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Advances in Bioresearch

ORIGINAL ARTICLE

Development and Validation of RP-HPLC Method for Estimation of Vildagliptin and Dapagliflozin in Bulk Drug and Dosage Form

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ABSTRACT
A simple, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of dapagliflozin and vildagliptin in bulk and pharmaceutical dosage forms. Initial method development involved evaluation of physicochemical properties, solvent selection, solubility studies, and determination of the analytical wavelength, which was optimized at 210 nm. Various mobile phase compositions were investigated to achieve optimal chromatographic separation, with methanol employed as the diluent. The optimized chromatographic conditions consisted of an Agilent Poroshell column using a mobile phase of acetonitrile and 0.1% trifluoroacetic acid in water (50:50 v/v) at a flow rate of 1.0 mL/min, with UV detection at 210 nm. The method was validated in accordance with ICH guidelines, assessing system suitability, specificity, linearity, accuracy, precision, robustness, and solution stability. The results demonstrated excellent linearity, high accuracy, and good precision across the studied concentration range. The validated method was successfully applied for the quantitative determination of dapagliflozin and vildagliptin in commercial formulations, confirming its suitability for routine quality control analysis.
Keywords: *RP-HPLC; dapagliflozin; vildagliptin; simultaneous estimation; method validation; ICH guidelines.*

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic and endocrine disorder with deranged glucose regulation that results in raised blood glucose levels secondary to the underproduction of insulin and reduced cell sensitivity to insulin's action.(1) Hyperglycaemia secondary to chronic elevation of blood glucose provokes extensive complications involving microvascular and macrovascular dysfunctions in the circulatory, nervous, and immune systems.(2) Once known as adult-onset diabetes, T2DM is increasingly being seen in young adults because of the world's pandemic of obesity, especially among children and adolescents.(3)

Good control of T2DM is most commonly achieved with combination pharmacotherapy that addresses several pathophysiologic pathways.(4) Metformin is also used as first-line therapy in general because it decreases blood glucose by activating AMP-activated protein kinase (AMPK), which inhibits hepatic glucose production, increases insulin sensitivity, and increases peripheral glucose uptake.(5) However, most of these patients require additional medications to achieve optimal glycaemic control.(6) Dapagliflozin is an SGLT2 inhibitor which reduces blood glucose levels regardless of insulin by inhibiting renal reabsorption of glucose and thereby enhancing the excretion of glucose in urine.(7) As dapagliflozin has an insulin-independent mechanism, it acts at all phases of T2DM progression.(8)

Vildagliptin is a dipeptidyl peptidase-4 inhibitor that acts by having strong and stable binding to the DPP-4 enzyme and hence increasing active incretin hormone levels such as glucagon-like peptide-1 (GLP-1).(9) It causes insulin release in response to glucose and reduces glucagon secretion during fasting and postprandial conditions.(10) The synergistic action of dapagliflozin and vildagliptin is a therapeutic approach to superior glycaemic control with minimal risk of hypoglycaemia.(11) This study will discuss the potential synergistic and action of benefit of combined administration of SGLT2 and DPP-4 inhibitors in the management of type 2 diabetes mellitus.(12)

Dapagliflozin and Vildagliptin have also been promising when combined with other drugs for type 2 diabetes mellitus (T2DM) management as they possess complementary activity.(12) Dapagliflozin, being an SGLT2i, reduces high blood glucose by enhanced urinary excretion of glucose, regardless of insulin secretion or sensitivity.(13) Alternatively, Vildagliptin, a dipeptidyl peptidase-4 (DPP4i) inhibitor, increases glycaemic control through inhibition of incretin hormone degradation with subsequent stimulation of insulin release in a glucose-dependent fashion and suppression of glucagon release.(14,15) A novel approach to treating type 2 diabetes mellitus, the synergistic use of medicines in this class offers a very favourable mode of glycaemic management that is highly successful in lowering blood glucose with a negligible risk of hypoglycemia.(16, 17)

