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STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND VILDAGLIPTIN IN PHARMACEUTICAL DOSAGE FORM

Vishva S. Patel [*1], Pankaj Kapupara [1], Jitendra O. Bhangale [2]

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Abstract

The objective of the study was to develop a reliable and consistent stability indicating RP-HPLC method for simultaneous determination of Dapagliflozin and Vildagliptin. By Utilizing a mobile phase of 0.05 M KH_2PO_4: Acetonitrile: Methanol (pH 6.13) (35:10:55% v/v/v), and detecting at 220 nm, the chromatographic separation of Dapagliflozin and Vildagliptin was achieved on an Anachrom Cosmosil C_{18} (250 × 4.6 mm. 5 µm) Column. The column temperature was maintained at 35°C, and the flow rate set a 1 ML/min. Retention times of 7.97 and 2.91 min were determined for Dapagliflozin and Vildagliptin, respectively. The developed method exhibited linearity, as evidenced by the regression analysis, which showed a strong correlation with a linear curve within the concentration range of 1-5 µg ml^{-1} for Dapagliflozin and 10-50 µg ml^{-1} for Vildagliptin. Forced deterioration experiments were carried out under various conditions, including thermal, photolytic, oxidative, acidic and basic environments. In accordance with ICH guidelines, the method's robustness, accuracy, precision, linearity, limit of detection, and specificity were all evaluated. Its adaptability, accuracy, and high precision make the developed method suitable for routine analysis of bulk and dosage forms.

Keywords: Dapagliflozin, Vildagliptin, RP-HPLC Method, Validation, Stability, Degradation.

[1*]Research Scholar, Department of Pharmaceutical Quality Assurance, School of Pharmacy, RK University, Rajkot, 360020, Gujarat, India.

[1]Professor, Department of Pharmaceutical Quality Assurance, School of Pharmacy, RK University, Rajkot, 360020, Gujarat, India.

[2]Professor, Department of Pharmacology, Smt. N. M. Padalia Pharmacy College, Ahmedabad, 382210, Gujarat, India,

Corresponding Author:

Vishva S. Patel, Research Scholar, Department of Pharmaceutical Quality Assurance, School of Pharmacy, RK University, Rajkot, 360020, Gujarat, India

vishva.patel22194@gmail.com

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INTRODUCTION

The third phase of the regulatory submission process may involve stress studies, also known as forced degradation, forced decomposition. These studies are crucial for the development of new drug substances and product, particularly when they are exposed to harsh conditions. Early forced degradation studies are conducted to identify degradants that may be present in the final drug product. The main factor that contributes to the degradation of drug compound and products are hydrolysis, oxidation, heat, and photolysis.

Dapagliflozin, $C_{21}H_{25}ClO_6$ (Figure 1), is gliflozin class of drug used in the treatment of diabetes mellitus type 2 as SGLT-2 inhibitor. It helps to improve glycemic control by inhibiting glucose reabsorption in the proximal tubule along with controlled diet and exercise.[1] Physicochemical properties of Dapagliflozin showed 408.87 g/mol molecular weight, 55°C-58°C of melting point, and soluble in Methanol, Acetone and Acetonitrile. Whereas, Vildagliptin , $C_{17}H_{25}N_3O_2$ (Figure 2), is DPP4 inhibitor indicated to reduce hyperglycemia in type 2 diabetes mellitus.[2] Physicochemical properties of Vildagliptin showed 303.39 g/mole molecular weight, 140°C-145°C of melting point and soluble in Acetone, Water, Methanol and Acetonitrile. Combination of Dapagliflozin and Vildagliptin is utilized to improve brain oxidative stress rather than single drug therapy and also provide better therapeutics approach for neuroprotection in obese-insulin resistant patients.[3]

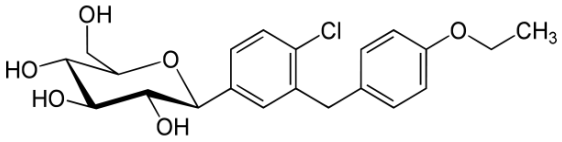

Figure 1: Structure of Dapagliflozin

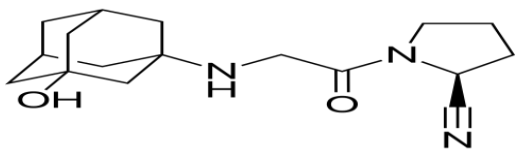

Figure 2: Structure of Vildagliptin

Based on literature, analysis has been performed on individual and other drug combination with Dapagliflozin and Vildagliptin using spectroscopic (UV and Mass spectroscopy) and Chromatographic (HPLC and HPTLC) techniques.[4-11] The main objective of the present study was to develop simple, rapid, precise, and accurate stability indicating RP-HPLC method for simultaneous estimation of Dapagliflozin and Vildagliptin in dosage form.

