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Original Article

Stability Indicating Method Development and Validation of Vildagliptin and Dapagliflozin in Bulk and in Marketed Formulation by UHPLC Method

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ABSTRACT

Aim: Vildagliptin and Dapagliflozin in fixed-dose combination is used for treatment of Type II Diabetes Mellitus. The key objective of the current research work is to develop a new stability-indicating UHPLC method for the simultaneous estimation of Vildagliptin and Dapagliflozin in tablet dosage form as no such method is available. **Materials and Methods:** A successful separation was achieved by using Agilent column C_{18} (4.6×100 mm) and mobile phase of Methanol: 0.1% Orthophosphoric acid (78:22) at a flow rate of 1.0 mL and detection wavelength of 234 nm. The forced degradation study was performed at extreme forced conditions such as hydrolysis with acid and base, peroxide oxidation and photolytic degradation, following ICH guidelines. **Results:** The retention time for Vildagliptin and Dapagliflozin was 2.3 and 4.7 min respectively. The suggested approach yields linear responses for Vildagliptin and Dapagliflozin in the concentration of the 50-250 µg/mL and 5-25 µg/mL, respectively. Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 1.04 µg/mL and 3.15 µg/mL for Vildagliptin and 0.24 µg/mL and 0.73 µg/mL for Dapagliflozin, respectively. In the exposed conditions, the drugs did not exhibit any significant degradation. **Conclusion:** The UHPLC approach that was recommended proved to be highly sensitive, accurate, precise, robust and stability indicating. The method can be successfully adopted for the routine analysis for the simultaneous estimation of the Vildagliptin and Dapagliflozin in bulk and pharmaceutical dosage form.

Keywords: UHPLC, Vildagliptin, Dapagliflozin, Stability indicating, ICH guidelines.

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INTRODUCTION

One of the most prevalent and complicated metabolic disorders is Type 2 Diabetes Mellitus (T2DM), which typically requires the combination of multiple pharmaceutical approaches to control hyperglycemia.[1] Two main reasons contribute to T2DM: The β-cells impaired insulin secretion and inadequate response of insulin-sensitive tissues to insulin. In India, T2DM accounts for over 90% of Diabetes cases.[2]

Vildagliptin is a potent di-peptidyl peptidase IV (dip-IV) inhibitor, which binds covalently to the catalytic sites of dipeptidyl peptidase-4(dpp-4), eliciting prolonged enzyme inhibition. DPP-4 inhibitors are a novel class of oral antihyperglycemic drugs used to treat type 2 diabetes mellitus.[3] Dapagliflozin (DAPA) in Biopharmaceutics Classification System is classified as Class II drug, being highly soluble and poorly permeable. DAPA is a Sodium-Glucose Co-Transporter 2 (SGLT2) inhibitor that is highly potent, selective and reversible. It enhances urine glucose excretion by decreasing the kidney's reabsorption of glucose, improving glycemic management in individuals with type 2 diabetes mellitus.[4]

A review of the literature indicates that there aren't many techniques for estimating dapagliflozin and vildagliptin simultaneously. The literature conveyed that one RP-HPLC and HPTLC have been reported for the estimation of the VILDA and DAPA simultaneously.[5,6] There have been publications of several novel UV spectroscopic techniques for simultaneous assessment of dapagliflozin and vildagliptin in combination dose forms.[7] Vildagliptin and Dapagliflozin, either alone or in combination with pharmaceutical moieties, could be analyzed using a number of analytical techniques outlined in the literature.[8-12]

This is the first publication, as far as we are aware, of a stability suggesting UHPLC approach for simultaneous estimation of dapagliflozin and vildagliptin in bulk and therapeutic dosage formulation. Therefore, in accordance with ICH recommendations, the current study work was focused on

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developing a stability-indicating UHPLC method for the simultaneous assessment of dapagliflozin and vildagliptin in bulk and pharmaceutical formulation.

MATERIALS AND METHODS

Instrumentation

Agilent UHPLC system connected with quaternary pumps, DAD detector, CHEMSTATION software and with autosampler is used for the study.

