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Development and Validation of HPTLC Method for Simultaneous Quantification of Dapagliflozin and Vildagliptin in Tablet Dosage Form

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KEYWORDS
Dapagliflozin, Vildagliptin, HPTLC, Analytical Method validation

ABSTRACT:
In recent times, the combination of Dapagliflozin and Vildagliptin has emerged as a therapeutic option for managing diabetes. This study presents the development of a rapid, selective, sensitive, and robust high-performance thin-layer chromatography (HPTLC) method for the simultaneous quantification of Dapagliflozin (DAPA) and Vildagliptin (VILG) in both their pure forms and in a commercially available formulation. The separation was successfully achieved on silica gel F254, utilizing a solvent system composed of Toluene: Ethyl Acetate: Methanol: Ammonia (6.0: 2.0: 2.0: 0.1, v/v/v/v). Chromatographic bands were visualized under short-wave ultraviolet light at 217 nm. The proposed technique demonstrated well-defined spots for DAPA and VILG, with retention factor Rf values of 0.54 and 0.28, respectively. The method exhibited good linearity within concentration ranges of 200–1400 ng/band for DAPA and 2000-14000 ng/band for VILG. The developed method was successfully applied to the marketed formulation of tablets containing combined dosage form of DAPA and VILG.

1. INTRODUCTION

Diabetes mellitus, characterized by chronic hyperglycemia, remains a global health challenge of unprecedented magnitude. In the year 2021, an estimated 537 million adults (aged 20-79) were reported to be affected by diabetes. Projections indicate a substantial increase in the global prevalence of diabetes, with an expected rise to 643 million individuals by the year 2030 and a further increase to 783 million by 2045. Diabetes imposes an ever-growing burden on healthcare systems and poses grave threats to patients' quality of life due to its associated complications, including cardiovascular disease, neuropathy, retinopathy, and nephropathy (1).

The combination of Vildagliptin (VILG) and Dapagliflozin (DAPA) in a fixed-dose formulation serves as an antidiabetic medication(2) . VILG functions as an antidiabetic agent by promoting insulin release and reducing the levels of hormones that elevate blood glucose (3) Meanwhile, DAPA acts as an antidiabetic medication by facilitating the excretion of excess sugar through urine(4). The chemical structures of VILG and DAPA are illustrated in Figure 1.

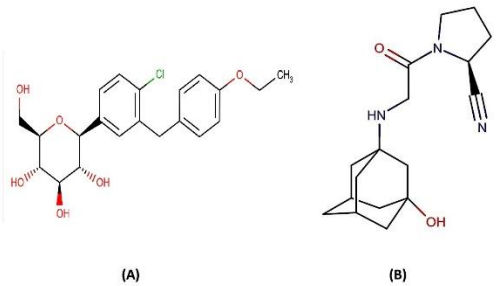

Figure 1 (A) Structure of Dapagliflozin and (B) Structure of Vildagliptin

Comparing the HPTLC approach to RP-HPLC, less organic solvent is used, and time-consuming procedures such solvent pretreatment and column washing before and after drug analysis are eliminated. (5).Dapagliflozin had been quantified alone by using various analytical techniques such as UV spectrophotometry, High Performance Liquid Chromatography and High-Performance Thin Layer Chromatography(6–9).

Journal of Chemical Health Risks
www.jchr.org
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Similarly,Vildagliptin (VILG) alone had also been quantified using various methods(10,11). Vildagliptin (VILG) andDapagliflozin (DAPA) have been also quantified in fixed-dose combinations with other medications using a variety of analytical techniques, such as spectrophotometry, RP-HPLC, and HPTLC(12-19). Despite existing methods, the literature review reveals an absence of an HPTLC approach for the simultaneous estimation of VILG and DAPA in their fixed-dose combination. The concurrent quantification of VILG and DAPA has thus led to the development of a reliable, accurate, and precise HPTLC method, whose validation was completed in compliance with ICH recommendations. (20).

2. EXPERIMENTAL

2.1 Chemicals and reagents

Pharmaceutical-grade standards for DAPA and VILG were generously provided by Glenmark Pharmaceutical (Mumbai, India). Analytical-grade Toluene, Ethyl Acetate, Methanol, and Glacial Acetic Acid were procured from Merck India Pvt. Ltd. (Mumbai, India).

2.1.1 Tablet formulation

A tablet formulation available in the market, with a labeled content of DAPA 10 mg and VILG 100 mg, was procured from a nearby pharmacy store.