Dapagliflozin

Adults with type 2 diabetes are treated with dapagliflozin, a family of SGLT2 inhibitors, particularly inconjunction with diet and exercise.(18, 19). By preventing glucose from being reabsorbed from the nephron's proximal tubule, it improves blood glucose regulation without the need for insulin and increases glucose excretion through urine.(20) It is chemically referred to as (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-6-(hydroxymethyl) oxane-3,4,5-triol.(21, 22)

Dapagliflozin has been investigated in large numbers as monotherapy, and together with other antidiabetic therapies like insulin and oral hypoglycaemic drugs, and was proved to be well effective for the glycaemia control and with an extremely good safety profile for the treatment of type 2 diabetes mellitus.(23-25)

Vildagliptin

Vildagliptin (LAF237) is an oral antidiabetic drug for the therapy of type 2 diabetes mellitus.(26) Its mechanism of action is through selective inhibition of the dipeptidyl peptidase-4 (DPP-4) enzyme involved in the degradation of incretin hormones such as glucagon-like peptide-1 (GLP-1).(27) Vildagliptin inhibits incretin degradation, resulting in increased insulin secretion and decreased glucagon secretion in a glucose-dependent manner and thereby increasing glycaemia control.(28) This activity is particularly beneficial in type 2 diabetes, an illness which is characterized by defective incretin action and insulinotropic effect.(29) Vildagliptin's chemical name is (2S)-1-{2-[(3-hydroxyadamantan-1-yl)amino]acetyl}pyrrolidine-2-carbonitrile and has been effectively employed as monotherapy or added to other antidiabetic therapy to ensure improved blood glucose control. (30, 31)

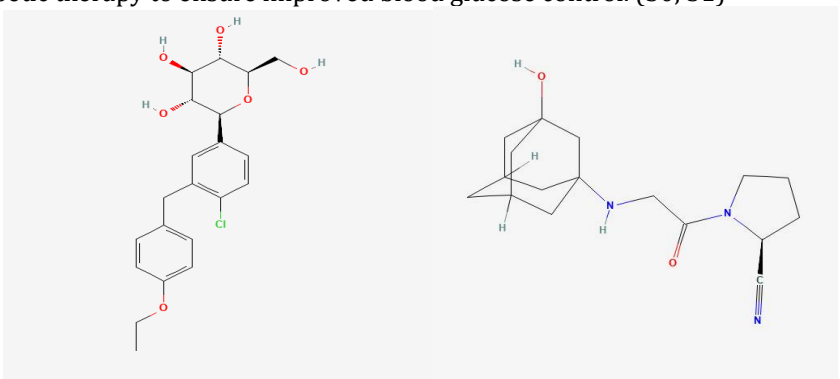


Fig. No. 1 Structure of Dapagliflozin Fig. No. 2 Structure of Vildagliptin

MATERIAL AND METHODS

Material, Reagent and Pharmaceutical Product

Vildagliptin, Dapagliflozin 100% were obtained from Vidisha Analytical. Reagents or Chemicals or Solvents like methanol, acetonitrile and water procured from Merk and siddhi lab.

Instrumentation

The instruments used during the experimental procedure consisted of both Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) and UV-Visible spectrophotometry. A UV-Vis spectrophotometer was used to determine the analytical wavelength by scanning standard solutions of

Vildagliptin and Dapagliflozin from 200 to 400 nm using methanol as the solvent. Both drugs showed a common absorbance peak (Q-point) at 210 nm, which was selected for further analysis.

For the RP-HPLC technique preparation, an Agilent Poroshell column (150 mm × 4.6 mm, 5 µm) was employed. The temperature of the column was kept at 40°C, and chromatographic analysis was carried out with the assistance of a UV detector under 210 nm conditions. Both times, the column injection was 20 µL, and the flow rate remained constant at 1.0 mL per minute. The mobile phase was also tuned to a 1:1 mixture of acetonitrile and 0.1% trifluoroacetic acid in water. The overall analysis time per run was 10 minutes.

