RESEARCH PAPER

Development and validation of HPLC method for simultaneous estimation of Dapagliflozin and Vildagliptin in their pharmaceutical dosage form

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Received: 16th Dec, 2025; Revised: 8th Feb 2026; Accepted: 12th Feb, 2026; Available Online: 28th Feb, 2026

ABSTRACT
The purpose of the present study was to develop a simple, precise and accurate HPLC method for simultaneous estimation of Dapagliflozin and Vildagliptin in their pharmaceutical dosage form. Symmetry C18 (250 x 4.6 mm, 5 µm) was used as stationary phase and Acetonitrile: phosphate buffer (60:40 v/v; pH 3.5 by OPA) was used as mobile phase and detection of wavelength was found at 218 nm. The retention time of Vildagliptin and Dapagliflozin was found to be 2.5 min and 5.5 min. The method was linear in the concentration in the range of 100-300 µg/ml for Vildagliptin and 10-30 µg/ml for Dapagliflozin with correlation coefficient (r2) 0.9989 for Vildagliptin and 0.9958 for Dapagliflozin. Accuracy of Vildagliptin and Dapagliflozin was found with in acceptance criteria 98%-102%. The proposed method was validated as per ICH Q2 (R1) guideline.
Keywords: *Vildagliptin, Dapagliflozin, HPLC, ICH Q2(R1), Validation*
How to cite this article: Bhavsar P*, Bera K, Patel Z, Singh S, Patel S, Patel J, Patel A, Development and validation of HPLC method for simultaneous estimation of Dapagliflozin and Vildagliptin in their pharmaceutical dosage form. Int J Drug Deliv Technol. 2026; 16(2): 397-404; DOI: 10.25258/ijddt.16.2.44
Source of support: Nil.
Conflict of interest: None

1 | INTRODUCTION

VILDAGLIPTIN (VLD)

Vildagliptin is chemically (2*S*)-1-[2-[(3-hydroxy-1-adamantyl) amino] acetyl] pyrrolidine-2-carbonitrile is a potent dipeptidyl peptidase IV (dip-IV) inhibitor, a drug for the treatment of diabetes (Figure 1). DPP-IV inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes. DPP IV inhibitors improve fasting and postprandial glycemic control without hypoglycemic or weight gain. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP IV, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas [1].

Dapagliflozin (DFZ)

Dapagliflozin is chemically (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4- ethoxy benzyl) phenyl]-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5- triol, as shown in (Figure 2). DAP is indicated for the management of diabetes mellitus Type 2, and functions to improve glycemic control in adults when combined with diet and exercise. DAP is an inhibitor of sodium-glucose cotransporter 2 (SGLT2) responsible for the majority of the reabsorption of filtered glucose from the tubular lumen. By inhibiting SGLT2, DAP reduces reabsorption of filtered glucose and lowers the renal threshold for glucose, and thereby increases urinary glucose excretion [2].

Literature survey reveals that various analytical method were reported like HPLC [3-20], UV [21-31], HPTLC [32], UPLC [33]in single or with combination with other drugs in pharmaceutical dosage form but no reported analytical method for simultaneous estimation of Vildagliptin and Dapagliflozin in their pharmaceutical dosage form.

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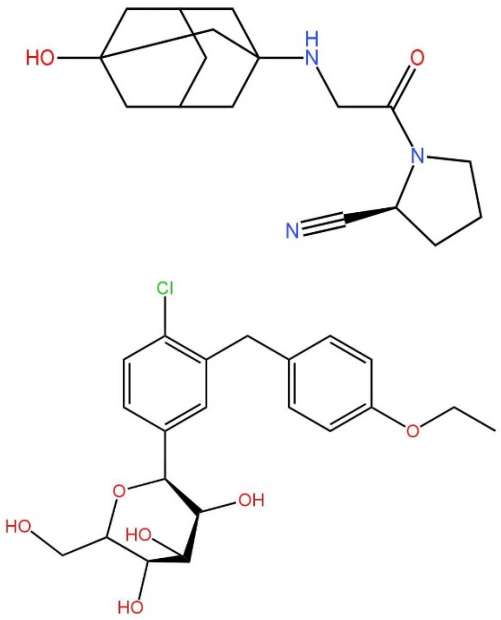

