RESEARCH ARTICLE

Stability-Indicating HPLC Method Development and Validation for the Simultaneous Determination of Vildagliptin and Dapagliflozin in Pharmaceutical Dosage Form

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ABSTRACT

Vildagliptin (VD) and Dapagliflozin (DP) were determined simultaneously by RP-HPLC using an ultraviolet (UV) detector, a Hypersil Gold C18 (250 × 4.6 mm) column, 5 µm and a mobile phase of acetonitrile: water (adjusted with O-Phosphoric acid to pH 3) in ratio 40: 60% v/v. The estimation wavelength was chosen to be 213 nm. VD and DP were shown to have retention times of 2.9 and 8.3 minutes, respectively. Q2 (R1) ICH guidelines were followed during the validation process. VD and DP correlation coefficient R^2 values are 0.9993 and 0.999, respectively. While DP's linearity range is 1 to 6 µg/mL, VD's is 10 to 60 µg/mL. The precision and accuracy studies show %relative standard deviation (%RSD) below 2%. Recovery% was assessed to meet the ICH Q2 (R1) guidelines criteria. The presented research on forced degradation showed stability indicating studies. The findings also demonstrated that the suggested method is suitable for determining VD and DP precisely and accurately.

Keywords: Vildagliptin, Dapagliflozin, Forced degradation studies, Validation.

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INTRODUCTION

Type 2 diabetes results from the body's diminished ability to control and burn glucose as fuel. Because of this chronic condition, too much glucose is continually circulating through the blood. In the end, high blood glucose levels may cause cardiovascular, immune, and nervous issues. Vildagliptin and dapagliflozin are oral antidiabetic medications that are administered in combination for the cure of type 2 diabetes.

Chemically (Figure 1A), vildagliptin (VD) is called (S)-1-[2-(3-Hydroxyadamantan1-ylamino) acetyl] pyrrolidine-2-carbonitrile. VD functions as a dipeptidyl peptidase-4 (CD26) inhibitor, which is used to treat diabetes. VD makes a covalent connection with the DPP-4 at a catalytic location, causing prolonged enzyme inhibition. This elevates both before and after meals and during fast GLP-1 levels.

Dapagliflozin (DP) is chemically (Figure 1B) called (2S, 3R, 4R, 5S, 6R) 3-[(4-ethoxyphenyl) methyl]-2-chloro-3-[phenyl] phenyl -6- (hydroxymethyl) Oxane-3, 4, 5-triol and is an effective and selective sodium glucose co-transporter 2 (SGLT-2) inhibitor acts by preventing glucose reabsorption.

An exhaustive literature survey found that few methods are available for vildagliptin and dapagliflozin as separate medications and in combination with other medications. Vildagliptin and dapagliflozin have been measured by HPLC[1-5], HPLC-MS,[6,7] UV spectrophotometry,[8,9] LC/ESI-MS, LC-UV and UHPLC-DAD-MS.[10] Some techniques have also been reported in conjunction with different drugs.[11-16] As a result, this research aimed to develop a simple isocratic RP-HPLC technique for simultaneously measuring VD and DP and investigating forced degradation studies of VD and DP.

MATERIALS AND METHODS

Chemicals and Reagent

Vildagliptin was purchased from Astitva Chemicals (Vapi, Gujarat). Dapagliflozin was obtained from Manus Aktteva Biopharma LLP, Gujarat. The HPLC-grade acetonitrile was acquired from Merck Ltd., Mumbai. Analytical grade reagents for example, NaOH, HCl, H_2O_2 and H_3PO_4 were procured from Merck Ltd, Mumbai. Zomelis D strip of tablets by Eris

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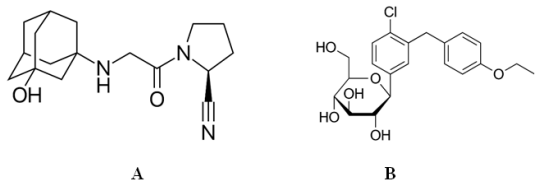


Figure 1 A and B: Chemical structure of vildagliptin and dapagliflozin

Lifesciences Pvt Ltd, Mumbai, was purchased from a local pharmacy store.

Instrumentation

Isocratic Jasco company HPLC contains pump PU- 2080 plus, a sample injector port, Jasco UV/VIS detector (UV-2075 plus) with the Jasco Borwin software version 1.5, and LC-Net II/ ADC system for data acquisition and processing used. For chromatographic separation, a Thermo Hypersil Gold C18 column (250, 4.6 mm, 5) was used.