Determination of solubility

At a target concentration of 3 mg/mL, dapagliflozin propanediol monohydrate and vildagliptin were soluble in both water and methanol. About 30 mg of Vildagliptin was carefully weighed for the aqueous solubility research, then added to a 10 mL volumetric flask with distilled water and sonicated for 5–10 minutes to dissolve it. Using distilled water, the same procedure was performed on 36.90 mg of dapagliflozin propanediol monohydrate, which is equal to 30 mg of dapagliflozin under the identical circumstances. With the exception of using methanol, the same methodology was applied in the methanolic solubility investigation. In this case, 10 mL of methanol was used to dissolve 30 mg of Vildagliptin and 36.90 mg of Dapagliflozin Propanediol Monohydrate, which were then sonicated for 5–10 minutes. The results of solubility derived using these protocols are reported in the Results.

Selection of Analytical Wavelength

Methanol was used as the typical solvent used to prepare Vildagliptin and Dapagliflozin standard solutions since it is compatible with good solubilizing ability and compatibility for UV spectrophotometry.

Development Method with RP–HPLC

Vildagliptin and dapagliflozin stock standard solutions were developed in order to establish the RP-HPLC analytical technique. Twenty milligrams of Vildagliptin were precisely weighed and put in a 20 millilitre volumetric flask. After adding around 15 mL of methanol, it was sonicated until it was dissolved. A stock solution containing 1000 µg/mL of methanol was then added to bring the volume up to 20 mL. A final concentration of 100 µg/mL was generated by diluting 1 mL of stock solution with 10 mL of the mobile phase to create a working solution. This was introduced into the HPLC system and used for all method development runs.

Similar to the last example, 24.60 mg, or 20 mg, of dapagliflozin propanediol monohydrate was precisely weighed and added to a 20 mL volumetric flask. To create a 1000 µg/mL stock solution, 15 mL of methanol was added, sonicated to dissolve it, and then the amount was increased to 20 mL using methanol. This was diluted with the mobile phase to create a working concentration of 100 µg/mL from a 1 mL aliquot. This solution was injected for chromatographic optimisation and used during mobile phase testing.

Optimization of HPLC Method

RP–HPLC optimization of the estimation of Vildagliptin and Dapagliflozin simultaneously was achieved with isocratic elution and UV detection at 210 nm. Mobile phase modification was tried in four trials. Standard solutions (100 µg/mL each) were injected under the same chromatographic conditions in an Agilent Poroshell column (150 mm × 4.6 mm, 5 µm), temperature 40°C, flow rate 1.0 mL/min and injection volume 20 µL.

Trial 4 with Acetonitrile:0.1% TFAA in Water (50:50) as mobile phase yielded better resolution and peak shape and was chosen as the final method.

Table No. 1: Optimized Chromatographic Conditions for RP–HPLC Analysis of Vildagliptin and Dapagliflozin

Parameter	**Condition**
Detector	UV Detector
Column	Agilent Poroshell
Column Dimensions	150 mm × 4.6 mm *i.d.,* 5 µm
Column Oven Temperature	40°C
Injection Volume	20 µL
Detection Wavelength	210 nm
Mobile Phase	Acetonitrile: 0.1% TFAA in Water (50:50)
Flow Rate	1.0 mL/min
Run Time	10 minutes

Method Validation

System suitability

System suitability test as per pharmacopeial specifications was conducted through the injection of five replicate injections of reference mixture. The following acceptance criteria were used. (1) Relative Standard Deviation (RSD) of not more than 2.0% for five injection replicates; (2) USP Tailing Factor (Asymmetry Factor) should not be higher than 2.0; and (3) Specificity

Specificity refers to the capability to identify the analyte clearly regardless of the presence of the other anticipated constituents, e.g., excipients.

