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Method Development and Validation for Simultaneous Estimation of Dapagliflozin and Vildagliptin in Pharmaceutical Dosage form Using RP-HPLC

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ABSTRACT:

A straightforward and accurate method was developed for the simultaneous estimation of Dapagliflozin and Vildagliptin in tablet dosage form. The chromatogram was passed through a standard Kromosil C18 column with dimensions of 4.6 x 150mm and a particle size of 5µm. A mobile phase consisting of a mixture of Acetonitrile and Na2hpo4 in a ratio of 70:30 was passed through a column at a flow rate of 0.9 ml/min. The method utilizes a buffer. The pH of the phosphate buffer is adjusted to 5.4 by adding 0.1% formic acid. The temperature was consistently maintained at 30°C. The spectrum chosen for optimization was 240 nm. The retention times of Dapagliflozin and Vildagliptin were determined to be 2.890 minutes and 2.349 minutes, respectively.The relative standard deviation (RSD) of Dapagliflozin and Vildagliptin were determined to be 0.3 and 1.0, respectively. Percent The recovery rates for Dapagliflozin and Vildagliptin were 99.95% and 100.07% respectively. The limits of detection (LOD) and limits of quantification (LOQ) values obtained from the regression equations for Dapagliflozin and Vildagliptin were as follows: 0.07 and 0.21 for Dapagliflozin, and 0.46 and 1.39 for Vildagliptin, respectively. The regression equation for Dapagliflozin is expressed as y = 8461.7x + 842.05. The equation y = 4162.9x + 747.14 represents the relationship between Vildagliptin and its corresponding value. The retention times and run time were minimized rendering the developed method simple and cost-effective for regular adoption in industrial quality control procedures.

Keywords: Dapagliflozin, Vildagliptin, RP-HPLC, Method Validation

Introduction:

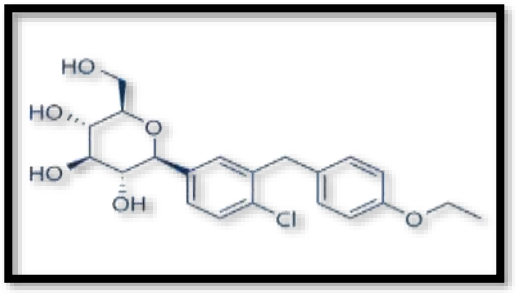
Type 2 Diabetes Mellitus a prevalent metabolic disorder, arises from the dual factors of impaired insulin secretion by pancreatic β-cells and the inadequate response of insulin-sensitive tissues to insulin.[1] It is a marked by increased blood glucose levels, resulting in long-term harm to the heart, blood vessels, eyes, kidneys, and nerves. T2DM, which accounts for more than 90% of diabetes mellitus cases, is characterized by a lack of insulin secretion from pancreatic islet β-cells, tissue resistance to insulin, and an insufficient compensatory response in insulin secretion.[2,3]The organs implicated in the development of type 2 diabetes mellitus (T2DM) encompass the pancreas (specifically β-cells and α-cells), liver, skeletal muscle, kidneys, brain, small intestine, and adipose tissue.[4] Emerging data indicate that adipokine dysregulation, inflammation, abnormalities in gut microbiota, immune dysregulation, and inflammation play significant roles in the development of this condition.[5]Endocrinologists commonly prescribe metformin as a treatment for type II diabetes mellitus, unless there are specific reasons not to.[6] It hinders the liver's mitochondrial respiratory chain, which triggers the activation of AMP-activated protein kinase (AMPK), improves insulin sensitivity (by affecting fat metabolism), and decreases cyclic adenosine monophosphate (cAMP) levels. As a result, it reduces the production of enzymes involved in glucose production and helps maintain normal blood sugar levels. Dapagliflozin hinders the activity of sodium-glucose co-transporter 2 (SGLT2), which prevents the reabsorption of glucose that has been filtered in the kidney. As a result, more glucose is excreted in the urine, leading to a decrease in blood glucose levels. The mechanism of action is not influenced by the function of pancreatic β cells or the modulation of insulin sensitivity.[7]Vildagliptin forms a strong chemical bond with the active site of dipeptidyl peptidase-4 (DPP-4), resulting in long-lasting inhibition of the enzyme. [8] This results in elevated levels of intact glucagon-like peptide-1 (GLP-1), both after consuming a meal and during periods of fasting. Research has demonstrated that it effectively triggers the release of insulin while suppressing the release of glucagon in response to glucose levels.[9]