MATERIALS AND METHODS

Chemicals and Reagents: Dapagliflozin and Vildagliptin were obtained from Emmennar Pharma Pvt. Ltd., Sanathnagar, Hyderabad, India and Livmore Life Sciences Pvt. Ltd. Vadodara, Gujarat, India respectively as a gift sample. Solvents used for the study were procured from Finar chemicals, Ahmedabad, Gujarat, India. Every day, fresh solutions were prepared for the study. Jalra- DP tablets (Combination of Dapagliflozin (10 mg) and Vildagliptin (100 mg)) was procured from the local drug store, Ahmedabad, Gujarat.

Equipment and Chromatographic Condition: Shimadzu LC-2010 CHT system equipped with a photodiode array detector, an autosampler injector, and an Anachrom Cosmosil C_{18} Column (250 × 4.6 mm, 4.6 µm) was used. The Lab Solution software was employed to monitor and integrate the data. The mobile phase containing isocratic mixture of 0.05 M KH_2PO_4: Acetonitrile: Methanol (pH 6.13) (35:10:55% v/v/v) was used, and detection was carried out at a wavelength 220 nm with 10 min run time.

Method for preparation of solutions: Stock Solution for Dapagliflozin I (100 µg ml^{-1}): A precise measurement of 10 mg of DAPA was taken and added to a volumetric flask (100 ml), volume was make up using Methanol; **Stock Solution for Dapagliflozin II (20 µg ml^{-1}):** Pipetted out 20 ml from Stock Solution for Dapagliflozin I, which was transferred into

a 100 ml volumetric flask and volume was make up using Methanol; **Stock Solution for Vildagliptin I (1000 µg ml^{-1}):** A precise measurement of 100 mg of VILDA was taken and added to a volumetric flask (100 ml), volume was make up using Methanol, **Stock Solution for Vildagliptin II (200 µg ml^{-1}):** Pipetted out 20 ml from Stock Solution for Vildagliptin I, which was transferred into a 100 ml volumetric flask and volume was make up using Methanol. The 0.05 M KH_2PO_4 solution (Buffer solution) is prepared as per Indian Pharmacopeia 2020.[12]

Preparation of Mobile Phase: A Mixtures of 0.05 M KH_2PO_4: Acetonitrile: Methanol (35:10:55 % v/v/v, pH 6.13) was thoroughly mixed, followed by filtration under vacuum through a 0.45 µ filter.

FORCED DEGRADATION STUDY

Acid Degradation: To Prepare 2 µg/ml of DAPA and 20 µg/ml of VILDA, 1 ml was pippeted out from the standard stock solution containing 20 µg/ml of DAPA and 200 µg/ml of VILDA respectively and added in a 10 ml volumetric flask, then solution was treated with 2 ml of 0.1 N HCl (prepared as per IP, 2018) and warmed at 60°C for 1 h. The solution was allowed to cool and neutralized with 2 ml of 0.1 N NaOH (prepared as per IP, 2018), before being made up to 10 ml using Mobile Phase. The same procedure was repeated with 0.1 N HCl for 3 h to achieve appropriate degradation.[13,17]

Base Degradation: To Prepare 2 µg/ml of DAPA and 20 µg/ml of VILDA, 1 ml was pippeted out from the standard stock solution containing 20 µg/ml of DAPA and 200 µg/ml of VILDA respectively and added in 10 ml volumetric flask, then solution treated with 2 ml of 0.1 N NaOH and warmed at 60°C for 1 h. The solution was allowed to cool and neutralized with 2 ml of 0.1 N HCl, before being made up to 10 ml using Mobile Phase. The same procedure was repeated with 0.1 N NaOH for 3 h to achieve appropriate degradation.[14,17]

Oxidative Degradation: A 1 ml solution was pipetted from a stock solution that contained 20 µg/ml of DAPA and 200 µg/ml of VILDA. This 1 ml solution was then placed in a 10 ml volumetric flask, followed by the addition of 2 ml of 3 % H_2O_2. The solution was subjected to sonication for 5 min, left to stand at room temperature for 1 h, and made up to 10 ml with mobile phase. This final solution contained 2 µg/ml of DAPA and 20 µg/ml of VILDA. The same process was repeated for 3 h.[14,17]

Thermal Degradation (Dry Heat Degradation): Solution Preparation for Thermal degradation study of DAPA: To prepare Stock I solution, a precisely measured amount of 10 mg of DAPA was added to a clean and dry volumetric flask (100 ml). The DAPA was then subjected to heat at 80°C for 1 and 3 h. Subsequently, around 75 ml of diluent was added to the flask, and the solution was sonicated. Finally, the diluent was added up to mark on the flask, resulting in a concentration of 100 µg/ml. Pipette out 2 ml of Stock I was moved into a volumetric flask (10 ml) and then diluted with diluent, resulting in a concentration of 20 µg/ml (Stock II).

