Research Article

Concurrent Estimation of Vildagliptin and Dapagliflozin in Tablet Dosage Form: A Rapid and Reliable RP-HPLC Method

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Received: 10-06-2025; **Revised:** 26-08-2025; **Accepted:** 05-09-2025; **Published online:** 20-09-2025.

ABSTRACT

The co-formulation of dapagliflozin and vildagliptin presents analytical challenges due to their distinct physicochemical properties, necessitating a robust and efficient quantification strategy. In this study, a novel, rapid, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for their simultaneous determination in tablet dosage form. Chromatographic separation was achieved on a Hemochrome BDS C18 column (250 × 4.6 mm, 5 µm) using a mobile phase of 25 mM disodium hydrogen orthophosphate buffer (pH 6.5) and acetonitrile (55:45, v/v) at a flow rate of 1.0 mL/min. UV detection was performed at 210 nm with an injection volume of 20 µL. The method afforded sharp, well-resolved peaks with retention times of 3.1 min for vildagliptin and 5.5 min for dapagliflozin. Validation, performed as per ICH Q2 (R1) guidelines, confirmed excellent linearity, precision, accuracy, and robustness. The method demonstrated high sensitivity and reliability, enabling its application for routine quality control, batch release, and regulatory compliance testing of combined formulations. The method demonstrates a moderate greenness profile in accordance with Green Analytical Chemistry principles, characterized by short analysis time, low solvent consumption, and minimal hazardous reagent use, thereby supporting sustainable pharmaceutical quality control and offering a cost-effective and scalable analytical tool for pharmaceutical industries engaged in the manufacture of fixed-dose antidiabetic combinations.

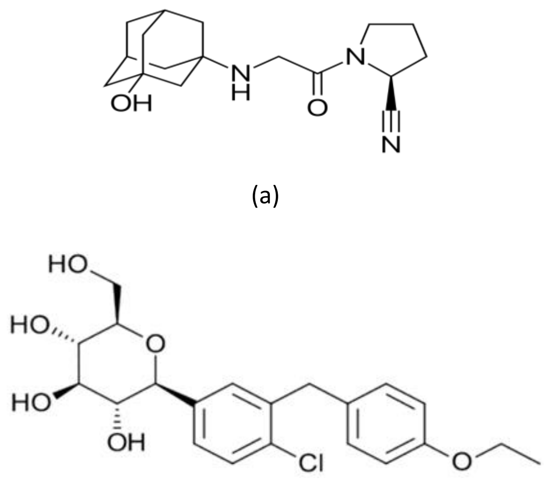
Keywords: RP-HPLC method development, simultaneous estimation, ICH Q2 (R1) validation, Vildagliptin, Dapagliflozin, fixed dose combination analysis.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder characterized by insulin resistance, β-cell dysfunction, and impaired glucose homeostasis[1]. Its increasing global prevalence has driven the need for effective therapeutic regimens capable of addressing multiple pathophysiological pathways simultaneously[2]. Fixed-dose combinations (FDCs) of oral antidiabetic agents have emerged as a rational approach to enhance glycemic control, improve patient compliance, and reduce pill burden compared to monotherapy[3].

Vildagliptin, a potent and selective dipeptidyl peptidase-4 (DPP-4) inhibitor, prolongs the activity of incretin hormones, thereby stimulating glucose-dependent insulin secretion and suppressing glucagon release[4]. Dapagliflozin, a sodium-glucose co-transporter 2 (SGLT2) inhibitor, promotes urinary glucose excretion independent of insulin action, offering an additive mechanism for glycemic control[5,6].

Figure 1 illustrates the chemical structures of Vildagliptin and Dapagliflozin, highlighting their distinct functional moieties pertinent to chromatographic method development. The complementary pharmacological profiles of these agents make their co-formulation highly effective in achieving better HbA1c reduction while minimizing the risk of hypoglycemia[7,8].



(a)

(b)

Figure 1: Structure of a) Vildagliptin and b) Dapagliflozin

From a pharmaceutical quality perspective, the simultaneous quantification of active pharmaceutical ingredients (APIs) in fixed dose combinations (FDCs) is critical for ensuring product uniformity, therapeutic efficacy, and regulatory compliance[9]. Reverse-phase high-performance liquid chromatography (RP-HPLC) remains the method of choice for such analyses due to its high resolution, reproducibility, and adaptability to routine quality control operations[10]. Although several analytical techniques have been reported for individual estimation of vildagliptin or dapagliflozin, there is limited literature describing a rapid, precise, and validated RP-HPLC method for their simultaneous determination in tablet dosage forms.