Background:

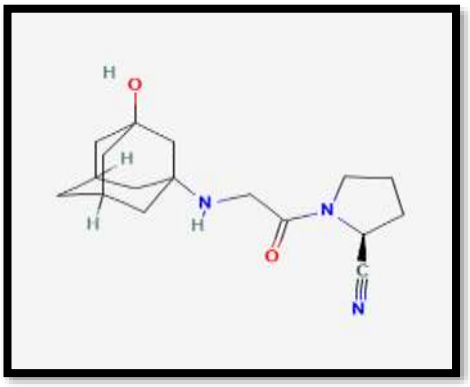
Dapagliflozin and Vildagliptin consists of the combination of sodium-glucose cotransporter type 2 inhibitors (SGT2i) and dipeptidyl peptidase-4 inhibitors (DPP4i) shows potential.[10] SGLT2i reduces high blood sugar levels by increasing the excretion of glucose in urine, without affecting the secretion or function of insulin. DPP4i, by inhibiting the degradation of active incretin hormones, not only improves glucose regulation but also stimulates insulin release and reduces glucagon release.[11]

Dapagliflozin-Chemically known as $C_{21}H_{25}ClO_6$, Chemical Nomenclature of the drug given by IUPAC-(2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4ethoxyphenyl) methyl] phenyl}-6-(hydroxymethyl) oxane-3,4,5-.Dapagliflozin is an inhibitor of the sodium-glucose cotransporter 2 (SGLT2), and it was the initial SGLT2 inhibitor to receive approval. Prescribed for the treatment of type 2 diabetes mellitus.[12] When used alongside diet and exercise in adults, dapagliflozin enhances glycemic control by blocking the reabsorption of glucose in the proximal tubule of the nephron, resulting in the excretion of glucose in the urine, it has been studied both as a standalone treatment and as a supplementary therapy alongside insulin or other oral hypoglycemic agents.[13,14]

Vildagliptin-Chemically known as $C_{17}H_{25}N_3O_2$, Chemical Nomenclature of the drug given by IUPAC-(2S)-1-{2-[(3-hydroxy-1 -adamantanyl)amino]acetyl}pyrrolidine-2-carbonitrile,Vildagliptin is a medication which is effective in reducing high blood sugar levels. It works by specifically blocking the activity of the dipeptidyl peptidase-4 (DPP-4) enzyme. This medication is utilized for the management of type II diabetes mellitus, specifically in cases where there is a deficiency in the secretion of GLP-1 and impaired insulinotropic effects. Dual therapy with this medication is recommended in adults who have inadequate glycemic control, sulphonyl urea, or a thiazolidinedione.



Dapagliflozin, Vildagliptin shown in (figure-1).



Dapagliflozin

Vildagliptin Fig1: Structures of Dapagliflozin, Vildagliptin.

A comprehensive literature review revealed that numerous analytical methods have been documented, with the identification of more cost-effective approaches. However, no method has been reported for estimating stability studies. Therefore, a straightforward and economical method for determining the stability of Dapagliflozin, Vildagliptin, and in a pharmaceutical dosage form using RP-HPLC is proposed.[19-30] must be developing and validated asper the guidelines of ICH (Q2 specification) [31].

Materials and Reagents

Dapagliflozin, Vildagliptin, the respective pure drugs were acquired from Spectrum Pharma research solutions. The Dapagliflozin, Vildagliptin, and Jalra-Dp combination tablet was purchased from India Mart in Hyderabad. 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:

The primary objective of this study is to create a highly reliable, exact, sensitive, specific, consistent, and expedient analytical method for concurrently determining the quantities of Dapagliflozin and Vildagliptin in both their pure form and tablet formulation.