Solution Preparation for Thermal degradation study of VILDA: To prepare Stock I Solution, a precisely measured amount of 10 mg of VILDA was added to a clean and dry volumetric flask (100 ml). The VILDA was then subjected to heat at 80°C for 1 and 3 h. Subsequently, around 75 ml of diluent was added to the flask, and the solution was sonicated. Finally, the diluent was added up to mark on the flask, resulting in a concentration of 100 µg/ml. Pipette out 20 ml of Stock I was moved into a volumetric flask (10 ml) and then diluted with diluent, resulting in a concentration of 200 µg/ml (Stock II)

Solution Preparation for Thermal degradation study of DAPA and VILDA: A 1 ml portion of the Stock solution of DAPA II and VILDA II was taken and added into volumetric flask (10 ml). The

flask was the filled up to mark with diluent to obtain concentration of DAPA (2 µg/ml) and VILDA (20 µg/ml).[15,17]

Photolytic Degradation: Solution Preparation for Photolytic degradation study of DAPA: A clean and dry petri dish was used to hold approximately 10 mg of DAPA. The dish was then placed in a UV cabinet and continuously exposed to UV light for 1 and 3 h. The DAPA was then transferred to a 100 ml volumetric flask and diluted with diluent, followed by sonication with intermittent shaking at a controlled Temp. until the DAPA was completely dissolved. Diluent was added to adjust the volume to the desired level until a concentration of 100 µg/ml was achieved, Pipette out 2 ml of Stock I was moved into a volumetric flask (10 ml) and then diluted with diluent, resulting in a concentration of 20 µg/ml (Stock II).

Solution Preparation for Photolytic degradation study of VILDA: A clean and dry petri dish was used to hold approximately 10 mg of VILDA. The dish was then placed in a UV cabinet and continuously exposed to UV light for 1 and 3 h. The VILDA was then transferred to a 100 ml volumetric flask and diluted with diluent, followed by sonication with intermittent shaking at a controlled Temp. until the VILDA was completely dissolved. Diluent was added to adjust the volume to the desired level until a concentration of 100 µg/ml was achieved, Pipette out 20 ml of Stock I was moved into a volumetric flask (10 ml) and then diluted with diluent, resulting in a concentration of 200 µg/ml (Stock II).

Solution Preparation for Photolytic degradation study of DAPA and VILDA: A 1 ml portion of the Stock solution of DAPA II and VILDA II was taken and added into volumetric flask (10 ml). The flask was the filled up to mark with diluent to obtain concentration of DAPA (2 µg/ml) and VILDA (20 µg/ml).[16,17]

VALIDATION OF RP-HPLC METHOD

This study aimed to develop a new, reliable, cost-effective, and practical technique for the simultaneous quantification of both drugs in a combined dosage form using RP-HPLC. The established method underwent validation for various factors, including system suitability, linearity, precision, detection and quantitation limit, accuracy, assay, and robustness.

System Suitability: For the system suitability test, six replicates of freshly prepared standard solutions of DAPA and VILDA were injected. The standard chromatogram was used to evaluate parameters such as theoretical plate, resolution, retention time, and tailing factor.

Specificity: Sample solutions of DAPA (2 µg/ml) and VILDA (20 µg/ml) were prepared and injected to verify the analysis of the drugs and check for any degradation or interferences. The presence of interferences from DAPA and VILDA was determined by analyzing a blank chromatogram.[17-18]

Linearity: Five separate volumetric flasks (10 ml) were used to pipette out Dapagliflozin (100 µg ml^{-1}) stock solution aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5, as well as Vildagliptin (1000 µg ml^{-1}) stock solution aliquots of 0.1, 0.2, 0.3, 0.4 and 0.5. These solutions were then diluted with mobile phase to obtain different concentrations 1, 2, 3, 4, and 5 µg ml^{-1} for Dapagliflozin and 10, 20, 30, 40, and 50 µg ml^{-1} for Vildagliptin. An RP-HPLC system with an injecting column was used to inject 20 µl of each solution using a Hamilton syringe for analysis. Standard solution calibration curves were plotted by comparing the corresponding concentrations with their response ratio.[19-20]