Pure Samples

Standard samples of Vildagliptin and Dapagliflozin were procured from Swapnroop Drugs and Pharmaceuticals, Sambhajinagar.

Formulation

Marketed formulation of VILDAR-D (100-10 mg) from Elder Pharma Pvt. Ltd., was employed in the research.

Reagents and Chemicals

Methanol, water (HPLC grade), hydrogen peroxide (AR grade), hydrochloric acid and sodium hydroxide chemicals were procured from Merck.

Mobile Phase Preparation

The mobile phase is made up of 0.1% OPA and methanol at a 78:22 ratio. To de-gas the mixture, a 0.45 µ membrane filter is used for filtration, followed by sonication.

Standard Stock Solution Preparation

Stock solutions of both drugs were prepared by dissolving 100 mg of Vildagliptin and 10 mg of Dapagliflozin in methanol and sonicated until fully dissolved. Using methanol in a 10 mL volumetric flask, the volume was further adjusted. According to the requirements, the stock solution was diluted.

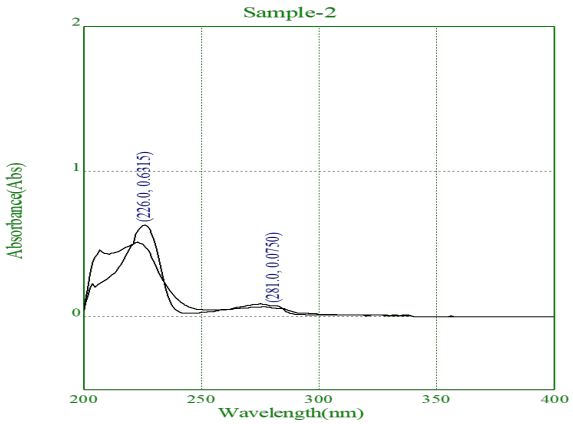

Figure 1: The isobestic point of both drugs in UV-visible Spectroscopy.

Preparation of Sample Solution

The equivalent weights of DAPA and vildagliptin were determined. Weight of powder equal to 100 mg of Vildagliptin and 10 mg of Dapagliflozin was taken in a 100 mL volumetric flask, and sonicated for 15 min. Volume was made up of methanol. The solution was filtered via a 0.45 µm membrane filter.

Selection of Working Wavelength

The UV spectrum of VILDA and DAPA in the range of 200-400 nm was recorded in order to determine the appropriate wavelength of chromatographic separation. The iso-absorptive point was found at 234 nm and 262 nm from the obtained overlaying spectrum given in Figure 1. The 234 nm wavelength was used for chromatographic due to better resolution.

Method Development

In order to improve chromatographic conditions and produce a symmetrical peak and improve drug resolution, a number of system appropriateness parameters were examined. The mobile phase including methanol and 0.1% OPA (78:22%, v/v) at a flow rate of 1 mL/min produced the more symmetrical and resolved peaks at 234 nm as given in Figure 2. This was achieved by combining several appropriate solvents in different ratios to optimize the mobile phase. Table 1 lists the ideal chromatographic conditions.

Method Validation

The suggested method's validation was completed in accordance with ICH Q2 (R1) requirements and validation metrics included system suitability parameters, LOD, LOQ, robustness, specificity, precision, accuracy and linearity.

Forced degradation experiments were conducted in accordance with ICH Q1A (R2) and Q1B recommendations, covering alkali hydrolysis, peroxide hydrolysis, acid hydrolysis, hydrolytic degradation and photolysis.

Linearity

The linearity denotes direct proportionality between the input concentrations and the peak regions of the technique. The existing approach produced linear graphs for both VILDA and DAPA between peak areas and employing concentrations between 50-250 mg/mL of VILDA and between 5-25 µg/mL of DAPA. The regression coefficient (r^2) values were evaluated.