Linearity and Range

Linearity of the reported RP-HPLC method was investigated to identify the capability of the method in providing results in direct proportion with the concentration of the analyte in a previously selected range of concentrations. The study was carried out at five concentrations within the range of 50% to 150% of the working levels of concentration.

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

The Limit of Detection (LOD) is the lowest level of analyte in a sample that can be detected reliably but not accurately quantitated, while the Limit of Quantitation (LOQ) is the lowest amount that can be quantitated with good accuracy and precision. LOD and LOQ were both established using the calibration curve method, as required by the provisions outlined in ICH Q2 (R1).

Accuracy (% Recovery)

Accuracy of an analytical method is the closeness of a test result to the accepted true value or reference standard. In order to estimate the recovery, recovery studies were performed by spiking known amounts of standard drug solutions into the placebo in concentration ranges comparable to 50%, 100%, and 150% of the working concentration. Triplicate levels of each strength were made. Percentage recovery of each sample was determined separately, as well as mean recovery for each level, total recovery, and percentage relative standard deviation (% RSD) to determine precision and accuracy of the process.

Precision

Precision of an analytical method is the nearness of agreement between two or more separate results from several analyses of a homogeneous sample under normal conditions, e.g., repeatability and intermediate precision. Precision was accessed in this validation by the commercially available tablet form of Dapagliflozin and Vildagliptin.

Robustness

Robustness of the method was ensured by running the standard solution under deliberately varying chromatographic conditions to demonstrate the robustness of the method for reuse. The variations involved ±10% adjustment of the flow rate (i.e., ±0.1 mL/min), ±2°C variation in column oven temperature, and ±3 nm variation in detection wavelength.

RESULT AND DISCUSSION

Preliminary Characterization and Identification of Drug

Colour, Odour, and Appearance

Their results are detailed in the table below:

Table No. 2: Colour, Odour, and Appearance of Drug Substances

Sr. No.	Name of Drug	Colour, Odour, and Appearance
1	Vildagliptin	White, odourless, and slightly amorphous powder
2	Dapagliflozin Propanediol Monohydrate	White, odourless, and amorphous powder

Solubility Study

The solubility of Vildagliptin and Dapagliflozin Propanediol Monohydrate was also assessed using water and methanol. Vildagliptin had been soluble in both water and methanol since no precipitate formed even after sonication. In contrast, Dapagliflozin had poor solubility in water with visible precipitates even after sonication, which means that water is not a good solvent for Dapagliflozin. However, both the drugs were found to be fully soluble in methanol and thus methanol proved a good common solvent for preparing stock solutions in analytical work.

Selection of Analytical Wavelength

Methanol was chosen as a solvent for Vildagliptin and Dapagliflozin because it has a superior solubilizing quality, as also established from the solubility test. A stock solution of known concentration of both drugs

was prepared at 500 PPM and then diluted to levels of 20 PPM for the UV spectroscopic determination. Blank methanol being used, the standard solutions were scanned from 200–400 nm to determine their maximum absorbance. It is evident from Figure No.3 that both Vildagliptin and Dapagliflozin showed several peaks of absorbance; a shared Q-absorption point was, however, identified to be 210 nm in both the drugs. Therefore, 210 nm was used as the wavelength of analysis for simultaneous estimation and further optimization of the method.