Precision: An analytical validation study was conducted to evaluate the precision and reliability of an analytical method for

quantifying DAPA and VILDA concentration in solution. The study consisted of three parts: an intraday study in which the same day triplicate analysis of DAPA solution (1, 2, and 3 µg ml^{-1}) and VILDA solution (10, 20, and 30 µg ml^{-1}) was performed, an interday study that analyzed the same solutions on three different days, and a repeatability study that analyzed DAPA (2 µg ml^{-1}) and VILDA (20 µg ml^{-1}) six times.[20]

Accuracy: To test the accuracy of the HPLC system, known amounts of DAPA and VILDA were added to a pre-tested at three concentration level (50%, 100%, and 150%). Triplicate samples were injected at each concentration level, and the average percentage recovery of both drugs was calculated.[21]

Detection Limit and Quantification Limit: The ICH guidelines provide an equation for calculating the Detection Limit and Quantification Limit.[22]

Robustness: Robustness was tested with the requirement that they should satisfy the system suitability criteria. Optimized method parameters such as Detection wavelength, and flow rate robustness was evaluated.[22]

RESULTS AND DISCUSSION

The system suitability was assessed by calculating various parameters, which are presented in Table 1.

Table 1: System suitability parameters for Dapagliflozin and Vildagliptin

Sr. No.	System suitability parameter	Dapagliflozin	Vildagliptin
1	Area	1372264	704656
2	Retention time	7.97	2.91
3	Theoretical Plates	54550	23340
4	Tailing Factors	0.722	0.924
5	Resolution	18.304	

The absorption maximum of both compounds was determined to be 220 nm utilized for the RP-HPLC System showed in Figure 3. Figure 4 provides evidence that the RP HPLC method is specific, as the drug's peak was not detected with any interferences from the mobile phase, placebo, or other excipients. In Figure 5, 6, and 7, the theoretical plates observed for DAPA and VILDA were 1372264 and 704656 respectively. The tailing factors for DAPA and VILDA were 0.722 and 0.924, and the retention time for DAPA and VILDA were 7.97 and 2.91 min, respectively.

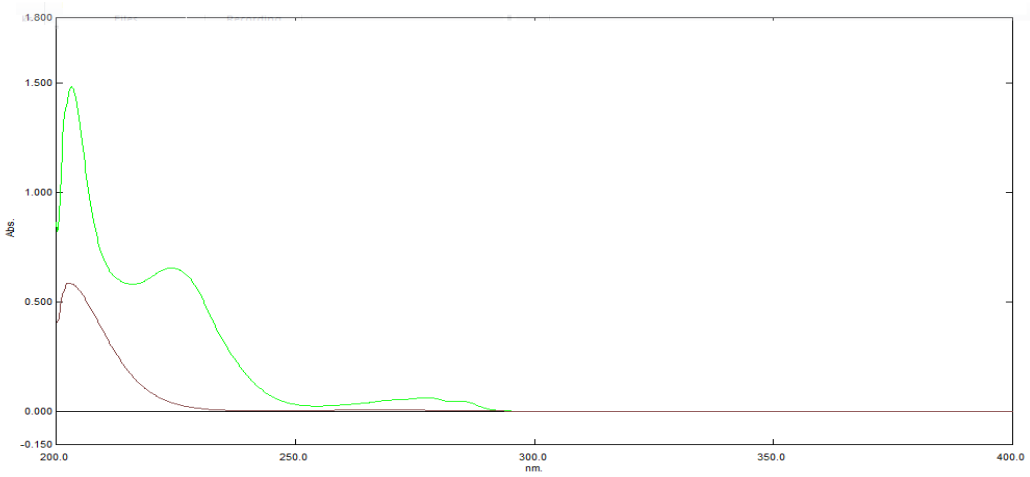

Figure 3: Overlain Zero order spectra of Dapagliflozin (2 µg/ml) and Vildagliptin (20 µg/ml) in Methanol