Preparation of Mobile Phase, Standard Solution and Sample solution

Mobile phase

Acetonitrile and Millipore Water (40: 60% v/v) mixture adjusted with O-H_3PO_4 to pH 3. Before use, the mobile phase was degassed and filtered using a 0.45 membrane filter.

Standard solution

10mg of VD and DP were individually weighed and added to 10 mL volumetric flasks. The weighed both powders were dissolved in a 10 mL diluent (Acetonitrile: Water - 70:30% v/v) to make a resultant conc. of 1000 µg/mL.

Sample solution

1-mL VD and 0.1 mL DP solution were withdrawn from the respective standard solution and diluted with diluent (Acetonitrile: Water - 70:30% v/v) up to 10 mL, which give the resulting conc. of 100 and 10 µg/mL correspondingly.

Method Optimization

The mobile phase consists of acetonitrile (ACN) and water (40: 60 % v/v) (adjusted with O-H_3PO_4 to pH 3). The flow rate of the mobile phase was kept at 1mL/min while the sample injection volume was 20 µL for both VD and DP, and the detecting wavelength was assigned 213 nm. The standard solution and sample solution of 20 µL volume were injected and chromatograms were recorded.

Forced Degradation Studies[17]

Acid degradation

An exact quantity of 25 mg of the drug was put into an RBF. About 5 mL of 1N HCl and 20 mL of a buffer were added in RBF. Refluxed the solution at 60°C for 8 hours on water bath. After that, the chromatogram was recorded by injecting the sample into the HPLC.

Base degradation

An exact quantity of 25 mg of the drug was put into a RBF. About 5 mL of 2N NaOH and 20 mL of a diluent were added in RBF. Refluxed the solution at 60°C for 8 hours on water bath. After that, the chromatogram was recorded by injecting the sample into the HPLC.

Oxidative degradation

An exact quantity of 25 mg of the drug was put into an RBF. About 5 mL of 6% H_2O_2 and 20 mL of a diluent were added in RBF. Refluxed the solution at 60°C for 8 hours on water bath. After that, the chromatogram was recorded by injecting the sample into the HPLC.

Wet degradation

About 25 mg of the drug in 25 mL diluent was kept at room temperature (25ºC) for 24 hours. Then, after injecting this solution into the HPLC, the chromatogram was taken.

Photolytic degradation

About 25 mg of drug with 25 mL diluent was exposed to direct UV light (sunlight) for 24 hours. The sample was injected in HPLC to procure the results.

Method Validation

The approach developed has been validated in accordance with the requirements established by ICH Q1 (R2).[18] The Validation parameters include LoD and LoQ, linearity, precision, accuracy, specificity, and robustness.

RESULT AND DISCUSSION

The maximum absorption (λmax) of vildagliptin and dapagliflozin was reported to be 213 nm. The drug demonstrated good absorption at the indicated (λmax), and the UV absorption spectra are shown in Figure 2.

System Suitability

Freshly made stock solutions underwent system suitability testing to see how effective the system works. Table 1 displays the obtained parameters.

The method was optimized for VD and DP at retention time of 2.9 and 8.3 minutes, respectively with maximum absorption at 213 nm.

Specificity

First, standard samples of VD and DP were injected to assess the method's specificity. Then, the instrument was used to run

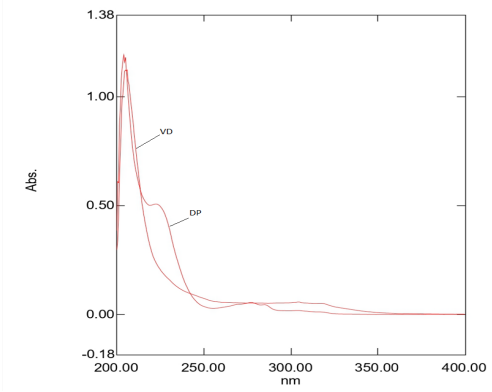


Figure 2: UV Spectrum of vildagliptin and dapagliflozin

Table 1: Parameters of System Suitability

Parameters	*VD*	*DP*
RT (t_R) in min.	2.9	8.3
Resolution (Rs)	0.00	14.53
Theoretical plates number (N)	24007.050	45270.230
Asymmetry factor	1.165	1.139

the blank, marketed product, and an excipient solution one after the other. Thus, the method turned out to be specific for VD and DP. Figure 3 (A) and (B) show the standard chromatograms of VD and DP demonstrating specificity studies.