The present work focuses on the development and validation of a simple, robust, and stability-indicating RP-HPLC method for concurrent estimation of vildagliptin and dapagliflozin in combined tablet formulations. The proposed method was optimized for short analysis time, sharp peak resolution, and minimal solvent consumption, and was validated in accordance with ICH Q2 (R1) guidelines. This analytical approach is intended to serve as a reliable tool for routine quality control, batch release testing, and regulatory submissions for fixed-dose antidiabetic combinations. By eliminating hazardous solvents and derivatization steps, the authors have implemented key green chemistry principles[11], employing a straightforward aqueous–organic sample preparation approach that enhances operator safety and environmental sustainability[12].

MATERIALS AND METHODS

Chemicals and reagents

Certified reference standards of vildagliptin (potency: 100% on dried basis) and dapagliflozin (potency: 80.9%) were kindly provided by the Mumbai Central Drug Testing Laboratory, India. A marketed fixed-dose combination tablet, *VILATIN DP* sustained-release formulation (Ajanta Pharma Ltd., India), containing 100 mg of vildagliptin and 10 mg of dapagliflozin per tablet, was used for analysis. HPLC-grade acetonitrile and analytical reagent (AR) grade triethylamine were procured from Merck Life Science Pvt. Ltd. (India). Orthophosphoric acid (AR grade) was obtained from SRL Chemicals (India). Ultra-purified water (HPLC grade) was prepared using a Milli-Q® purification system, and filtration was performed through a 0.45 µm high-flow nylon membrane filter (Axiva Sichem Pvt. Ltd., India) prior to use.

Instrumentation

UV–visible spectrophotometric measurements were carried out using a Lab India UV–Vis spectrometer equipped with WinLab® software for data acquisition and analysis. Chromatographic separations were performed on a Thermo Scientific Dionex Ultimate 3000 HPLC system controlled by Chromeleon™ software (version 7.2.6) for instrument operation and data processing. Analytical weighing was conducted on a Sartorius analytical balance with high precision. pH adjustments of buffer solutions were performed using a calibrated digital pH meter. The chromatographic column employed was a Hemochrome BDS C18 (250 × 4.6 mm, 5 µm particle size), chosen for its inert stationary phase and suitability for reproducible, high-resolution separations.

Choice of Diluent

In view of the physicochemical properties and solubility profiles of vildagliptin and dapagliflozin, a mixture of HPLC-grade water and acetonitrile (50:50, v/v) was selected as the diluent for preparation of both standard and sample solutions, ensuring optimal solubility and chromatographic compatibility[13].

Selection of Detection Wavelength

Individual standard solutions of vildagliptin (200 µg/mL) and dapagliflozin (20 µg/mL) were prepared using the selected diluent and scanned in the range of 200–400 nm. The overlay spectrum indicated maximum absorbance at 210 nm for the combined analyte mixture, which was chosen as the detection wavelength.

Preparation of Standard Solutions

Accurately weighed 50 mg each of vildagliptin and dapagliflozin reference standards were transferred into separate 50 mL volumetric flasks to obtain stock solutions of 1000 µg/mL. From these, working standard solutions were prepared by appropriate dilution with the diluent, followed by sonication to ensure complete dissolution. For mixed standard preparation, 2 mL of vildagliptin (1000 µg/mL) and 2 mL of dapagliflozin (100 µg/mL) stock solutions were combined and diluted to 10 mL with the diluent, yielding final concentrations of 200 µg/mL and 20 µg/mL, respectively.

Analysis of Marketed Formulation

A quantity of the marketed combination tablet equivalent to 100 mg vildagliptin and 10 mg dapagliflozin was transferred to a 100 mL volumetric flask, sonicated for 20 min, and volume made up with the diluent. An aliquot of 10 mL from this solution was further diluted to 50 mL with diluent and filtered through a 0.45 µm nylon membrane filter prior to HPLC analysis.

Chromatographic Conditions

Chromatographic separation was achieved on a Hemochrome BDS C18 column (250 × 4.6 mm, 5 µm particle size) maintained at ambient temperature. The mobile phase consisted of 25 mM disodium hydrogen orthophosphate buffer (pH adjusted to 6.5 with orthophosphoric acid) and acetonitrile in a 55:45 (v/v) ratio, delivered at a flow rate of 1.0 mL/min. Detection was performed at 210 nm using a UV–Vis detector, with an injection volume of 20 µL. The total run time per sample was less than 7 minutes, yielding retention times of approximately 3.1 minutes for vildagliptin and 5.5 minutes for dapagliflozin. Prior to use, all mobile phase components were filtered through a 0.45 µm high-flow nylon membrane filter and degassed to ensure baseline stability.