Figure 1 Chemical Structure of Vildagliptin and Dapagliflozin

2 | MATERIAL AND METHOD

2.1 | CHEMICAL

Both API Vildagliptin and Dapagliflozin were obtained as gift sample from Exemed Pharmaceutical, Vadodara, Gujarat. Marketed formulation (brand name: Voage-V 100/10 manufacture by Alembic Pharmaceutical Ltd.) was purchased from local pharmacy store. Acetonitrile (Hplc grade,99.80%), HPLC- grade water and Orthophosphoric acid (Hplc grade) from were purchased from Thermo fisher scientific India Pvt Ltd. Potassium dihydrogen phosphate were purchased from Sisco Research laboratories Pvt Ltd.

2.2 | INSTRUMENTATION

The HPLC system consist of a Shimadzu LC-20 AT Liquid Chromatograph (Japan). The system was equipped with a UV-visible detector (SPD-20A, Japan). UV- visible spectrophotometer (Shimadzu -1800), Analytical balance (Mettler Toledo, ML 204/A01, Switzerland), pH meter (Elico-L1 610) were used in this work.

2.3 | CHROMATOGRAPHIC CONDITION

Chromatographic separation was achieved on Shimadzu C18 column (250 x 4.6mm,5 µm) with UV detection at 218 nm and mobile phase consist of Acetonitrile and phosphate buffer (60:40 %V/V) pH 3.5 adjusted by 10% orthophosphoric acid was used. The flow rate was maintained at 1.0 ml/min.

2.4 | PREPARATION OF STOCK SOLUTION

VLD and DFZ were weighed (10 mg VLD and 10 mg DFZ) and transferred into two separated 10 ml volumetric Flasks and dissolved in mobile used as diluent. Volume was made up to the mark to yield a stock solution containing 1000 µg/ml VLD and 1000 µg/ml DFZ, respectively. From the stock solution, the final concentration was made for VLD in range of 100-300 µg/ml and DFZ in the range of 10-30 µg/ml from the stock solution.

2.5 | VALIDATION

The method was validated as per ICH Q2 (R1) guideline for parameter like linearity, precision, LOD, LOQ, accuracy, robustness and system suitability [34].

2.5.1 | SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

2.5.2 | SYSTEM SUITABILITY

A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of VLD and DFZ to be performed. The system suitability test of the chromatography system was performed with Six replicate injections of Standard solution of both drug in mixture. Retention time, tailing factor, resolution factor, and theoretical plates were determined.

2.5.3 | LINEARITY

The linearity of an analytical method was carried out to check its ability to give test results within given range. Different concentration of VLD (100-300 µg/ml) and DFZ (10-30 µg/ml) were prepared in to 10 ml volumetric flask with mobile phase as a diluent and inject each concentration in mixture form in HPLC system and chromatogram were recorded. A calibration graph was plotted as concentration (µg/ml) verses chromatographic peak area (mV).

2.5.4 | PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

2.5.6 | ROBUSTNESS

The robustness of the method was confirmed by making small deliberate changes in the flow rate, mobile phase, pH, temperature and wavelength. The effect of these changes was recorded and % relative standard deviation was calculated.

2.5.7 | LOD and LOQ

The LOD is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels and LOQ of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy.

LOD = 3.3 x σ/S

LOQ = 10 x σ/S

where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

2.5.8 | ACCURACY

The accuracy of the method was determined by percentage recoveries of VLD and DFZ. The standard drugs were spiked at 50%,100% and 150% of the target concentration (150,200 and 250 µg/ml for VLD and 15, 20 and 25 µg/ml for DFZ). The solution were injected and peak area was recorded to

2.5.9 | ASSAY

Twenty tablets of marketed formulation were weighed and powdered. Powder equivalent to 100 mg VLD and 10mg DFZ was taken in a 100 ml volumetric flask add 70 ml of methanol and sonicated for 30 min. The solution was filtered using Whatman filter paper and the volume was made up to mark with methanol. The final concentration was made up of 100 µg/ml for VLD and 10 µg/ml for DFZ in 10 ml volumetric flask and make up volume with mobile phase as diluent. The solution were injected and the peak area is determined.