Chromatographic Conditions:

Flow rate: 1ml/min

Column: Kromosil C18 (4.6 x 150mm, 5µm)

Buffer: Di sodium phosphate buffer

Detector: 220.0 nm

Temperature: Ambient

Injection volume:10.0µL

Run time: 5.0 mins

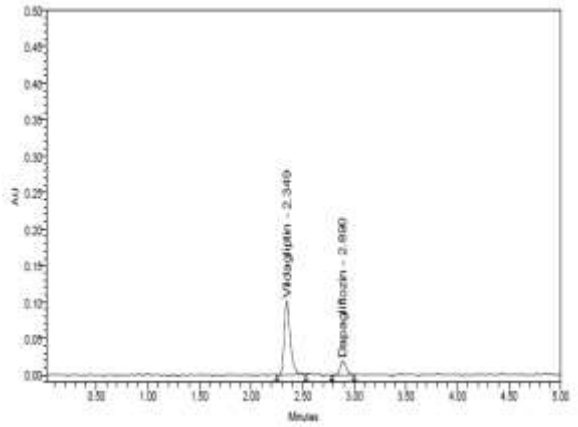


Fig 2 Optimized Chromatogram

Preparation of Buffer

Preparation of 0.01N di-sodium hydrogen phosphate Buffer: Weighed precisely 1.41 grams of Sodium dihydrogen Ortho phosphate and added it to a 1000 milliliter Volumetric flask. Approximately 900 milliliters of milli-Q water were then added and degassed through sonication. The flask was filled with water to reach the desired volume, and the pH was adjusted to 5.4 using diluted Formic acid.

Preparation of Standard solution: Precisely measured 10mg of Dapagliflozin and 100mg of Vildagliptin, and then transferred them into separate 100ml volumetric flasks. Three-fourths of the diluents was added to the flask and subjected to sonication for a duration of 10 minutes. The flasks were prepared by combining diluents and labeled as Standard stock solution 1. The concentration of Dapagliflozin is 100µg/ml and the concentration of Vildagliptin is 1000µg/ml.

Preparation of Standard working solution:1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (10 µg/ml of Dapagliflozin and 100µg/ml of Vildagliptin)

Preparation of Sample solution: 10 tablets were weighed and the mean weight of each tablet was determined. Then, the weight corresponding to one tablet was transferred into a 100 ml volumetric flask. 50 ml of diluent was added, and the mixture was subjected to sonication for 25 minutes. Subsequently, the volume was adjusted with diluent and filtered using HPLC filters. The concentration of Dapagliflozin is 100µg/ml and the concentration of Vildagliptin is 1000µg/ml.

Preparation of Sample working solution: 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (10 µg/ml of Dapagliflozin and 100µg/ml of Vildagliptin)

Method Validation

The HPLC method was validated to simultaneously estimate the drug substances Dapagliflozin and Vildagliptin, following the ICH guidelines. This was done to show that the method is suitable for routine analysis.

System suitability:

The system suitability parameters were determined by preparing standard solutions of Dapagliflozin (10ppm) and Vildagliptin (100ppm). These solutions were then injected six times to determine parameters such as peak tailing, resolution, and USP plate count. The relative standard deviation (RSD) for the area of six standard injections should not exceed 2%. System suitability chromatogram was shown in figure 3 and values are mentioned in the table 1.

Specificity (Selectivity):

Checking of the interference in the optimized method. We haven't found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific. Representative chromatogram is shown in Figure 4 and experimental data is given in Table 2.

Table 1:S ystem suitability results

	Peak Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Vildagliptin	2.349	417912	8221.5		1.3
2	Dapagliflozin	2.890	87187	11763.5	5.1	1.1

Fig 3: System suitability Chromatogram of Dapagliflozin and Vildagliptin.

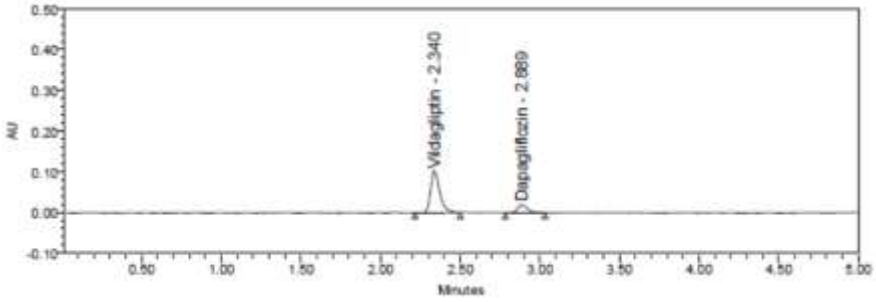


Table 2: Specificity data

Sample name	**Retention time(mins)**	**Area**
Dapagliflozin	2.890	82345
Vildagliptin	2.349	417648