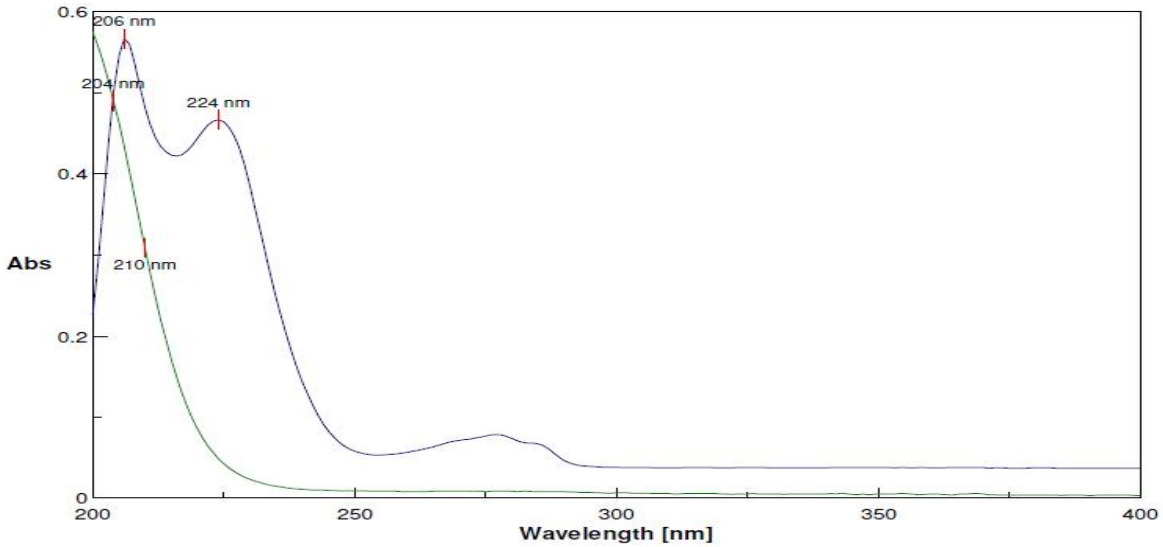
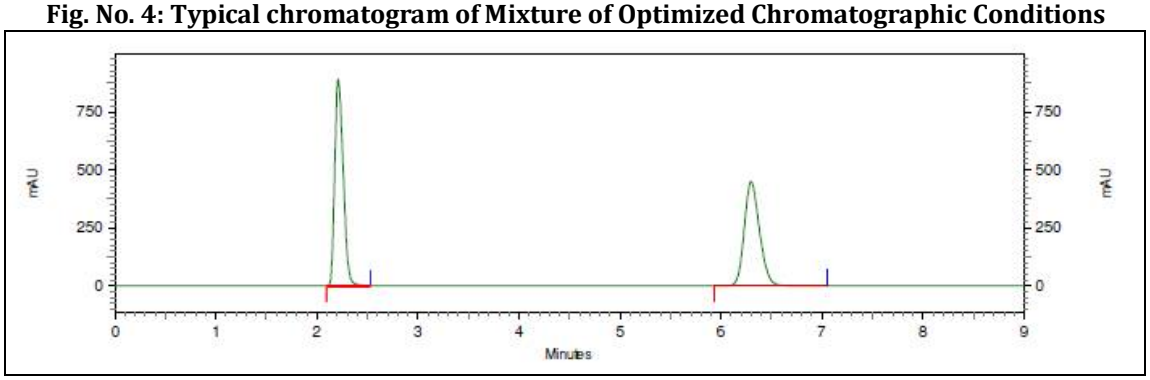


Fig. No. 3: Overlay UV spectrum of Vildagliptin & Dapagliflozin

RP-HPLC Method Development and Optimization

A successful optimized RP–HPLC method was established for co-estimation of Vildagliptin and Dapagliflozin. Both drugs were dissolved as stock solution in methanol in a concentration of 1000 µg/mL and working standard of 100 µg/mL was used throughout method development experiments. Various combinations of mobile phase were tried to attain optimal chromatographic performance. Out of the four trials, the fourth trial with a mobile phase of Acetonitrile and 0.1% Trifluoroacetic Acid (TFAA) in Water in the ratio of 50:50 proved to have better resolution, peak shape, and reproducibility of both analytes. The ultimate chromatographic conditions were Agilent Poroshell column (150 mm × 4.6 mm, 5 µm), flow rate of 1.0 mL/min, column oven temperature of 40°C, and UV detection at 210 nm. These were all established as the ultimate conditions to utilize for future method validation and analysis.