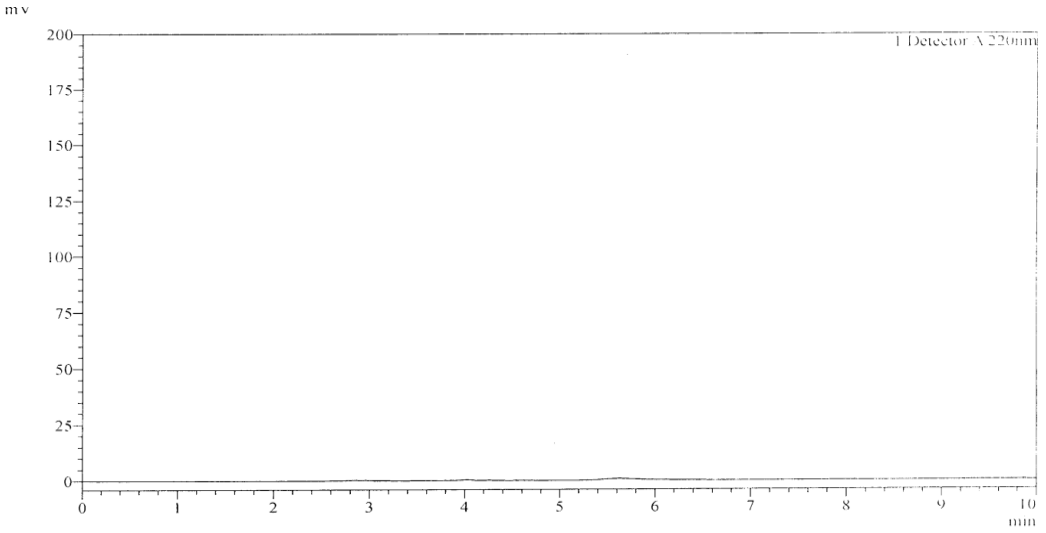

Figure 4: RP-HPLC Chromatogram of Blank in Phosphate Buffer: Acetonitrile: Methanol (35: 10: 55 %v/v/v) Flow rate: 1 ml/min 220 nm

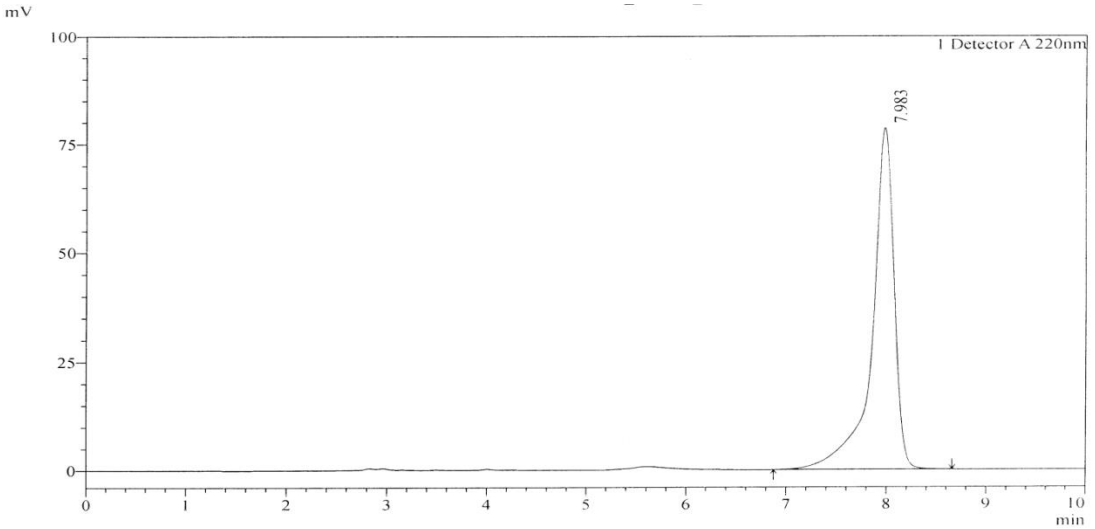

Figure 5: RP-HPLC Chromatogram of Dapagliflozin (2 µg/ml) in Phosphate Buffer: Acetonitrile: Methanol (35: 10: 55 %v/v/v) Flow rate: 1 ml/min 220 nm