Precision

The precision of the method was assessed by using intra- and inter-day precision. Three distinct concentrations were analyzed in order to assess the intra-day precision of VILDA (100, 150, 200 µg/mL) and DAPA (10, 15, 20 µg/mL) using UHPLC on the same day. Similarly, the same concentrations were analyzed

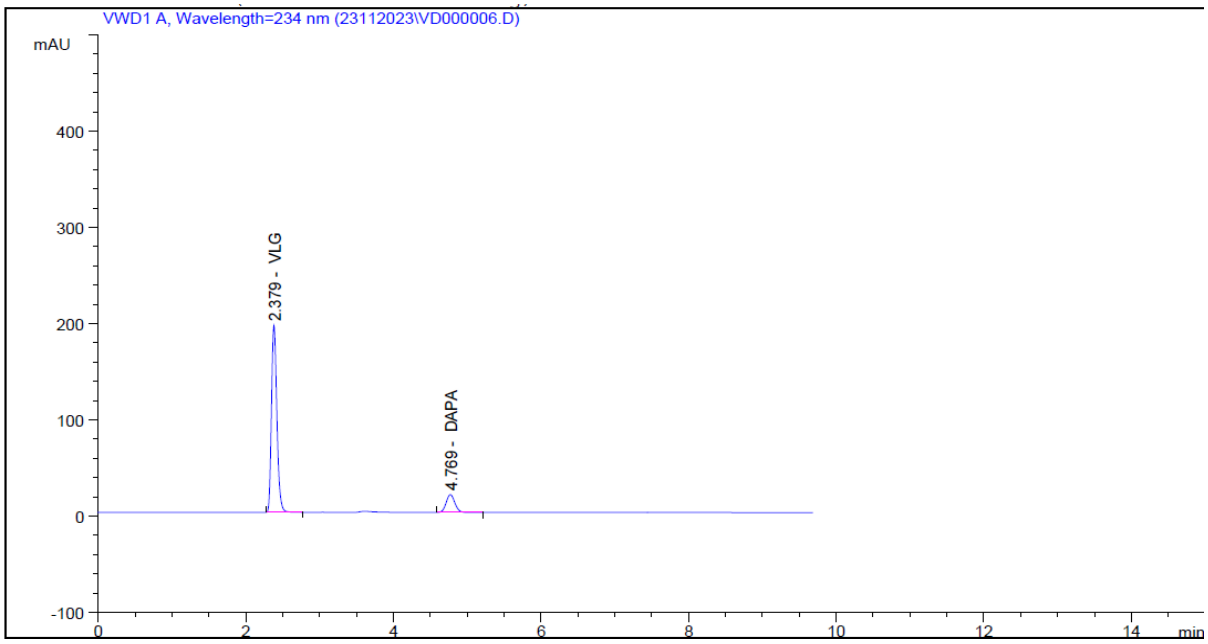

Figure 2: The developed chromatogram of the optimized method for VILDA and DAPA.

Table 1: Optimum conditions for chromatography.

Parameter	Optimized condition
Mobile phase	Methanol: 0.1% OPA (78:22).
Column	Agilent C18 (4.6 ×100 mm).
Flow rate	1.0 mL
Column temperature	25°C
Wavelength	234 nm
Injection volume	20 µL
Elution	Isocratic
Runtime	9.5 min
Retention time	2.379 for VILDA and 4.769 for DAPA.

for inter-day precision. Relative standard deviation was used to express precision.

Accuracy

Accuracy is expressed as % recovery. The percentage recovery was obtained by adding a known amount of vildagliptin and dapagliflozin standard at three different concentrations (80%, 100% and 120%), to pre-analyzed samples.

Limit of Detection and Limit of Quantification

The standard deviation and the slope of the calibration curve were used to determine the LOD and LOQ. By using the slope approach, the method's sensitivity to the LOD and LOQ for Vildagliptin and Dapagliflozin was determined. LOD and LOQ were computed with LOD=3.3σ/S and LOQ=10σ/S, where S is the slope of the standard curve and σ is the standard deviation.

Robustness

The robustness of the approach was confirmed by deliberately altering the process specifications, including the mobile phase, detecting wavelength and flow rate.

Assay

The marketed formulation, VILDAR-D, including 100 mg of Vildagliptin and 10 mg of Dapagliflozin, was assayed. To record the chromatogram, 20 µL of the sample solution and standard solution were introduced into the UHPLC independently. The peak area of the chromatograms that were recorded was used to calculate the drug concentrations that were present.