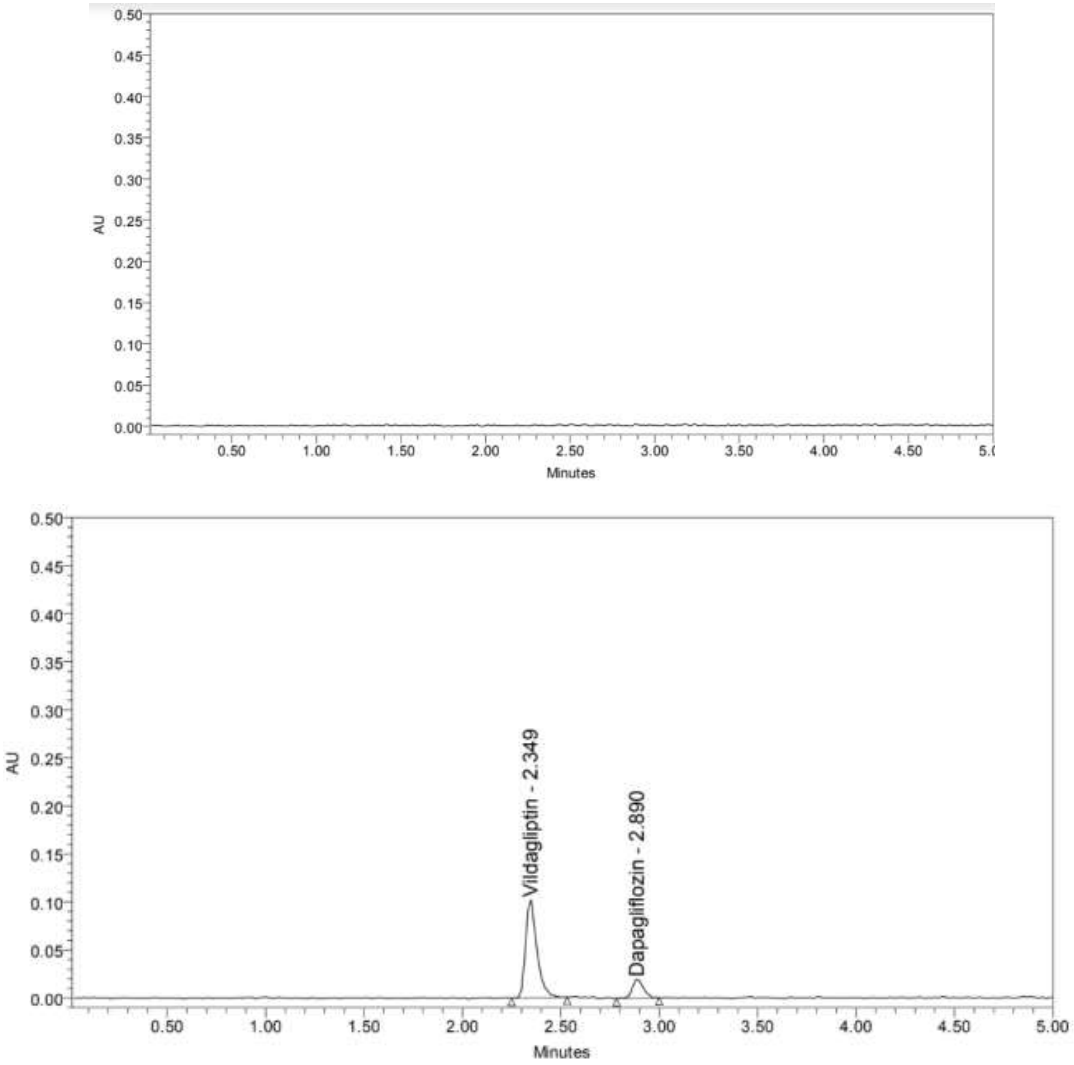


Fig 4: Specificity Chromatogram of Dapagliflozin, and Vildagliptin.