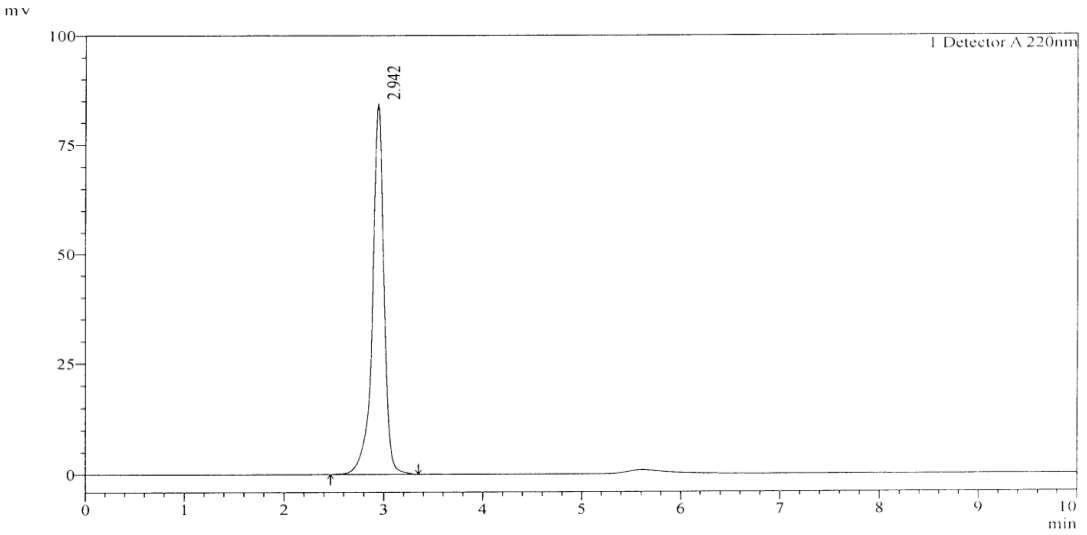

Figure 6: RP- HPLC Chromatogram of Vildagliptin (20 µg/ml) in Phosphate Buffer: Acetonitrile: Methanol (35: 10: 55 %v/v/v) Flow rate: 1 ml/min 220 nm

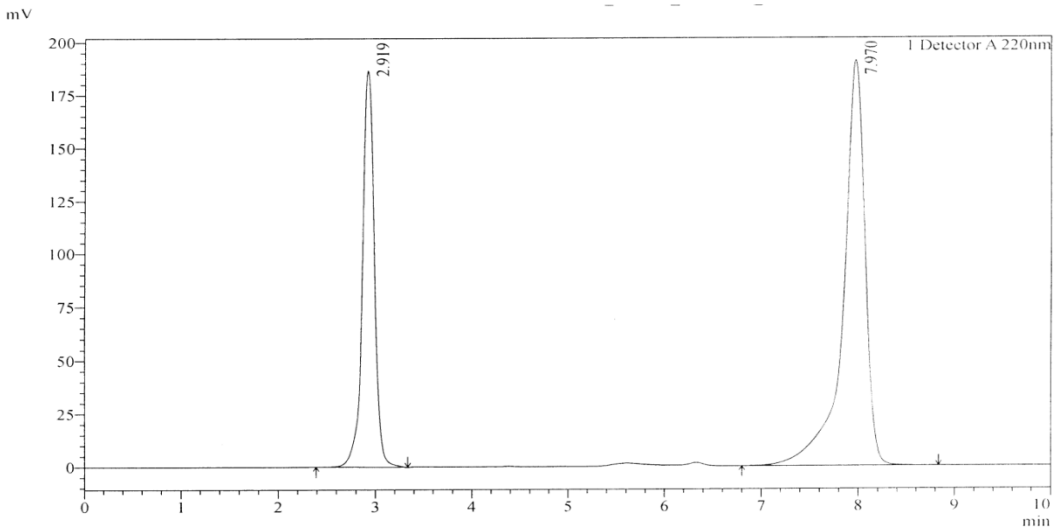

Figure 7: RP- HPLC Chromatogram of Dapagliflozin (2 µg/ml) and Vildagliptin (20 µg/ml) in Phosphate Buffer: Acetonitrile: Methanol (35: 10: 55 %v/v/v) Flow rate: 1 ml/min 220 nm

FORCED DEGRADATION STUDIES

Peak area of Dapagliflozin and Vildagliptin were found to be 1372264 and 704656, respectively. % degradation of Dapagliflozin and Vildagliptin were calculated using this equation,

$$\%\, degradation = \left(\frac{Standard\ area - Degraded\ area}{Standard\ area}\right) \times 100$$

Acid degradation study: The combination showed sufficient degradation within 3 h with 0.1 N Hydrochloric acid at 60˚C. Dapagliflozin showed 8.57 and 10.50 % degradation at 1 and 3 h, respectively; whereas Vildagliptin showed 9.73 and 9.86 % degradation at 1 and 3 h, respectively.

Base degradation study: Similar to acid, sufficient degradation was observed within 3 h with 0.1 N Sodium Hydroxide at 60˚C. Dapagliflozin showed 4.00 and 10.06 % degradation at 1 and 3 h, respectively; whereas Vildagliptin showed 12.68 % and 23.56 % degradation at 1 and 3 h, respectively.

Oxidative degradation study: Degradation was observed within 3 h after heating with 3 % Hydrogen peroxide at

room temperature. Dapagliflozin showed 2.13 and 5.15 % degradation at 1 and 3 h, respectively; whereas Vildagliptin showed 1.27 and 5.71 % degradation at 1 and 3 h, respectively.