Along with the forced degradation experiment, the developed method was validated. Stability chromatograms are mentioned in Figure 4 while the results of degradation are mentioned in Table 2.

Forced Degradation Studies

Missing text

Method Validation

LoD and LoQ

The calibration curve slope and the standard deviation approach were used to determine LoD and LoQ for VD and DP. LoQ and LoD solutions were injected in triplicate. The LoD and LoQ for VD are 10 and 50 µg/mL, while for DP has 0.4 and 0.6 µg/mL.

Linearity

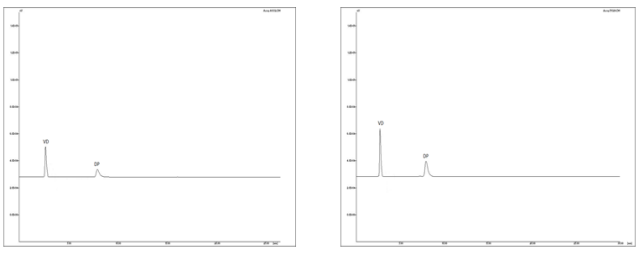
Linearity was performed from (10–60) and (1–6) µg/ mL for VD and DP separately. For each concentration, three replicate measurements were used to define the concentration range. The linearity curve of VD and DP is in Figure 5 respectively.

Precision

Three replicate injections of various concentrations were injected. Studies utilised VD and DP concentrations of 20, 40, 60 and 2, 4, 6 µg/mL. The intra-day precision for three concentration determinations in one day. Three days of triplicate trials with identical concentrations tested the method's inter-day precision. Method precision was measured by % RSD. The findings are in Table 3.

***Accuracy* (Recovery)**

A recovery experiment was performed by spiking the samples at about 80 , 100 and 120% of the specification level. Samples



(A) (B)

Figure 3 A and B: Chromatograms of Standard drugs and formulation

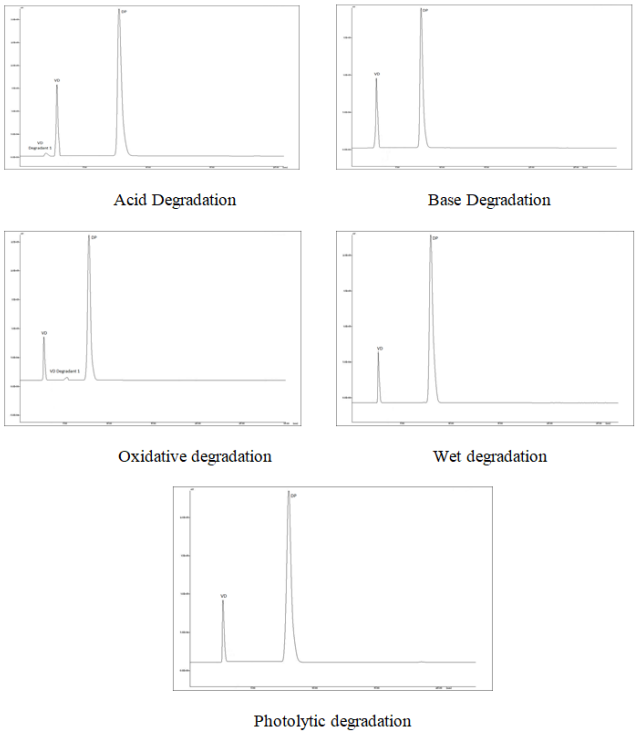


Figure 4: Chromatograms of forced degradation studies

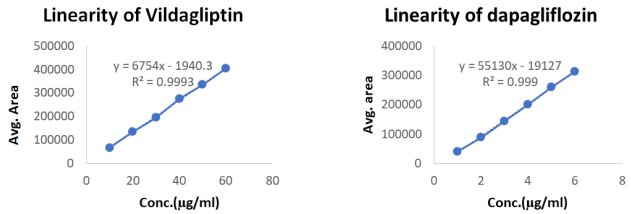


Figure 5: Linearity curve of VD and DP

were suitably diluted and injected at each concentration level in triplicate. The average percent recovery for VD and DP was determined. Table 4 summarizes the results.