Method Development and Validation

The RP-HPLC method was systematically developed to achieve sharp, well-resolved peaks for vildagliptin and dapagliflozin within a short run time (Figure 2a and 2b), ensuring suitability for routine quality control analysis. Several mobile phase compositions, pH conditions, and organic solvent ratios were evaluated to optimize resolution, peak symmetry, and baseline stability. The finalized chromatographic conditions employed a mobile phase of 25 mM disodium hydrogen orthophosphate buffer (pH 6.5, adjusted with orthophosphoric acid) and acetonitrile (55:45, v/v) at a flow rate of 1.0 mL/min on a Hemochrome BDS C18 column (250 × 4.6 mm, 5 µm). UV detection at 210 nm was selected based on the λ_{max} obtained from UV spectral scans of both analytes, providing optimal sensitivity.

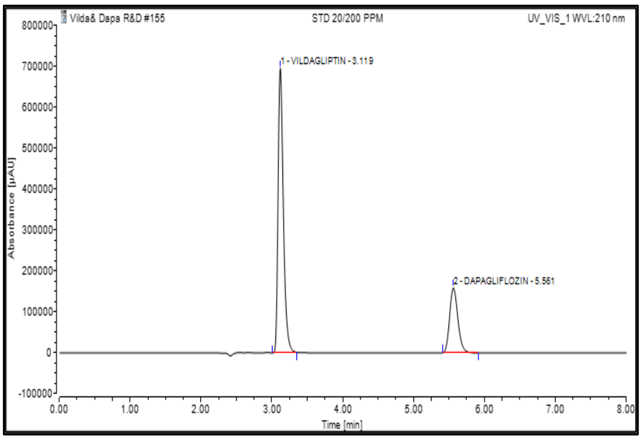


Figure 2a: Chromatogram of standard solution of vildagliptin and dapagliflozin

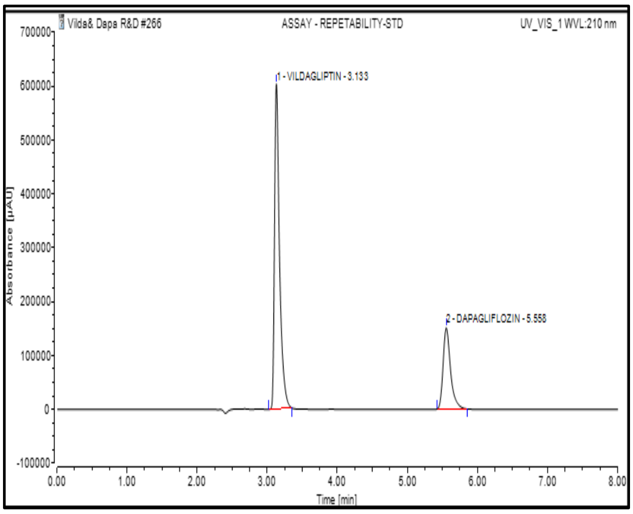


Figure 2b: Chromatogram of sample solution of vildagliptin and dapagliflozin

Method validation was conducted in accordance with ICH Q2(R1) guidelines, assessing the following parameters:

System Suitability – Evaluated by analyzing six replicate injections of standard solutions to determine parameters such as retention time, peak area, theoretical plates (N), tailing factor (T), and resolution (Rs).

Linearity – Established by preparing calibration curves for vildagliptin and dapagliflozin over an appropriate concentration range, with regression analysis performed to obtain slope, intercept, and correlation coefficient (R^2).

Accuracy – Assessed through recovery studies at three concentration levels (100%, 110%, 120% and 130% of target concentration) by standard addition to pre-analyzed samples. Mean percentage recovery and %RSD were calculated.

Precision – Determined as repeatability (intra-day) and intermediate precision (inter-day) by analyzing multiple replicates under similar conditions and calculating %RSD values.

Limit of detection (LOD) and Limit of quantitation (LOQ) – Calculated using the standard deviation of the response and the slope of the calibration curve (LOD = 3.3σ/s, LOQ = 10σ/s)(14).