Photolytic degradation study: Drugs were exposed to direct UV light for 3 h. Dapagliflozin showed 2.89 and 3.50 % degradation at 1 and 3 h, respectively; All the results are summarized in Table 2. whereas Vildagliptin showed 2.10 and 9.40 % degradation at 1 and 3 h, respectively.

Thermal degradation study: Drugs were exposed under heat at 80 ºC for 3 h. Dapagliflozin showed 0.126 and 9.66 % degradation at 1 and 3 h, respectively; whereas Vildagliptin showed 8.80 and 9.21 % degradation at 1 and 3 h, respectively.

Table 2: Degradation Study of Dapagliflozin and Vildagliptin

Standard Area of Dapagliflozin			1372264	
Standard Area of Vildagliptin			704656	
Acid Degradation Study				
	Area		**% Degradation**	
	DAPA	**VILDA**	**DAPA**	**VILDA**
0.1 N HCl for 1 h	1254656	636058	8.57	9.73
0.1 N HCl for 3 h	1228149	635118	10.50	9.86
Base Degradation Study				
0.1 N NaOH for 1 h	1317279	615281	4.00	12.68
0.1 N NaOH for 3 h	1234192	538609	10.06	23.56
Oxidative Degradation Study				
3% H_2O_2 for 1 h	1342978	695645	2.13	1.27
3% H_2O_2 for 3 h	1301569	664386	5.15	5.71
Thermal Degradation Study				
After 1 h	1370524	642586	0.126	8.80
After 3 h	1239682	639744	9.66	9.21
Photo Degradation Study				
After 1 h	1332503	689809	2.89	2.10
After 3 h	1324135	638383	3.50	9.40

VALIDATION OF RP HPLC METHOD

Linearity: At various concentration levels of 1-5 µg ml^{-1} for DAPA and 10-50 µg ml^{-1} for VILDA, the developed method exhibited a linear correlation. Correlation coefficients of DAPA was observed as 0.999 whereas for VILDA was 0. 997 (Table 3).

Table 3: Linearity of Dapagliflozin and Vildagliptin

Concentration (µg/ml)		Area ± SD (n=6)		% RSD	
DAPA	**VILDA**	**DAPA**	**VILDA**	**DAPA**	**VILDA**
1	10	784659.8± 8109.62	354877.5 ± 4646.72	1.03	1.30
2	20	1373960.0± 12384.54	704427.5 ± 8732.69	0.90	1.23
3	30	1931911.0 ± 15775.89	1028892.0 ± 11072.65	0.81	1.07
4	40	2444925.0 ± 18290.29	1318423.0 ± 12851.88	0.74	0.97
5	50	2984477.0 ± 20007.78	1581963.0 ± 14429.93	0.67	0.91

Precision: The % RSD for Intraday Precision of Dapagliflozin and Vildagliptin was found to be 0.57 % - 0.78 % and 0.70 % - 1.08 %, respectively. Also % RSD for Interday Precision of Dapagliflozin and Vildagliptin was found to be 0.66 % - 0.84 % and 0.76 % - 1.19 %, respectively. Whereas, % RSD for Repeatability of Dapagliflozin and Vildagliptin was found to be 0.84 % and 0.96 %, respectively (Table 4-5).

Table 4: Precision Study of Dapagliflozin

Intraday Precision of Dapagliflozin		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
1	785243 ± 6138.926	0.78
2	1378761 ± 8640.548	0.62
3	1935228 ± 11134.34	0.57
Interday Precision of Dapagliflozin		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
1	785909 ± 6669.913	0.84
2	1376094 ± 10008.22	0.72
3	1931894 ± 12804.05	0.66
Repeatability of Dapagliflozin		
Conc. (µg/ml)	**Mean Area ± SD (n=6)**	**% RSD**
2	1373627 ± 11591.28	0.84

Table 5: Precision Study of Vildagliptin

Intraday Precision of Vildagliptin		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
10	353265 ± 3844.883	1.08
20	703032 ± 5655.106	0.80
30	1024979 ± 7249.011	0.70
Interday Precision of Vildagliptin		
Conc. (µg/ml)	**Mean Area ± SD (n=3)**	**% RSD**
10	353498 ± 4227.553	1.19
20	704365 ± 6618.778	0.93
30	1027319 ± 7842.942	0.76
Repeatability of Vildagliptin		
Conc. (µg/ml)	**Mean Area ± SD (n=6)**	**% RSD**
20	705927 ± 6831.585	0.96

Accuracy: Recovery studies were conducted at 50 %, 100 %, and 150 %. Concentration levels, which Three samples from each concentration level were injected, and the mean percentage recoveries were calculated. The mean percentage recoveries for DAPA and VILDA were found to be in the range of 99.21-99.62 % and 99.84-99.92 % respectively (Table 6).