2.2 Instrumentation

Chromatographic analysis was conducted using a high-performance thin-layer chromatograph. The HPTLC chromatograph was equipped with a LINOMAT-V automatic spotter device and Scanner IV, manufactured by CAMAG. The 20 x 10 cm twin-trough glass chamber was used to develop the chromatogram, and the ultraviolet (UV) chamber was used to visualize the bands Application of bands onto the TLC plates was performed using a 100 µL Hamilton syringe.

2.3 Chromatographic condition

Separation was conducted on aluminum plates (10 x 10 cm) coated with silica gel 60 F254. A semi-automatic spotting device, Linomat V, equipped with a 100 µL syringe, was utilized for sample spotting on the TLC plate. The mobile phase for TLC plate development consisted of Toluene: Ethyl Acetate: Methanol: Ammonia (6.0: 2.0: 2.0: 0.1, v/v/v/v). The migration distance was set at 90 mm, and a saturation period of 15 minutes was employed. Subsequently, the bands on the air-dried plate were scanned at 217 nm using Scanner IV, As the overlay UV spectrum shown isosbestic point at 217 nm. The Overlay UV spectrum has been illustrated in Figure 2.

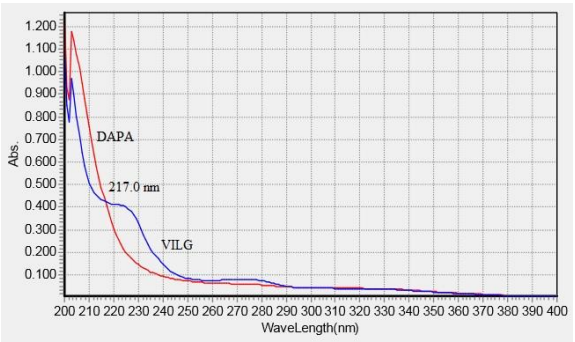

Figure 2 Overlain UV spectra of Dapagliflozin and Vildagliptin

2.4 Preparation of DAPA and VILG Mixed Standard Stock Solutions

The stock solution was prepared by accurately weighing and dissolving 10 mg each of DAPA and VILG into separate volumetric flasks, and the volume was adjusted to 10 mL, resulting in a concentration of 1000 µg/mL for each drug. From the standard stock solutions of DAPA (1000 µg/mL), 0.4 mL was taken, and from the standard stock solutions of VILG (1000 µg/mL), 4 mL was taken. These volumes were diluted to 10 mL with methanol, yielding solutions with concentrations of 40 µg/mL for DAPA and 400 µg/mL for VILG. Subsequently, 5 µL of each solution was applied, providing 200 ng/band and 2000 ng/band for DAPA and VILG, respectively.

2.5 Preparation of Sample Solutions

Twenty tablets, each containing 10 mg of DAPA and 100 mg of VILG, were collectively weighed, and their average weight was determined before triturating. The tablet powder equivalent to 4 mg of DAPA was precisely weighed and transferred to a 10 ml volumetric flask. Subsequently, 5 ml of water was added to the flask, followed by 15 minutes of sonication. The volume of the solution was adjusted to 10 ml, resulting in a solution with concentrations of 400 µg/ml for DAPA and 4000 µg/ml for VILG. The filtered solution,

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(6), 3643-3649 | ISSN:2251-6727

passed through a 0.2 µm filter, was further diluted by taking 10 ml of the solution and diluting it to 100 ml with methanol, yielding solutions with concentrations of 40 µg/ml for DAPA and 400 µg/ml for VILG.

3. METHOD VALIDATION (20,21)

The validation of the developed methods was conducted in accordance with the validation parameters outlined by the International Conference on Harmonization (ICH) guideline Q2(R1

3.1 Linearity

Calibration curves were constructed over a concentration range of 200 - 1400 ng/band for DAPA and 2000-14000 ng/band for VILG. Standard working solutions of DAPA and VILG were applied to the plate in specific aliquots. The peak area versus concentration relationship was established using the winCATS software to develop the calibration curves.

3.2 Accuracy (recovery)

Accuracy assessment was performed through the standard addition method by determining the recoveries of DAPA and VILG. Known quantities of a mixed standard solution containing DAPA (400, 800, 1200 ng/band) and VILG (4000, 8000, 12000 ng/band) were added to pre-quantified sample solutions of the test substances. The determination of DAPA and VILG amounts was accomplished by applying the peak area values to the regression equations derived from the calibration curve

3.3 Precision

Precision evaluations were conducted through both intraday and interday studies by injecting mixed standard solutions of DAPA and VILG featuring three distinct concentrations (400, 800, 1200 ng/band) for DAPA and (4000, 8000, 12000 ng/band) for VILG onto a plate using the same syringe. Peak area and the relative standard deviation as a percentage (% RSD) were then computed.