Robustness – Examined by introducing small deliberate variations in chromatographic parameters such as mobile phase composition, flow rate, and detection wavelength, with evaluation of their impact on system suitability(15).

Specificity – Confirmed by comparing chromatograms of standard solutions, sample solutions, and blank mobile phase to ensure no interference from excipients or baseline noise at the retention times of the analytes.

The method demonstrated compliance with all acceptance criteria specified by ICH Q2(R1), confirming its reliability, reproducibility, and suitability for the intended analytical application.

RESULTS AND DISCUSSION

System Suitability

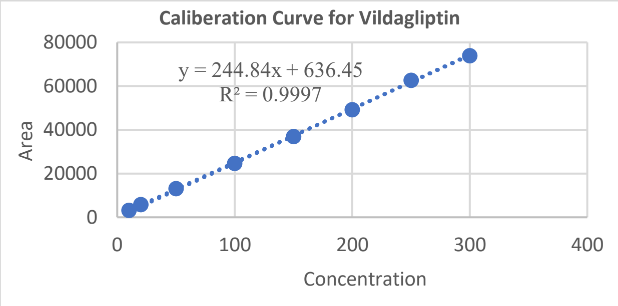
System suitability testing confirmed that all chromatographic parameters were within acceptance limits for both vildagliptin and dapagliflozin (Table 1). Theoretical plate counts exceeded 2000, tailing factors were < 2.0, and %RSD values for retention time and peak area were below 2%, indicating good column efficiency and consistent system performance[13].

Table 1: Result of system suitability study

Parameters	Vildagliptin 200 µg/mL			Parameters	Dapagliflozin 20 µg/mL
	Mean (n=5)	%RSD		Mean (n=5)	%RSD
Retention time (t_R)	3.106	0.18	Retention time (t_R)	5.50	0.08
Peak area (A)	50302.6	0.06	Peak area (A)	18719.2	1.20
Tailing factor (T)	1.632	1.91	Tailing factor (T)	1.398	1.85
No. of theoretical plates (N)	7337	0.31	No. of theoretical plates (N)	10470	0.72

Linearity

The method exhibited linearity over the range of 1–30 µg/mL for dapagliflozin and 10–300 µg/mL for vildagliptin. Calibration curves showed correlation coefficients (R^2) of approximately 0.999 for both drugs (Figure 3), confirming an excellent linear relationship between concentration and peak area[16].



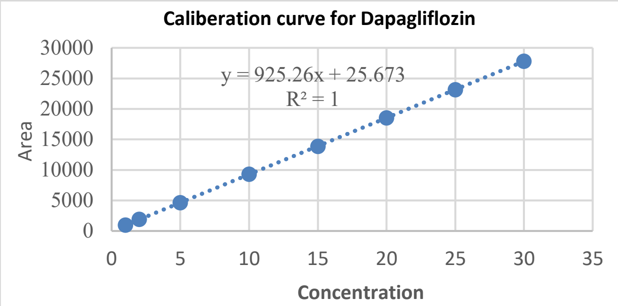


Figure 3: Calibration curve for a) vildagliptin and b) dapagliflozine

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

LOD and LOQ values, calculated from the slope and standard deviation of the calibration curve, were sufficiently low for both analytes, enabling detection and quantification at trace levels (Table 2). LOD and LOQ values indicated high sensitivity of the method, which is crucial for its industry application[14].

Accuracy

Recovery studies at 100%, 110%, 120% and 130% spiking levels yielded mean recoveries between 98% and 102% for both vildagliptin and dapagliflozin, with %RSD values < 2% (table III), indicating accuracy (recovery) was within the ICH acceptance range (98–102%)[17].

Precision

Intra-day (repeatability) and inter-day (intermediate precision) studies showed % RSD values below 2% for both drugs, demonstrating consistent peak areas and retention times across replicates and days (tables 4a and 4b). Precision studies showed %RSD values within acceptable limits[16,18].

Assay of Marketed Formulation

Analysis of *VILATIN DP* tablets (100 mg vildagliptin + 10 mg dapagliflozin) yielded assay results within pharmacopeial limits and close to label claims (Table 5).

Robustness

Deliberate variations in mobile phase composition, flow rate, and detection wavelength did not produce significant changes in retention time, peak area, or system suitability parameters. All %RSD values remained < 2% (Table 6), thus confirming minimal effect of small deliberate variations in chromatographic parameters[19], indicating method resilience across laboratory settings[20].

