	1425629	162926
5.	1422935	164523
6.	1418175	162963
Mean	1422078	163202
S.D	2904.8	724.3
%RSD	0.2	0.4

From the above results, the % RSD of method precision study was within the limit for Vildagliptin and Dapagliflozin.

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.0ml/min), mobile phase minus (50A:50B), mobile phase plus (60A:40B), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 9: Robustness data for Vildagliptin and Dapagliflozin.

Condition	%RSD of Vildagliptin	%RSD of Dapagliflozin
Flow rate (-) 0.9ml/min	0.3	0.3
Flow rate (+) 1.1ml/min	0.4	0.5
Mobile phase (-) 50A:50B	0.4	0.9
Mobile phase (+) 60A:40B	0.3	0.9
Temperature (-) 27°C	0.6	0.9
Temperature (+) 33°C	0.3	0.5

Sensitivity:

Table 10: sensitivity of Vildagliptin and Dapagliflozin

Molecule	LOD	LOQ
Vildagliptin	0.33 µg/ml	1.00 µg/ml
Dapagliflozin	0.01 µg/ml	0.3 µg/ml

Force Degradation Studies: table 11 shows degradation conditions and table 10 shows the obtained degraded data and purity plot chromatogram in figure 8, 9.

Table 11: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	60 mins
Base	2N NAOH	60^{0}c	60 mins
Oxdation	20% H_2O_2	60^{0}c	60 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	60 mins

Table 12: degradation data

Type of degradation	Vildagliptin			Dapagliflozin		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	1382586	96.52	3.48	160896	97.66	2.34
Base	1381245	96.43	3.57	160553	97.45	2.55
Peroxide	1390653	97.09	2.91	158832	96.40	3.60
Thermal	1398756	97.65	2.35	162368	98.55	1.45
Uv	1420254	99.15	0.85	163378	99.16	0.84
Water	1421657	99.25	0.75	163893	99.47	0.53