3.4 Limit of detection and limit of quantification

The following formulas were used to calculate the Limit of Quantification (LOQ) and Limit of Detection (LOD) for both drugs:

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

where σ = SD of the response and S = SD of the y-intercept of the regression line.

3.5 Specificity

To evaluate specificity blank, standard solution containing DAPA and VILG and sample solution were injected. Any interference from blank, sample excipients and mobile phase to the peak of interest was checked.

3.6 Robustness

By systematically changing the saturation period (13 min and 17 min), wavelength (215 nm and 219 nm), and distance from solvent front (8.5 cm and 9.5 cm), the robustness of the developed method was evaluated. Three duplicates of a single standard concentration (8000 ng/band of VILG and 800 ng/band of DAPA) were used in the evaluation. Mean area values and the percentage relative standard deviation (% RSD) were calculated, and the observed effects of parameter changes were reported.

3.7 Assay of DAPA and VILG in dosage form

Sample solution containing 40 and 400 µg/ml solutions for DAPA and VILG respectively was applied. The quantification of DAPA and VILG was achieved by determining their respective amounts through the application of peak area values to the regression equations derived from the calibration curve.

4. RESULTS AND DISCUSSIONS

4.1 Optimization of HPTLC chromatographic conditions

The goal of obtaining high-resolution and repeatable peaks for HPTLC tests was investigated by experimenting with different mobile phase proportions. Using Toluene: Ethyl Acetate: Methanol: Ammonia (6.0: 2.0: 2.0: 0.1, v/v/v/v)as the mobile phase allowed for the successful achievement of this goal. The studies used a solvent migration distance of 90 mm and a chamber saturation duration of 15 minutes at room temperature (25 ± 2 °C). With symmetrical and sharp peaks and Rf values of 0.28 and 0.54 for VILG and DAPA, respectively, the method showed effective resolution.

Journal of Chemical Health Risks
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4.2 Linearity curve

As Figure 3 illustrates, the linear regression data derived from the calibration curves demonstrated a strong linear correlation within the concentration range of 200–1400 ng/band for DAPA and 2000–14000 ng/band for VILG. The regression equations, along with the coefficient of determination (r^2) values, demonstrated strong linearity, with r^2 values of 0.9968 for DAPA (Figure 4) and 0.9977 for VILG (Figure 5), as summarized in Table I.

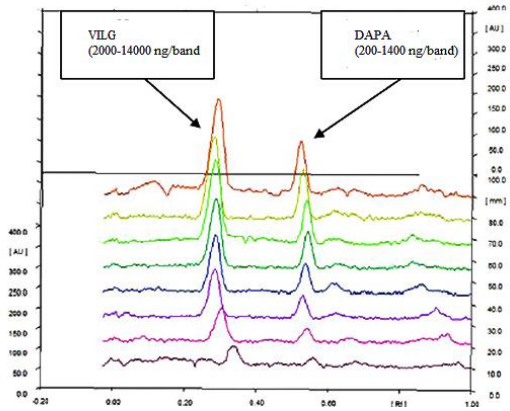

Figure 3 3D chromatogram of linearity for DAPA and VILG

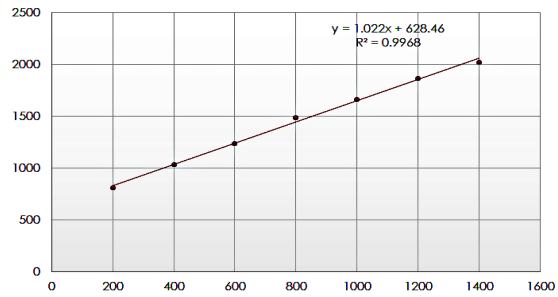

Figure 4 Calibration curve for linearity study of DAPA

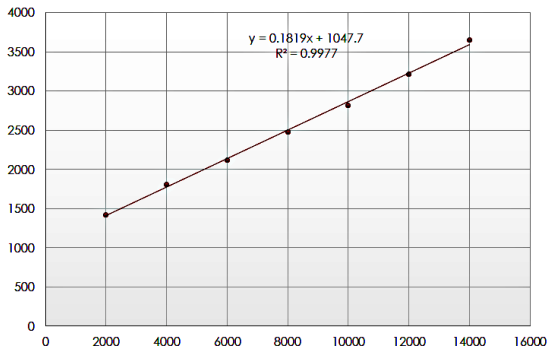

Figure 5 Calibration curve for linearity study of VILG

4.3 Accuracy

The recovery findings were within the acceptable range as defined by the ICH guideline. The range of recovery percentages for VILG was 99.01% to 99.69%, and for DAPA it was 98.87% to 99.71%. As shown in Table II, these values were calculated for formulation samples as well as pure drug samples at 50%, 100%, and 150% levels.