3 | RESULTS AND DISCUSSION

3.1 | Selection of wavelength

The selection of proper wavelength is essential for the sensitivity of the method. In the present study, standard solutions of VLD and DFZ were scanned in the UV region of 400– 200 nm. The overlay spectra showed that both the drugs absorb appreciably at 218 nm. So, it was selected as the detection wavelength (Figure 2).

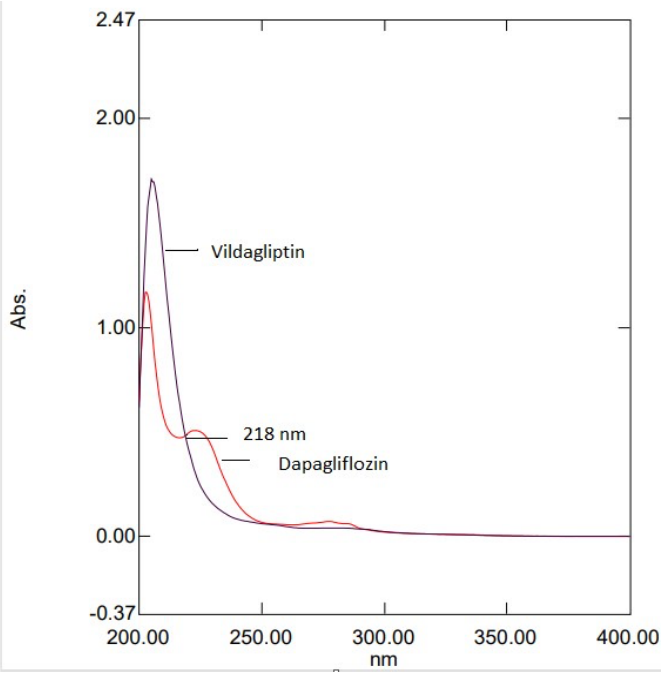

Figure 2: UV overlay spectra of Vildagliptin (VLD) & Dapagliflozin (DFZ)

3.2 | Optimization of mobile phase

The mobile phase of acetonitrile and phosphate buffer in the ratio of 60:40 v/v, pH 3.5 adjusted by orthophosphoric acid was found perfect for method development with well resolved peaks for VLD and DFZ with resolution 3.3. The retention time for VLD and DFZ were found 2.5 min and 5.5 min respectively and all parameter within range (Figure 3).

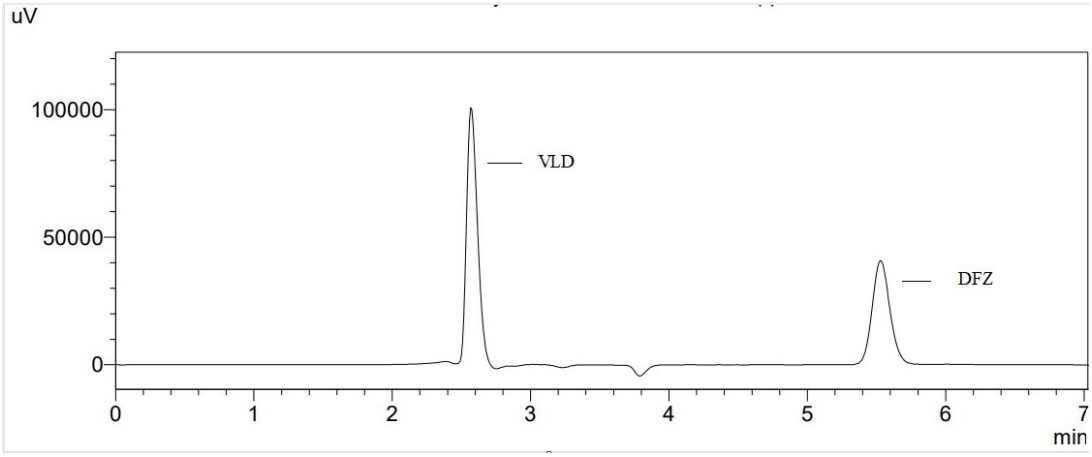

Figure 3: Chromatogram of Vildagliptin (VLD) and Dapagliflozin (DFZ) at Optimize condition of mobile phase