Table 3: Dapagliflozin Linearity

% Level	**CONC**	**Area**
0	0	0
25%	2.5	21625
50%	5	43735
75%	7.5	65149
100%	10	86006
125%	12.5	107556
150%	15	126061
R^2 value		0.999

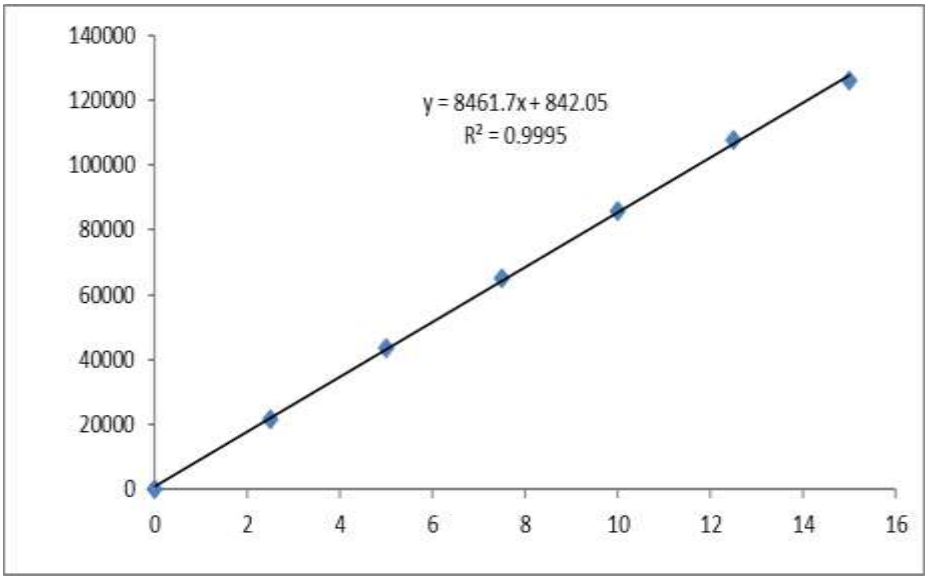


Fig 5: Dapagliflozin Calibration curve

Table 4: Vildagliptin Linearity

% Level	CONC	Area
0	0	0
25%	25	105881
50%	50	209019
75%	75	314876
100%	100	411857
125%	125	524386
150%	150	624721
R^2 value		0.999

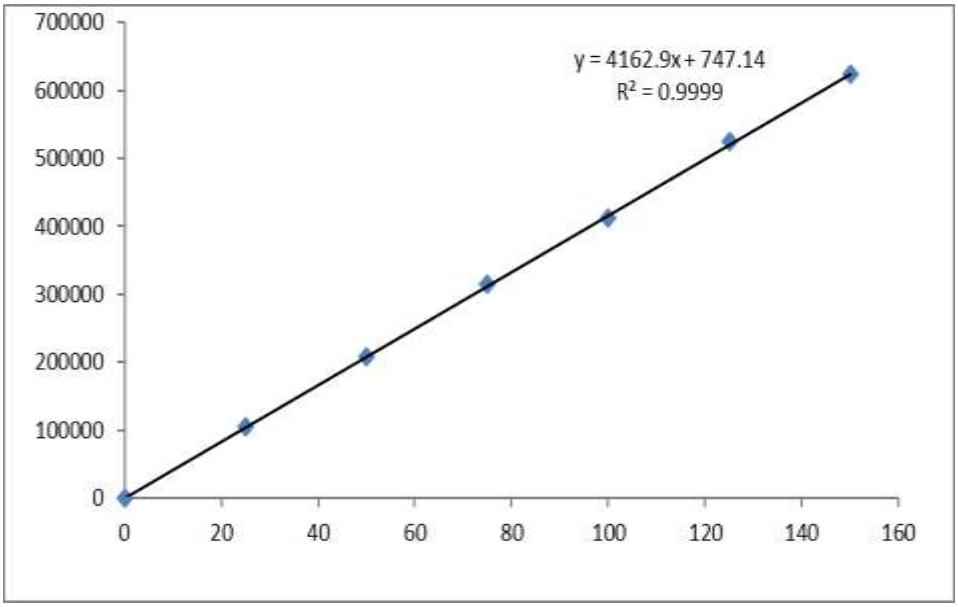


Fig 7: Vildagliptin Calibration curve

Table 6:Accuracy (%Recovery data)