Table 6: Recovery of Dapagliflozin and Vildagliptin

Name of Drug	**% Level of Recovery**	**Test Amount (µg/ml)**	**Amount of drug taken (µg/ml)**	**Spiked Std Amount (µg/ml)**	**Total amount Recovered (µg/ml)**	**% Recovery ± S.D (n=3)**
Dapagliflozin	50	2	1	3	2.97	99.21±0.509
	100	2	2	4	3.98	99.50±0.250
	150	2	3	5	4. 98	99.62±0.205
Vildagliptin	50	20	10	30	29.94	99.84±0.182
	100	20	20	40	39.96	99.90±0.028
	150	20	30	50	49.96	99.92±0.052

Detection Limit (LOD) and Quantitation Limit (LOQ)

The LOD is used for limit testing to determine if the analyte concentration is within the specified limit. On the other hand, the LOQ is a quantitative assay parameter that establishes

the minimum concentration of analyte present in a sample, making it useful for detecting impurities. The LOD and LOQ for DAPA were found to be 0.039 and 0.128 µg/ml, respectively; whereas LOD and LOQ for VILDA were found to be 0.585 and 1.930 µg/ml, respectively (Table 7).

Table 7: LOD and LOQ for Dapagliflozin and Vildagliptin

Parameter	Dapagliflozin	Vildagliptin
LOD (µg/ml)	0.039	0.585
LOQ (µg/ml)	0.128	1.930

Robustness

Changes were made in the flow rate and detection wavelength, and the corresponding results were summarized in Table 8. % RSD of Dapagliflozin and Vildagliptin was found after variation in Flow rate to be 0.069 % – 0.099 % and 0.108 % – 0.093 % respectively, whereas and % RSD of Dapagliflozin and Vildagliptin after variation in Detection wavelength was found to be 0.056 % - 0.088 % and 0.094 % - 0.081 % respectively.

Table 8: Robustness data for Dapagliflozin and Vildagliptin

SN	Parameter	Variation	Area ± S.D (n=3)		% RSD	
			DAPA	VILDA	DAPA	VILDA
1	Flow rate (1 ml/min)	0.9 ml/min	1374960 ± 953.939	705050 ± 763.959	0.069	0.108
2		1.0 ml/min	1375060 ±624.500	704994 ± 602.771	0.045	0.085
3		1.1 ml/min	1375393 ±1365.04	705027 ±655.744	0.099	0.093
1	Detection wavelength (220 nm) (± 2 nm)	218 nm	1375093 ±776.745	705164 ±669.353	0.056	0.094
2		220 nm	1375160 ±519.615	704758 ±310.227	0.037	0.044
3		222 nm	1375560 ± 1216.55	704760 ± 577.35	0.088	0.081

Assay

The system recorded chromatograms from three independent injections of the sample solution from the same sample were made into the system. From the resulting % purity values, the % RSD was determined and reported. DAPA showed 99.30 and VILDA showed 99.75, which exhibited higher recovery showed in Table 9.

Table 9: Analysis of Pharmaceutical Dosage form

Name of Drug	Amount taken (µg/ml)	Amount Found (µg/ml)	%Assay± S.D (n=3)	% RSD
Dapagliflozin	2	1.95	99.30 ± 0.700	0.704
Vildagliptin	20	19.95	99.75 ± 0.132	0.132

CONCLUSION

The safety of patients depends on the quality of API, making it crucial to identify and degradation impurities that may compromise the drug product's efficacy. These proposed methods were suitable for simultaneous estimation of Dapagliflozin and Vildagliptin in dosage form without any interference. This established RP-HPLC stability indicating method address this concern. Comprehensive stress testing to mixture of Dapagliflozin and Vildagliptin was carried out according to ICH guideline Q1A (R2) under various stress conditions in the presence of degradation products. During degradation study, the results obtained were found within the acceptance criteria. Validation of proposed methods was also carried out according to ICH guideline Q2 (R1). Hence, the proposed stability indicating RP-HPLC assay method might be applied and utilized for the routine analysis for the estimation of Dapagliflozin and Vildagliptin in combination.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ICH: International Council for Harmonization; **RP-HPLC:** Reverse phase High Performance liquid chromatography; **API:** Active Pharmaceutical Ingredient; **HCl:** Hydrochloric acid; **NaOH:** Sodium Hydroxide; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **RSD:** Relative Standard deviation; **KH_2PO_4:** Potassium Dihydrogen ortho phosphate buffer; **DAPA:** Dapagliflozin; **VILDA:** Vildagliptin.

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