Forced Degradation

Studies on forced degradation were carried out to evaluate the proposed method's stability-indicating property. The standard was subjected to a range of deterioration conditions, including acidic stress (0.1 N), alkaline stress (0.1 N NaOH), peroxide (3% H_2O_2) and photolytic stress (1 day). The system appropriateness characteristics were examined along with the percentage degradations after the exposed solutions were injected into duplicates

RESULTS

Specificity

The developed chromatogram of the optimized method for Vildagliptin and Dapagliflozin, as illustrated in Figure 2, shows that the blank has no peak at the retention time of Vildagliptin and Dapagliflozin, indicating that the peaks obtained in the standard solutions at working concentrations are solely due to the drugs.

Linearity

Aliquots at 50, 100, 150, 200, 250 µg/mL for VILDA and 5, 10, 15, 20 and 25 µg/mL for DAPA were prepared for the determination of linearity. Vildagliptin and Dapagliflozin obeyed the linearity for the concentration range of 50-250 µg/mL and 5-25 µg/mL correspondingly. The regression equations for VILDA and DAPA were discovered to be y=11.1686x+113.413 and y=19.08x+18.027 respectively. The correlation coefficient was discovered to be 0.9995 and 0.9999 for Vilda and DAPA respectively. Linearity graphs of VILDA and DAPA are shown in Figures 3 and 4 respectively. Results are represented in Table 2.

Precision

The inter-day and intra-day precisions were estimated by calculating the percentage RSD. The %RSD for intra-day and inter-day for Vilda and DAPA are given in Table 3. All the %RSD values was inside the range, demonstrating the precise method.

Accuracy

As per ICH, three criteria were used to evaluate the proposed method's accuracy. The outcomes demonstrate the accuracy of the proposed method. It was discovered that Vilda's percentage recovery fell between 100.56-102.28%, whereas DAPA's percentage recovery fell between 100.05-102.87%. Table 4 represents the results for accuracy.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

For VILDA and DAPA, the limit of detection was determined to be 1.0419 µg/mL and 0.2435 µg/mL, respectively. The limit of quantification was determined to be 3.1574 µg/mL and 0.7381 µg/mL for VILDA and DAPA respectively.

Robustness

The robustness study was done by modifying the flow rate, 0.9 mL/min (-) and 1.1 mL/min (+); mobile phase,77+23(-) and 79+21(+), detection wavelength, 233 nm (-) and 235 nm (+). The