Acid degradation chromatogram

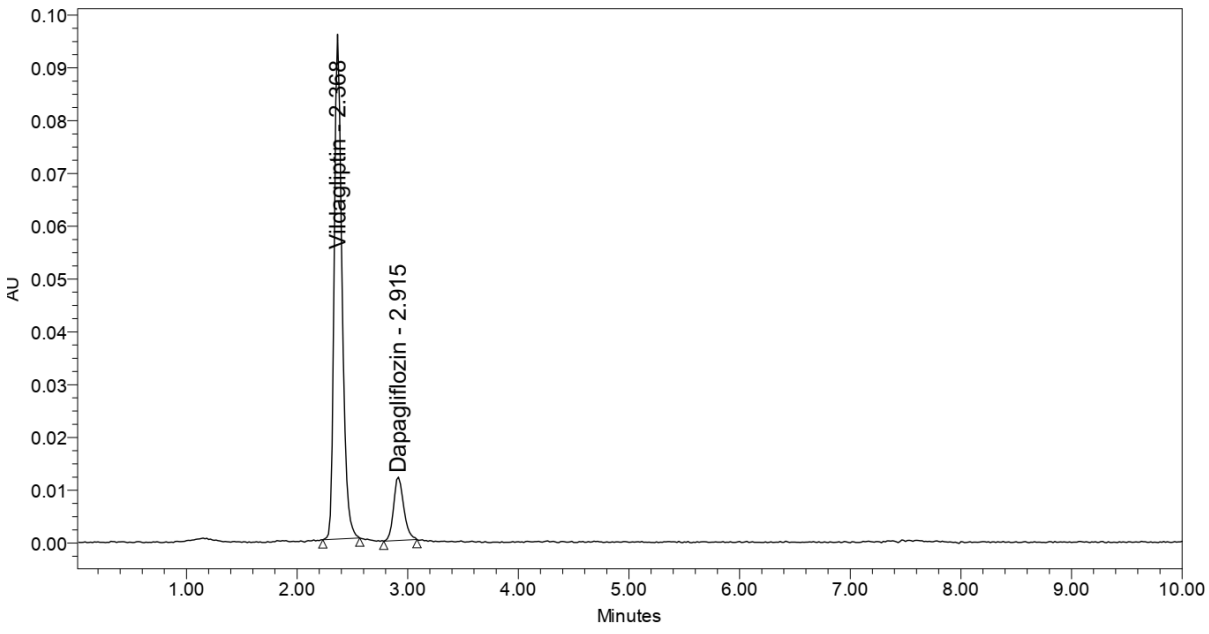

Fig 7 acid

Base degradation chromatogram

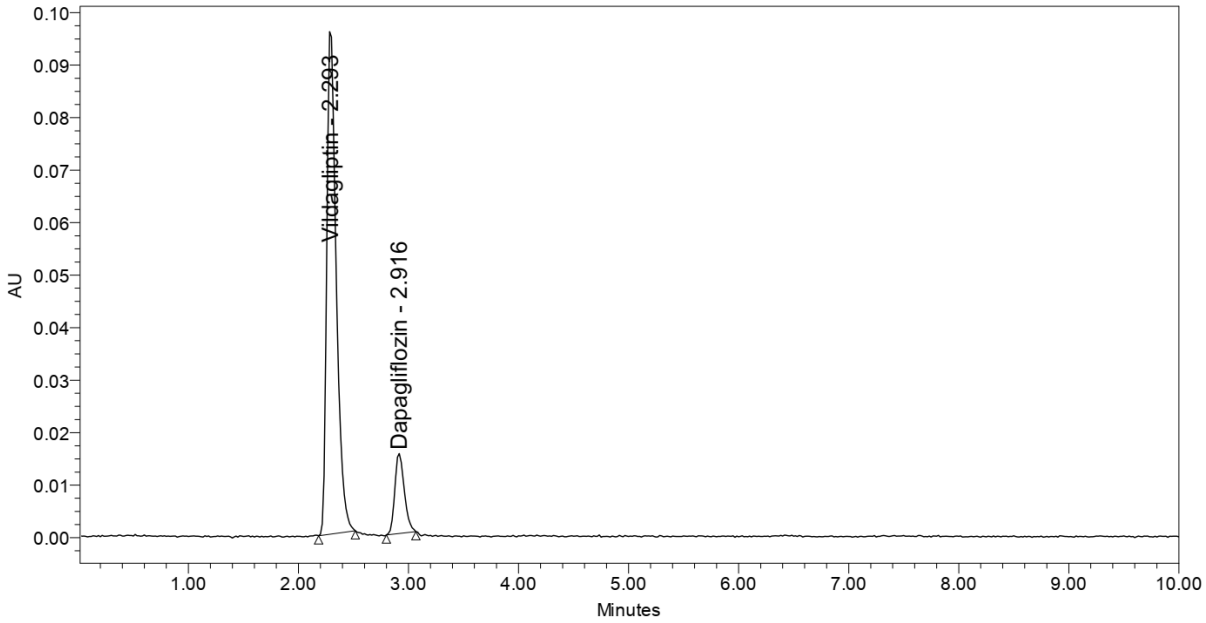

Fig 8 base

Peroxide degradation chromatogram

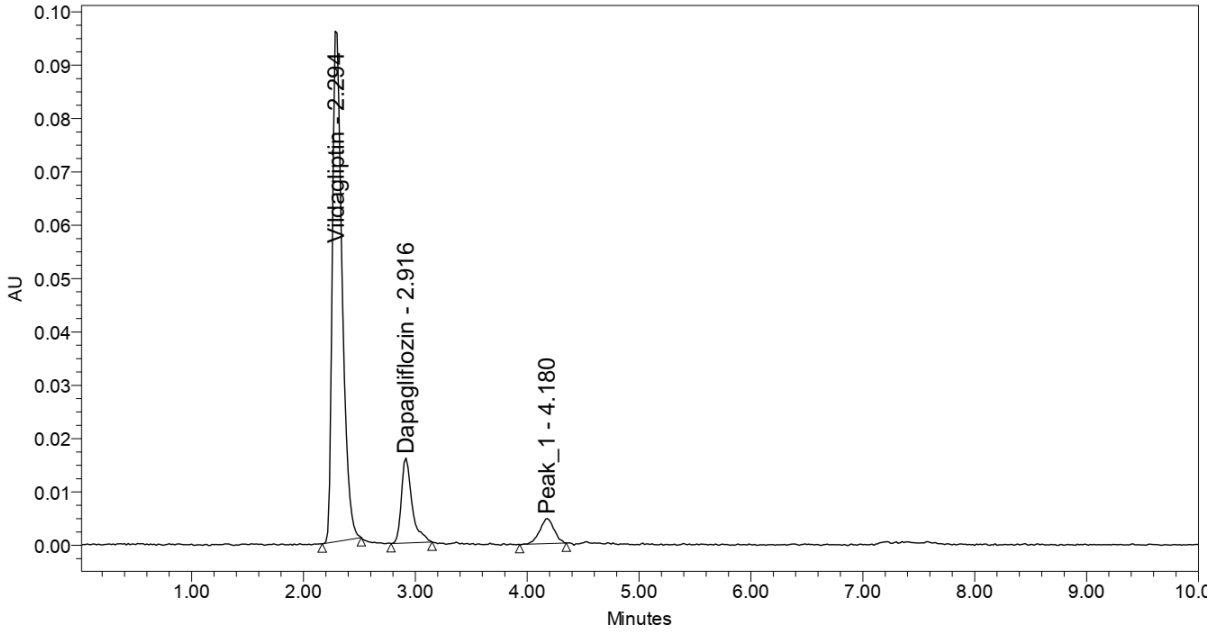

Fig 9 peroxide

Thermal degradation chromatogram

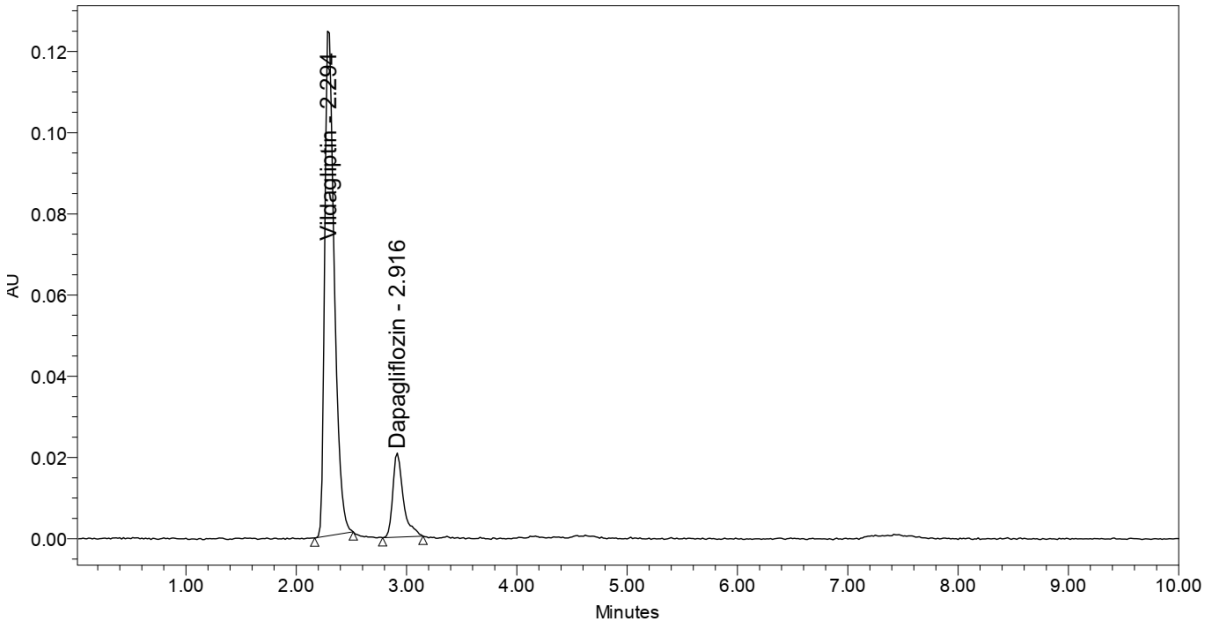

Fig 10 thermal

UV degradation chromatogram

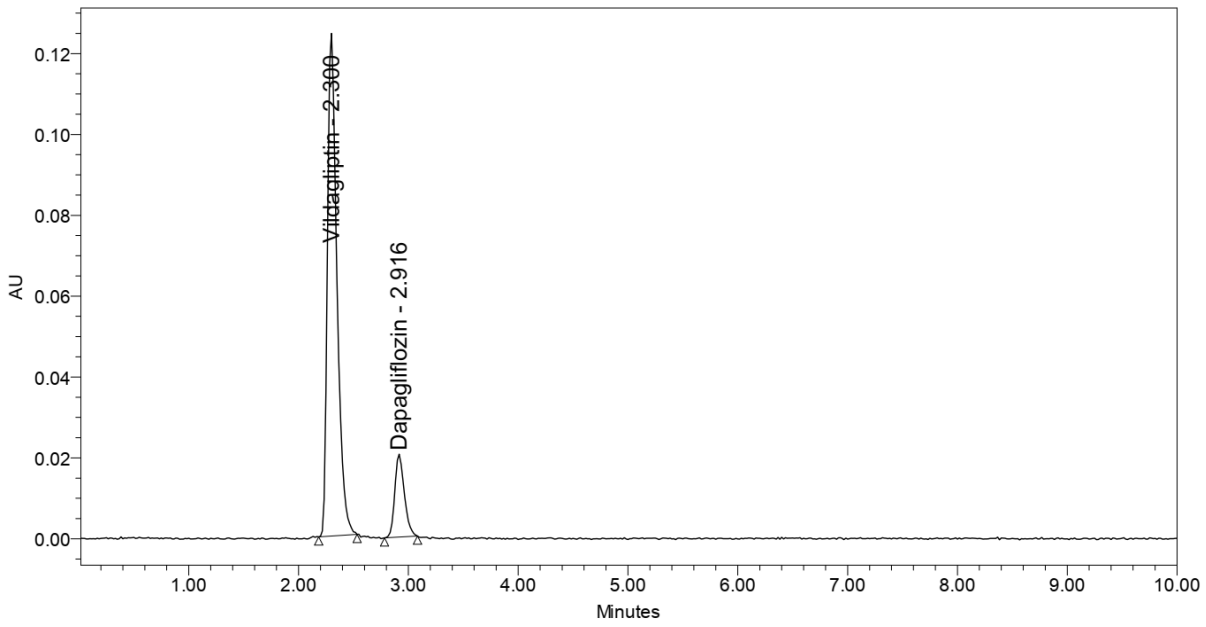

Fig 11 UV

Water degradation chromatogram

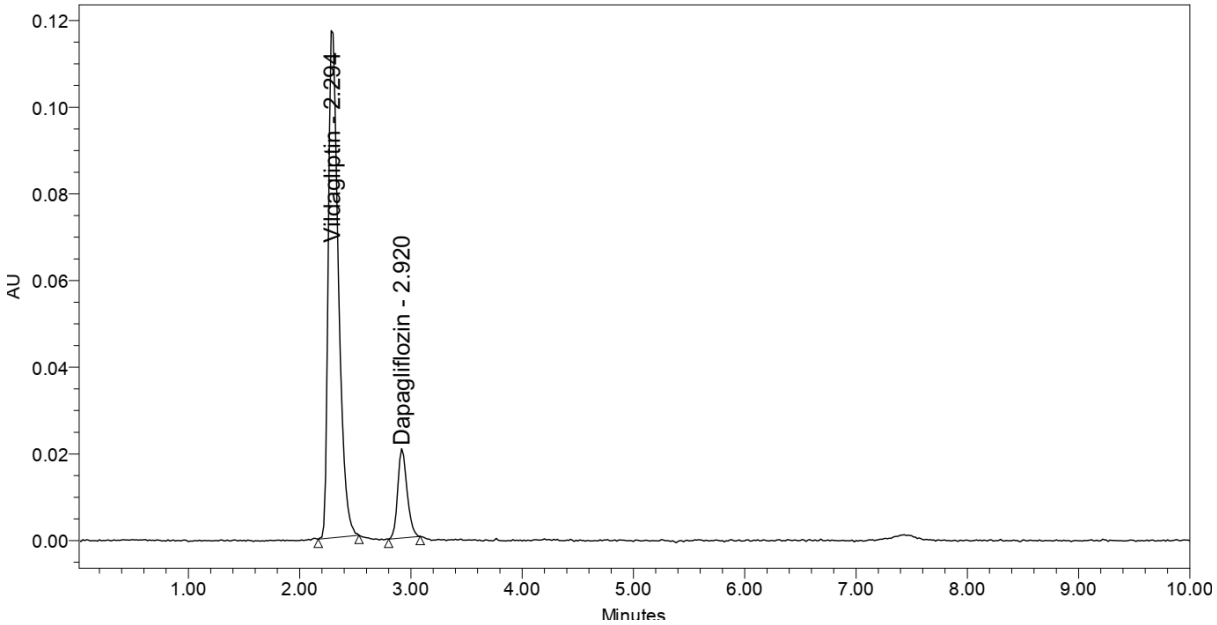

Fig 12 water

Assay: Vildaily - DZ, bearing the label claim Vildagliptin 100mg, Dapagliflozin 10mg. Assay was performed with the above formulation. Average % Assay for Vildagliptin and Dapagliflozin obtained was 99.97% and 100.15% respectively.

Table 13: assay data

Formulation	Label claim(mg)	% Assay*
Vildaily - DZ	Dapagliflozin 10mg.	99.05%w/w
	Vildagliptin 100mg	99.28%w/w

Conclusion:

The simultaneous analysis of Dapagliflozin and Vildagliptin in pharmaceutical formulations. The optimized conditions produced clear, well-separated peaks with good symmetry and a short analysis time. The method showed good linearity over the selected concentration ranges with acceptable correlation values. Precision and accuracy results were within limits, and recovery studies confirmed the accuracy of the method. Small changes in chromatographic conditions did not significantly affect the results, proving the method to be robust. Hence, this HPLC method is suitable for routine quality control and stability analysis of Dapagliflozin and Vildagliptin in combined dosage forms.

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