Table 2: Result of regression analysis

Parameters	Vildagliptin	Dapagliflozin
Linearity range (µg/ml)	100 - 300	10 - 30
Regression equation (y=mx+c)	y = 246.92x -405.21	y = 926.51x – 11.911
Slope (m)	246.92	926.51
Intercept (c)	405.21	11.911
Correlation coefficient (R^2)	0.9993	0.9999
Limit of detection (µg/ml)	9.9	0.580527
Limit of quantification (µg/ml)	30	1.759171

Table 3: Accuracy data for vildagliptin and dapagliflozin

Accuracy studies as per standard addition method for vildagliptin					Accuracy studies as per standard addition method for Dapagliflozin			
Level (%)	Average	Standard deviation	% RSD	Mean Recovery (%)	Average	Standard deviation	% RSD	Mean Recovery (%)
100	100.5	0.051	0.050	100.44	100.5	0.005	0.005	100.3
110	100.6	0.120	0.119		100.6	0.003	0.003	
120	100.6	0.142	0.141		100.0	0.065	0.065	
130	100.3	0.111	0.110		100.1	0.025	0.025	

Table 4(a): Precision (Repeatability)

Standard	Vildagliptin	Dapagliflozin
1	50286	18343
2	50299	18813
3	50348	18859
4	50314	18891
5	50266	18690
Average	50303	18719
Std Deviation	30.900	223.739
% RSD	0.061	1.195

Table 4(b): Intermediate Precision

	Day-1		Day-2	
Sr. No	Vildagliptin	Dapagliflozin	Vildagliptin	Dapagliflozin
1	99.79	100.30	98.94	99.91
2	100.54	100.53	98.59	101.27
3	100.81	100.35	98.52	101.04
Average	100.38	100.39	98.68	100.74
Standard Deviation	0.53	0.12	0.23	0.73
% RSD	0.526	0.123	0.231	0.724
Limit: -	NMT 2.0%		NMT 2.0%	

Table 5: Result of analysis of formulation

Formulation name	Label claim 100 mg for VILDA	Amount found in VILDA Containing 100 mg	% Label claim VILDA	Label claim 10 mg for DAPA	Amount found in DAPA Containing 10 mg	% Label claim DAPA
VILATIN DP	100	99.79	99.79	10	10.03	100.30
	100	100.54	100.54	10	10.05	100.53
	100	100.81	100.81	10	10.03	100.35
	100	100.43	100.43	10	9.99	99.93
	100	100.60	100.60	10	10.01	100.11
	100	100.28	100.28	10	9.93	99.32
		Mean	100.41		Mean	100.09
		SD	0.319		SD	0.393
		%RSD	0.32		%RSD	0.39

Table 4: Robustness data for both drugs

Vildagliptin						
Parameter	Change in parameter	% Estimation	Mean	SD	% RSD	Limit
Flow mL/min	0.8	99.23	98.89	0.62	0.62	NMT 2.0%
	1	99.25				
	1.2	98.18				
Temperature (º C)	33	99.30	99.79	0.85	0.85	NMT 2.0%
	35	100.77				
	37	99.29				
Wavelength nm	208	100.67	100.64	1.83	1.81	NMT 2.0%
	210	98.80				
	212	102.45				

Dapagliflozin						
Parameter	Change in parameter	% Estimation	Mean	SD	% RSD	Limit
Flow mL/min	0.8	101.54	100.28	1.45	1.45	NMT 2.0%
	1	98.69				
	1.2	100.62				
Temperature (º C)	33	99.30	99.59	0.78	0.79	NMT 2.0%
	35	99.00				
	37	100.48				
Wavelength nm	208	95.58	97.05	1.56	1.61	NMT 2.0%
	210	98.69				
	212	96.88				

CONCLUSION

The developed RP-HPLC method offers a simple, precise, accurate, and robust approach for the simultaneous estimation of dapagliflozin and vildagliptin in bulk and combined tablet dosage form. The method demonstrated excellent linearity, acceptable precision, high recovery values, and compliance with ICH validation parameters. Its low LOD and LOQ values confirm its sensitivity, while robustness studies underscore its reliability under minor deliberate variations. Given its performance characteristics, the method is well-suited for routine quality control analysis in pharmaceutical industries.

ACKNOWLEDGEMENT

The authors sincerely thank the Central Drug Testing Laboratory (CDTL), Mumbai, for generously providing the samples and reference standards of vildagliptin and dapagliflozin, as well as extending the necessary facilities and resources essential for the successful completion of this project.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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