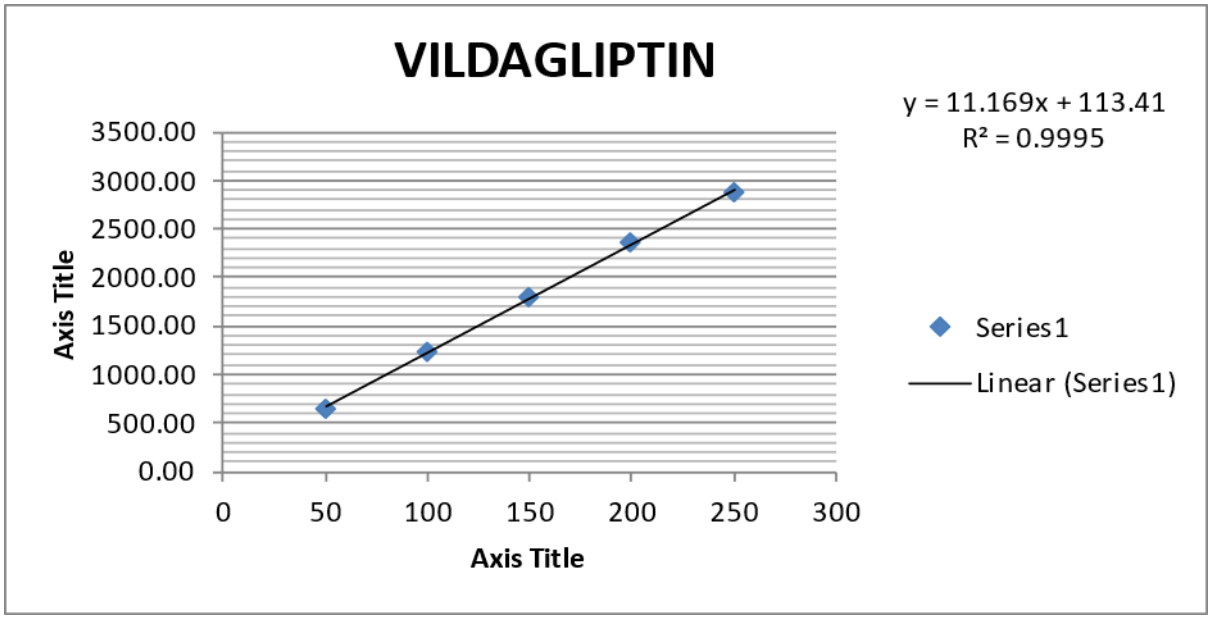

Figure 3: The calibration curve of Vildagliptin at 234 nm.

Table 2: Linearity Data of VILDA and DAPA.

Sl. No.	Vildagliptin		Dapagliflozin	
	Concentration (µg/mL)	Area± SD	Concentration (µg/mL)	Area± SD
1	50	650.20±3.05	5	111.73±0.41
2	100	1243.34±4.53	10	210.33±1.29
3	150	1808.09±3.20	15	305.67±1.25
4	200	2355.71±3.88	20	399.72±1.09
5	250	2886.16±2.95	25	494.21±3.01

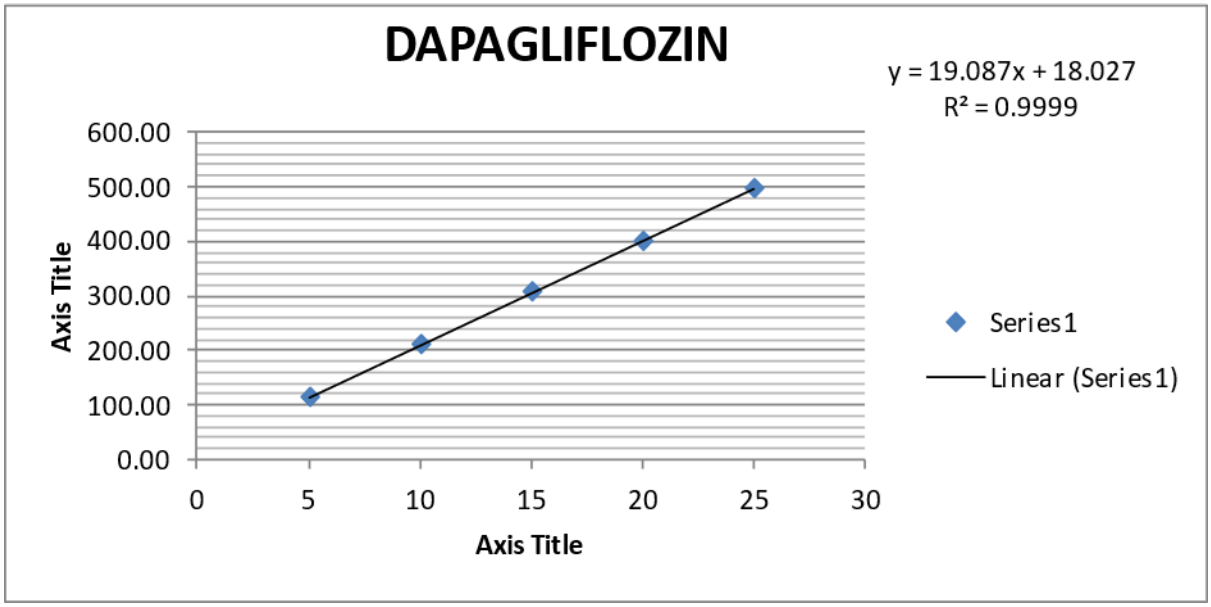

Figure 4: The calibration curve of Dapagliflozin at 234 nm.