4.4 Precision

The repeatability of the procedure was used to evaluate its precision. Three different concentration levels were used to measure intra- and inter-day variations for VILG and DAPA:4000, 8000, and 12000 ng/band for VILG, and 400, 800, and 1200 ng/band for DAPA. For all of these concentration levels, the percentage relative standard deviation (%RSD) was determined to be less than 2%. Table III presents the specific findings.

4.5 LOD and LOQ

The method's sensitivity was evaluated through the determination of Limit of Detection (LOD) and Limit of Quantification (LOQ), resulting in values of 2.34 ng and 7.10 ng for DAPA, and 5.82 ng and 17.63 ng for VILG, respectively, as illustrated in Table I.

4.6 Specificity

The R_f values corresponding to the chromatographic peaks of DAPA and VILG in the sample matched those in the standard chromatogram for DAPA and VILG. As shown in Figure 6, these experimental results demonstrate the purity of the chromatographic peaks for DAPA and VILG in both the sample and standard.

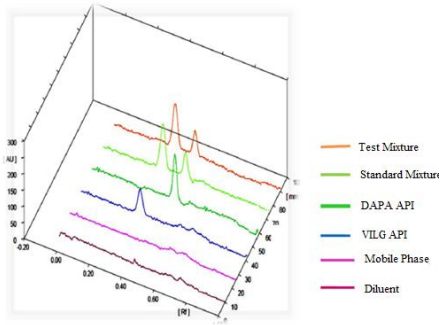

Figure 6 3D Chromatogram for specificity study

4.7 Robustness

A percentage relative standard deviation (%RSD) of less than 2% was obtained by calculating the standard deviation of peak areas for each parameter, as shown in Table IV.

Journal of Chemical Health Risks

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JCHR (2023) 13(6), 3643-3649 | ISSN:2251-6727

4.8 Assay of DAPA and VILG in dosage form

Table V displays the results of the quantification of the marketed tablet. After analysis, the percentages of VILG and DAPA were found to be 98.99% and 99.26%, respectively. The percentage relative standard deviation (%RSD) was found to be less than 2%.

5. CONCLUSIONS

The HPTLC method developed facilitated rapid separation within a brief timeframe, enabling the simultaneous measurement of numerous samples with good accuracy, sensitivity, and precision. This characteristic is particularly vital in quality control processes. The method, noted for its simplicity, speed, sensitivity, accuracy, precision, specificity, and efficiency, underwent validation in accordance with ICH guidelines.

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Table I Linearity, Rage, LOD and LOQ for DAPA and VILG

Parameter	**DAPA**	**VILG**
Concentration range	200–1400ng/spot	2000–14000ng/spot
Slope	1.0252	0.1823
Intercept	628.27	1045.40
Correlationcoefficient	0.9968	0.999
LOD	2.34 ng	5.82 ng
LOQ	7.10 ng	17.63 ng

Table II Accuracy study of DAPA and VILG

Drug	**Level (%)**	**Amount of sample (ng/band)**	**Amount of std. spiked (ng/band)**	**Total amount (ng/band)**	**% Recovery (avg.)**
DAPA	50	200	400		99.71
	100		800		98.87
	150		1200		99.29
VILG	50		4000		99.69

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(6), 3643-3649 | ISSN:2251-6727

	100	2000	8000		99.01
	150		12000		99.36

Table III Precision study of DAPA and VILG

Drug	Conc. (ng/band)	Intraday %RSD (Area) (Day 1)	Intraday %RSD (Area) (Day 1)	Interday %RSD (Area) (Day 2)	Interday %RSD (Area) (Day 3)
DAPA	400	0.1672	0.2782	0.1591	0.2804
	800	0.2237	0.1770	0.1989	0.0997
	1200	0.1130	0.1421	0.1645	0.1729
VILG	4000	0.1964	0.2170	0.1370	0.2302
	8000	0.1159	0.1408	0.1699	0.0687
	12000	0.1121	0.1094	0.1153	0.1324

Table IV Robustness study for DAPA and VILG

Parameter	Condition	%RSD DAPA	VILG
Unaltered		0.1488	0.1178
Change in saturation time	13 min	0.2992	0.0868
	17 min	0.2571	0.0754
Change in wavelength	248 nm	0.1873	0.1469
	252 nm	0.1102	0.1285
Change in distance travelled	8.5 cm	0.1305	0.1451
	9.5 cm	0.2826	0.1444

Table V Assay of DAPA and VILG in the marketed dosage form

Drug	Label claim	Amount obtained	% Assay
DAPA	10 mg	9.926 mg	99.26
VILG	100 mg	98.99 mg	98.99