%Level	**Recovery Data**					
	Dapagliflozin			**Vildagliptin**		
	Amt added	Amt found	%Rec	Amt added	Amt found	%Rec
50% Level	5	5.01	100.11	50	49.93	99.86
	5	4.95	99.00	50	49.97	99.93
	5	5.04	100.73	50	50.19	100.38
100%Level	10	10.00	99.98	100	100.82	100.82
	10	9.96	99.61	100	100.38	100.38
	10	9.99	99.86	100	99.23	99.23
150%Level	15	14.91	99.42	150	150.59	100.40
	15	15.11	100.75	150	149.27	99.52
	15	15.01	100.07	150	150.26	100.17
Mean%			**99.95**			**100.07**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Dapagliflozin andVildagliptin. Results of peak area are summarized in Table 7

Table 7: System precision data

Injection	**Dapagliflozin**	**Vildagliptin**
1	86817	410360
2	87387	419921
3	87015	416488
4	87650	411516
5	87067	419680
6	87159	418223
Avg	87183	416031
Std dev	295.3	4147.4
%RSD	0.3	1.0

The % RSD for the peak areas of Dapagliflozin andVildagliptinobtained 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 Dapagliflozin andVildagliptin. (Six individual sample preparations). Data obtained is summarized in Table 8.

Table 8: Method precision data

Injection	**Dapagliflozin**	**Vildagliptin**
1	87355	414521
2	87079	419480
3	87355	415163
4	87768	417535
5	87974	417219
6	87647	417724
Avg	87530	416940
Std dev	326.2	1817.0
%RSD	0.4	0.4

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

Table 9: Robustness results:-

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65B:35A), mobile phase plus (75B:25A), temperature minus (25°C) and temperature plus(35°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.

Chromatographic condition	**Dapagliflozin (RSD)**	**Vildagliptin (RSD)**
Flow(-)	0.3	0.8
Flow(+)	0.4	0.5
Temp(Ambient-)	0.4	0.6
Temp(Ambient+)	0.3	0.4
Mobile phase(-)	0.4	0.5
Mobile phase (+)	0.4	0.6

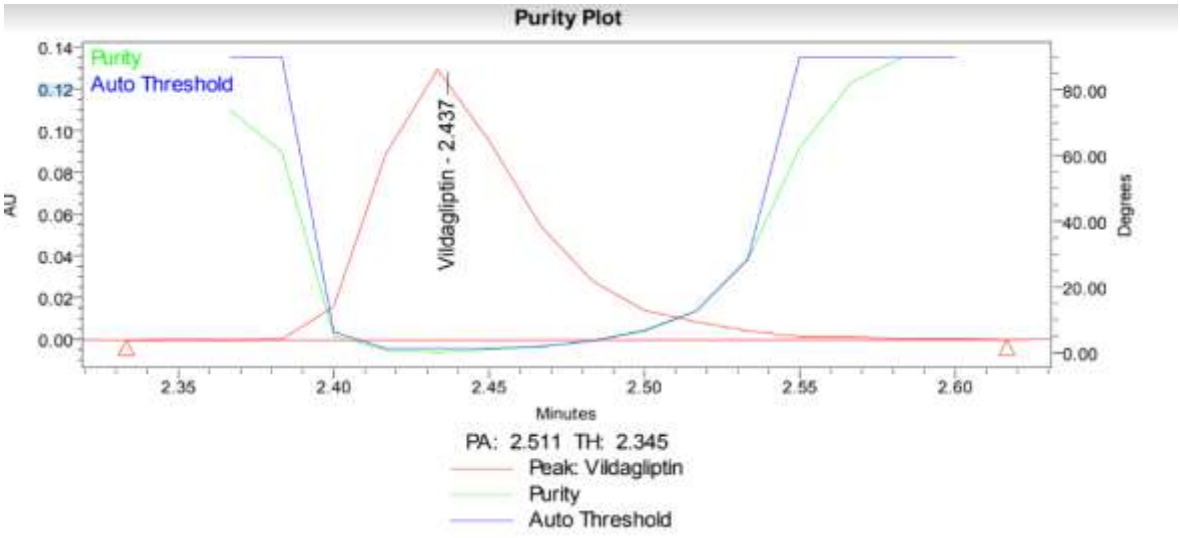
Table 10: Forced degradation conditions for Dapagliflozin and Vildagliptin.