Table 3: **Precision results of optimized method.**

Concentration (µg/mL)	Vildagliptin		Concentration (µg/mL)	Dapagliflozin	
	%RSD			%RSD	
	Intra-day	Inter-day		Intra-day	Inter-day
100	0.032	0.20	10	0.05	1.48
150	0.36	0.12	15	0.07	0.39
200	0.157	0.09	20	0.18	0.18

Table 4: **The accuracy data of drugs.**

Drug	% level	Analyte amount(mg)	Recovery amount(mg)	Mean % recovery	% RSD
Vildagliptin	80%	40	40.22	100.56	0.16
	100%	50	51.14	102.28	0.14
	120%	60	60.87	101.45	0.06
Dapagliflozin	80%	4	4.03	100.81	1.05
	100%	5	5.12	102.49	0.53
	120%	6	6.11	101.28	0.08

Table 5: **Outcome for the robustness analysis.**

Chromatographic condition	Variations	Peak Area		%RSD	
		VILDA	DAPA	VILDA	DAPA
Flow rate±0.1 (mL/min)	0.9	2214.54	396.90	0.15	0.59
	1.1	2354.31	374.90	0.15	0.68
Mobile phase±1	77+23	2332.6	393.2	0.10	0.74
	79+21	2321.20	386.90	0.10	0.97
Detection wavelength±1 nm	233	2358.1	391.4	0.13	0.42
	235	2356.63	388.37	0.13	0.68

Table 6: Result of Forced Degradation.

Stress Degradation	%Degradation	
	VILDA	DAPA
Acidic degradation	3.06	3.58
Alkali degradation	2.10	1.19
Peroxide degradation	9.31	3.58
Neutral degradation	0.31	1.19
Photo degradation	4.27	100.00

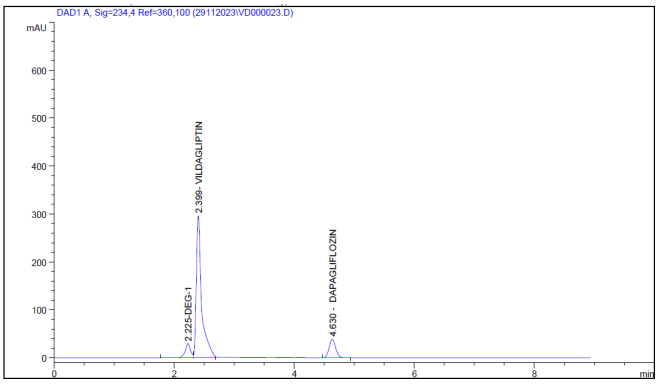

Figure 5a: Alkali degradation.

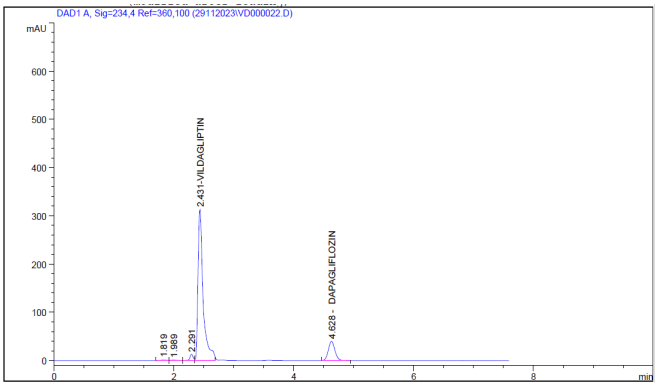

Figure 5b: Acid degradation.

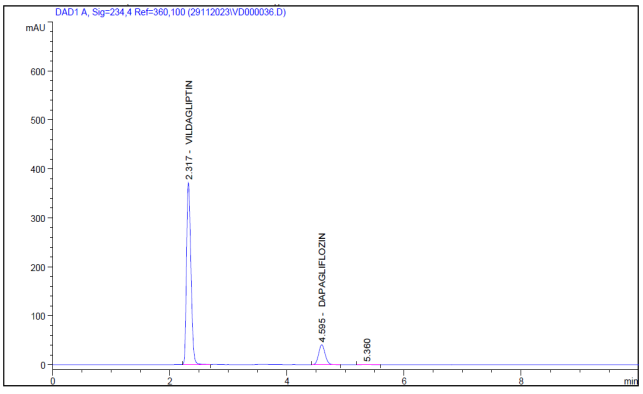

Figure 5c: Neutral degradation.