Assay

The marketed formulation was examined using VD and DP tablets (Zomelis D tablet contains 100 mg VD and 10 mg DP). 20 tablets were weighed, averaged, and crushed. The powder was weighed using the weight equivalent method and dissolved

Table 2: An overview of forced degradation studies

Condition	*%Degradation of VD*	*%Degradation of DP*
Acid degradation (1N Hcl, 60ºC, 7 hours)	7.3% (RT 2.3 minutes)	No degradation
Base degradation (2N NaOH, 60ºC, 8 hours)	No degradation	No degradation
Peroxide degradation (3% H_2O_2, 60ºC, 8 hours)	18% (RT 6.4min)	No degradation
Room temperature (25ºC)	No degradation	No degradation
Direct sunlight	No degradation	No degradation

Table 3: Inter-day and intra-day precision studies (n = 3)

Conc. (µg/mL)	*Intra-day precision*			*Inter-day precision*		
	Measured conc.	*%RSD*	*Recovery (%)*	*Measured conc.*	*%RSD*	*Recovery (%)*
VD						
20	19.95	0.6630	99.75	19.91	0.3657	99.55
40	39.99	0.3907	99.97	39.7	0.5867	99.25
60	59.86	0.1774	99.76	59.9	0.0958	99.833
DP						
2	1.99	0.6226	99.50	2	0.3843	100
4	3.99	0.1433	99.75	3.99	0.0812	99.75
6	5.99	0.2386	99.83	5.99	0.1380	99.83

Table 4: Recovery study

Label claim (mg/tablet)	*Amt. added (%)*	*Total amount (mg)*	*Amount recovered (mg)*	*%Recovery*	*Mean (%) Recovery*
VD 100	80	180	179.75	99.90	99.65
	100	200	199.41	99.70	
	120	220	218.60	99.36	
DP 10	80	18	17.7	98.69	98.3
	100	20	19.6	98.06	
	120	22	21.6	98.35	

Table 5: Robustness test of VD and DP

Conditions	*Level*	*VD*		*DP*	
		Peak Area	*%RSD*	*Peak Area*	*%RSD*
A. Flow rate (± 0.2 mL/min)					
0.8	-0.2	296777	1.0916	199077	0.2319
1	0.2	198203	0.7189	144077	0.2134
1.2	+0.2	196765	0.6629	129569	0.4566
Mean		230581	0.8245	156574	0.3006
B. Mobile phase (Acetonitrile: Water) (± 5 mL)					
35:65	-5	250043	0.9169	144432	1.7855
40:60	5	198203	0.7189	143895	0.2134
45:55	+5	233485	0.5241	143904	0.2237
Mean		227244	0.7200	167981	0.7409
C. Wavelength (± 2 nm)					
211	-2	291894	0.5509	171861	0.5688
213	2	198203	0.7189	144077	0.2134
215	+2	163079	0.8074	149977	1.6333
Mean		217725	0.6924	155305	0.8051
D. pH of mobile phase (± 0.2)					
2.8	-0.2	282742	0.6006	164065	0.4866
3.0	0.2	256562	0.7312	144077	0.2134
3.2	+0.2	257823	0.7312	157149	0.3220
Mean		265709	0.6877	155097	0.3407

in a 50 mL diluent (Acetonitrile: Water-70:30 %v/v). Three duplicate combination determinations were carried out. The percent content was found to be 99.28% for VD and 99.32% for DP.

Robustness

Robustness is determined by making minor or intended changes to the optimum method. A study was performed by varying the optimal method flow rate, wavelength, pH, and mobile phase. The results are revealed in Table 5.

CONCLUSION

The vildagliptin and dapagliflozin standards were identified by UV spectroscopy. The detection wavelength for Vildagliptin and Dapagliflozin was found to be 213 nm. The HPLC method was optimized using Acetonitrile: Water (adjusted with O-H_3PO_4 to pH 3) as a mobile phase in the ratio of 40: 60% v/v where the vildagliptin and dapagliflozin peak is eluting at a retention time of 2.9 and 8.3 minutes separately. The optimized method was found to be reliable, specific, economical and reproducible. Hence, this method can be routinely used to simultaneously estimate vildagliptin and dapagliflozin. The developed HPLC method was validated and extended for forced degradation studies to determine drug stability.

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