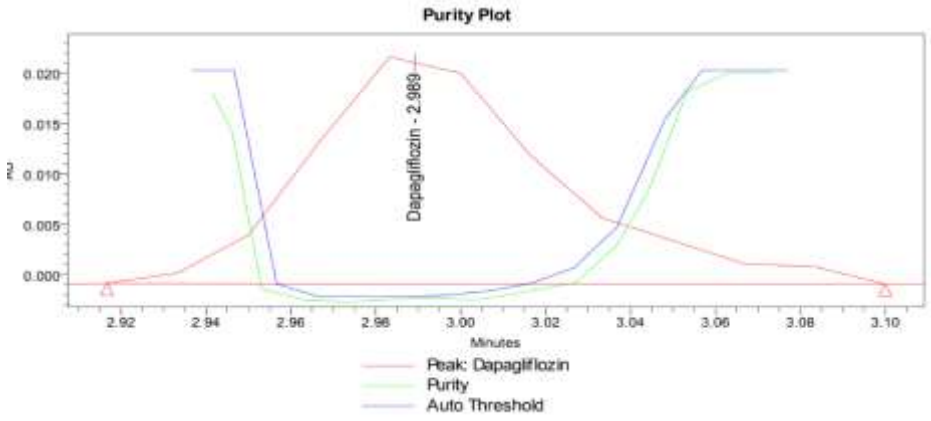
Stress condition	**Solvent**	**Temp(^{0}C)**	**Exposed time**
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxdation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

From the results, no degradation was observed when the samples were exposed to acid, base, hydrolysis, thermal, light and water. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 11: Degradation profile results

Type of degradation	**Vildagliptin**			**Dapagliflozin**		
	Area	%Recovered	%Degraded	Area	%Recovered	% Degraded
Acid	395386	94.85	5.15	82532	94.29	5.71
Base	397700	95.40	4.60	82834	94.63	5.37
Peroxide	398862	95.68	4.32	83201	95.05	4.95
Thermal	408108	97.90	2.10	85052	97.17	2.83
UV	410833	98.55	1.45	86583	98.92	1.08
Water	414097	99.34	0.66	87319	99.76	0.24





Purity Plot of Degraded of peroxide Sample

Table 12: Assay results for Dapagliflozin and Vildagliptin

	Label claim dose	**%Assay**
Dapagliflozin	10mg	100.00
Vildagliptin	100mg	100.02

(Cabenuva), bearing the label claim Dapagliflozin and Vildagliptin100MG, 10MG. Assay were performed with the above formulation.

Assay was performed by:-

The weight of 10 tablets was measured and the average weight of each tablet was calculated. Subsequently, the weight attributed to a single tablet was transferred into a volumetric flask with a capacity of 100 ml. A total of 50 milliliters of diluent was added, and the mixture underwent sonication for a duration of 25 minutes. Afterwards, the volume was modified with a diluent and then passed through HPLC filters for filtration. The Dapagliflozin concentration is 100µg/ml and the Vildagliptin concentration is 1000µg/ml. Preparation of the working solution sample: 1 milliliter of the filtered sample stock solution was transferred into a 10-milliliter volumetric flask and then filled with diluent to the top. The concentration of Dapagliflozin is 10 µg/ml and the concentration of Vildagliptin is 100 µg/ml. After injecting six samples of the formulation, the relative standard deviation (RSD) for the area of the six standard injections should not exceed 2%.

CONCLUSION

A novel stability indicating analytical approach was developed and validated using RP-HPLC methodology. The study's findings will greatly aid in the quality monitoring of Dapagliflozin and Vildagliptin in pharmaceutical dosage forms. This is due to the study's straightforward sample preparation method, which utilizes a minimal amount of mobile phase and requires only a brief analysis time. Upon analyzing two medications from a combination dose form, the results yielded a close approximation to 100% efficacy using the recently developed methodology. The recovery studies were successful, indicating that there is no influence from the excipient.

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