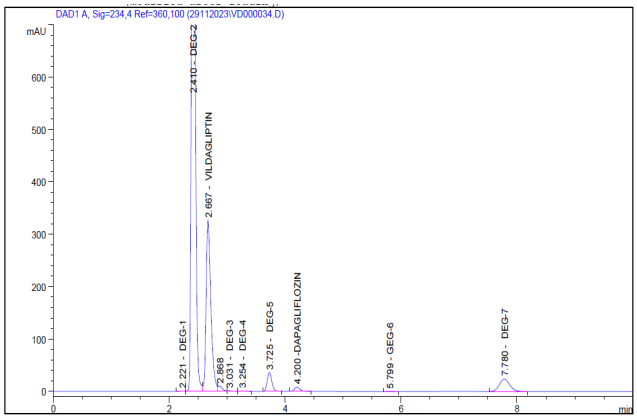

Figure 5d: Peroxide degradation.

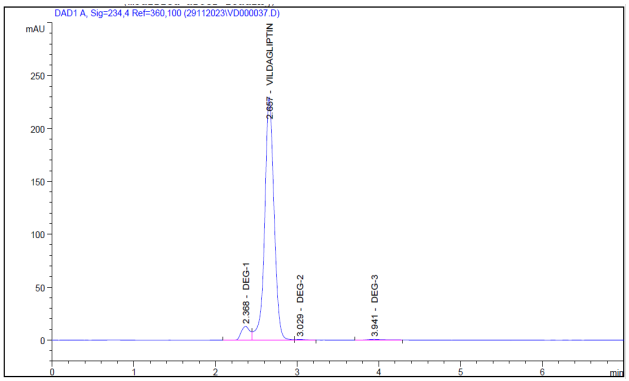

Figure 5e: Photolytic Degradation.

%RSD values were found to be less than 2, therefore the method is regarded as robust (Table 5).

Assay

The percentage purity of VILDA and DAPA was found to be 100.93% and 100.35%, respectively.

Forced degradation studies

According to degradation studies in exposed acidic, alkaline, peroxide and neutral environments, neither Vildagliptin nor Dapagliflozin underwent significant degradation. In a photolytic environment, DAPA shows significant degradation. Table 6 presents the force degradation results, whereas Figure 5a-e displays the chromatograms.

DISCUSSION

Extensive experimentation was conducted by changing the chromatographic parameters like buffer pH, mobile phase and flow rate in order to create a simple UHPLC method for the simultaneous estimation of Vildagliptin and Dapagliflozin. Initially, using mobile phase methanol and 0.1% OPA in the ratio 80:20 and flow rate of 0.7 mL, failed to achieve satisfactory separation. The best chromatographic results were obtained by

applying a mobile phase consisting of methanol and 0.1% OPA in a 78:22 ratio and flow rate of 1.0 mL\min. It was discovered that both drugs had an excellent resolution, and retention time of less than 4 min. The stability studies and validation of the method were conducted in compliance with ICH guidelines. The validation parameters, which include accuracy and precision, were determined to be within the acceptable range, suggesting that the approach is precise and accurate. A method's robustness research revealed that both intentional and minor alterations had no effect on the system suitability parameters' results. The degradation analysis revealed that, with the exception of photolytic degradation, there was a lower proportion of degradation under other conditions. All the parameters related to system suitability did not change throughout the forced degradation study.

CONCLUSION

In order to estimate Dapagliflozin and Vildagliptin simultaneously in pharmaceutical formulations, the current stability-indicating UHPLC method was developed and validated. The proposed method is easy to use, quick, affordable, precise, accurate and still stability indicating. The established UHPLC method can be successfully used to analyze dapagliflozin and vildagliptin in pharmaceutical formulations on a regular basis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UHPLC: Ultra High-Performance Liquid Chromatography; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **VILDA:** Vildagliptin; **DAPA:** Dapagliflozin; **nm:** Nanometre; **ug/mL:** Microgram per millimetre.

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