3.3 | Validation

Specificity:
The chromatograms of standard and blank were identical to each other. The blank injections were also identical without any interference from the optimize condition **(**Figure 4&5**).**

3.3.1 | Linearity:
The calibration curve for VLD was found to be linear in range of 100-300 µg/ml with correlation coefficient of 0.9989 and for DFZ was found to be a linear in range of 10-30 µg/ml with correlation coefficient of 0.9958 (Table 1 & Figure 5).

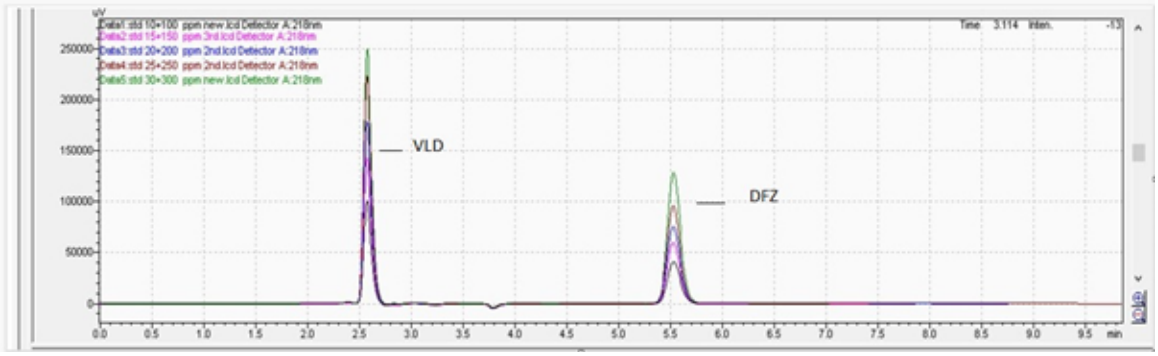

Figure 5: Linearity of Vildagliptin and Dapagliflozin in mixture

Table 1: Data of linearity

Sr. no	Vildagliptin		Dapagliflozin	
1	100	567142	10	406179
2	150	795428	15	592258
3	200	983660	20	775128
4	250	1227322	25	952528
5	300	1429541	30	1081761
Regression Equation	y = 4313.4x + 137942		y = 4313.4x + 137942	
R^2	0.9989		0.9958	

3.3.2 | System suitability
System suitability of both the drugs was injected in mixture form and all parameter like theoretical plates, tailing factor, resolution was determine as shown in (Table 2 & Figure 6).

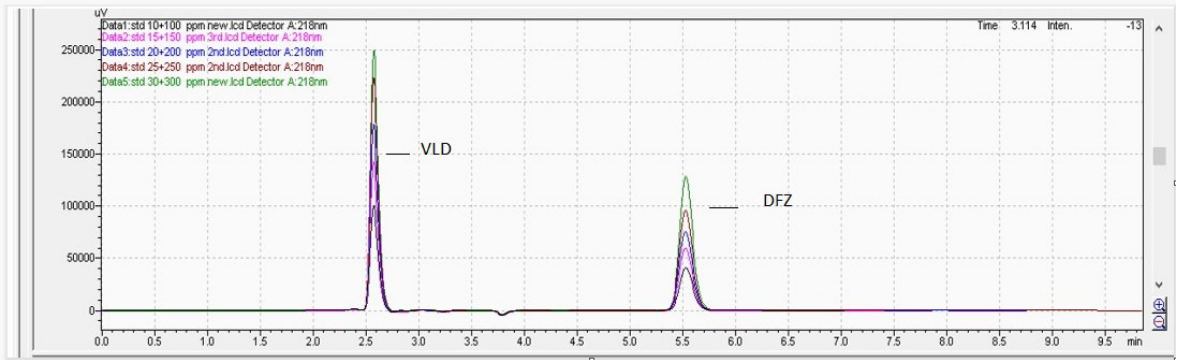

Figure 6: Chromatogram of System suitability

Table 2: System

SR no	Parameter	Results	
		Vildagliptin	Dapagliflozin
1	Tailing factor	1.24	1.13
2	Resolution	-	3.3
3	Theoritical plates	3914	7766
4	Retention times	2.5	5.5

SUITABILITY RESULTS

3.3.3 | Accuracy

The accuracy of the method was determined by calculating % recovery. Known amount of standard spike at three different level (50%, 100%, 150 %) to a pre quantified sample solution and percentage recovery was found with in range at 98%-102 % (Table 3). Calculated the percentage recovery.

Table 3: Data of Accuracy

Drug	Level	Amount of sample µg/ml	Amount of Std. spike µg/ml	Total amount µg/ml	Amount found µg/ml	% Recovery
Vildagliptin (n=3)	50%	100	50	150	148.11	98.74
	100%	100	100	200	199.24	99.62
	150%	100	150	250	248.39	99.35
Dapagliflozin (n=3)	50%	10	5	15	14.83	98.86
	100%	10	10	20	19.81	99.05
	150%	10	15	25	24.79	99.16

n = number of replicate

3.3.4 | Robustness:

The change was done in wavelength of detection (±1 nm) and flow rate (±0.1 mL/min). The % RSD for area was calculated (Table 4).

Table 4: Data of Robustness

Parameter		Vildagliptin			Dapagliflozin		
		Conc µg/ml	Peak area	%RSD	Conc µg/ml	Peak area	%RSD
Flow rate (ml/min)	0.9	150	805040	1.72	15	571781	1.77
	1.0	150	815428		15	592258	
	1.1	150	787930		15	584804	
Wavelength (nm)	217	150	805849	0.59	15	574400	1.64
	218	150	815428		15	592258	
	219	150	810417		15	589518	

Table 5: Data of precision

Parameter	%RSD	
	VLD	DFZ
Repeatability	1.67	0.36

Inter-day	0.92	0.85
Intraday	0.71	1.77

RSD = Relative standard deviation

Table 6: Summary of validation parameter

Parameter	Results	
	Vildagliptin	**Dapagliflozin**
Linearity (µg/ml)	100-300	10-30
LOD (µg/ml)	19.65	2.17
LOQ (µg/ml)	59.55	6.59
Accuracy (%)	98.74-99.35	98.86-99.16
Precision (%RSD)		
Intra-day (n=3)	0.71	1.77
Inter-day (n=3)	0.92	0.85
Repeatability (%RSD)	1.67	0.36
Assay %	99.89-99.36	99.60-99.12

RSD = Relative standard deviation, n= number of replicate

3.3.5 | Precision (repeatability)
The repeatability was performed by injecting middle concentration of the linearity range by six times i.e 200 µg/ml for VLD and 20 µg/ml for DFZ. The peak area was recorded and percentage relative standard deviation was determined (Table 5).

3.3.6 | Interday precision
Inter-day precision was performed by injecting standard preparations (150-250 µg/ml VLD and 15-25 µg/ml for DFZ) 3 times into the chromatographic system on 3 different days by maintaining the optimized chromatographic conditions and %RSD of peak areas for both VLD and DFZ calculated as shown in (Table 5).

3.3.7 | Assay:
Applicability of proposed method was assessed by analyzing marketed combined dosage forms of Vildagliptin and Dapagliflozin (tablet). The amount of Vildagliptin and Dapagliflozin were calculated from their respective calibration curves. The assay value for both Vildagliptin and Dapagliflozin was found with in acceptance criteria 98%-102%.

4 | CONCLUDING REMARKS

From this study it is concluded that the proposed HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Vildagliptin and Dapagliflozin in bulk and pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The method is validated as per ICH Q2(R1) guideline with all the parameter within acceptance criteria.

ACKNOWLEDGMENTS

All authors are very thankful Parul Institute of Pharmacy, Parul University, Vadodara, Gujarat, India for providing the necessary facilities to carry out research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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LIST OF FIGURE