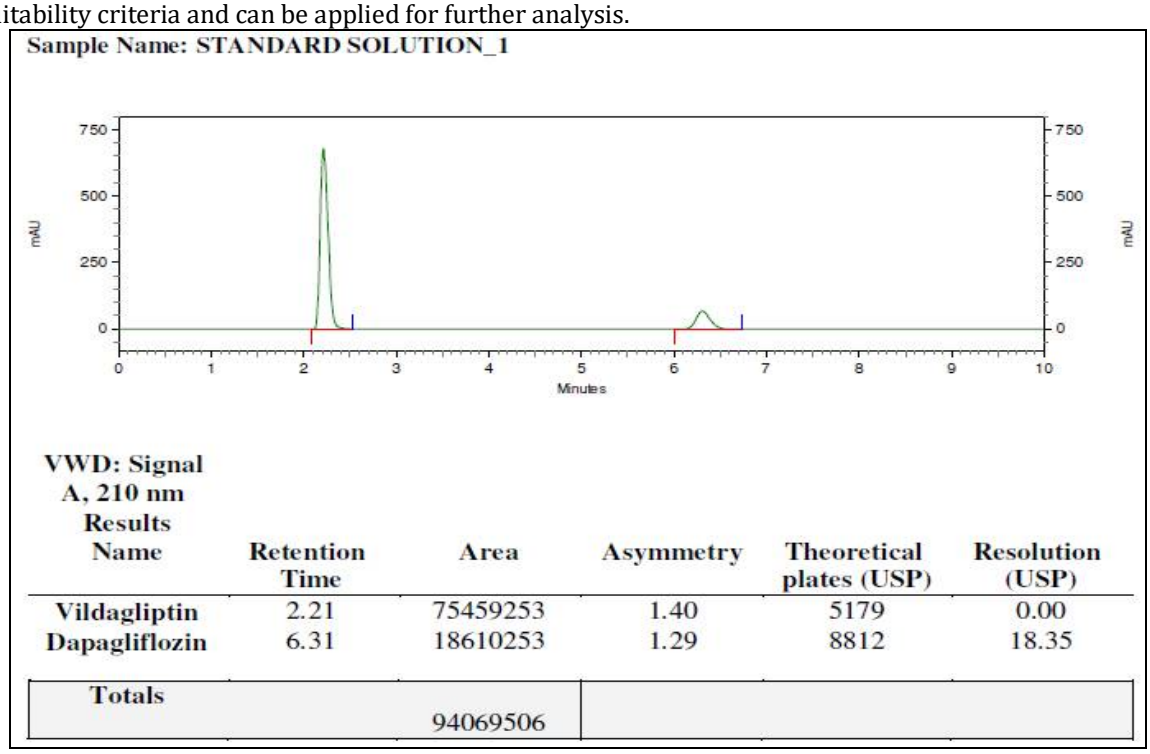
Fig. No. 4: Typical chromatogram of Mixture of Optimized Chromatographic Conditions



System Suitability

System suitability test was performed for the determination of the efficiency and reproducibility of RP–HPLC method standardized for the simultaneous quantitation of Vildagliptin and Dapagliflozin. Five repeated injections of standard solution were scanned and quantitated, and area, asymmetry (tailing factor), and theoretical plates were measured. For Vildagliptin, peak area %RSD was 0.28%, mean asymmetry was 1.40, and mean theoretical plates were 5171, showing column efficiency and peak symmetry to be good. For Dapagliflozin too, %RSD was 0.35%, mean asymmetry was 1.29, and theoretical plates were 8829, all of which were within acceptable limits.

System suitability acceptance criteria were: %RSD ≤ 2.0, theoretical plates ≥ 2000, and asymmetry < 2.0. It is evident from the chromatogram that there is good peak separation with retention times of 2.21 min for Vildagliptin and 6.31 min for Dapagliflozin from Figure No. 5. The resolution between both peaks was 18.35, ensuring perfect separation. Therefore, the new chromatographic method satisfies all the system suitability criteria and can be applied for further analysis.



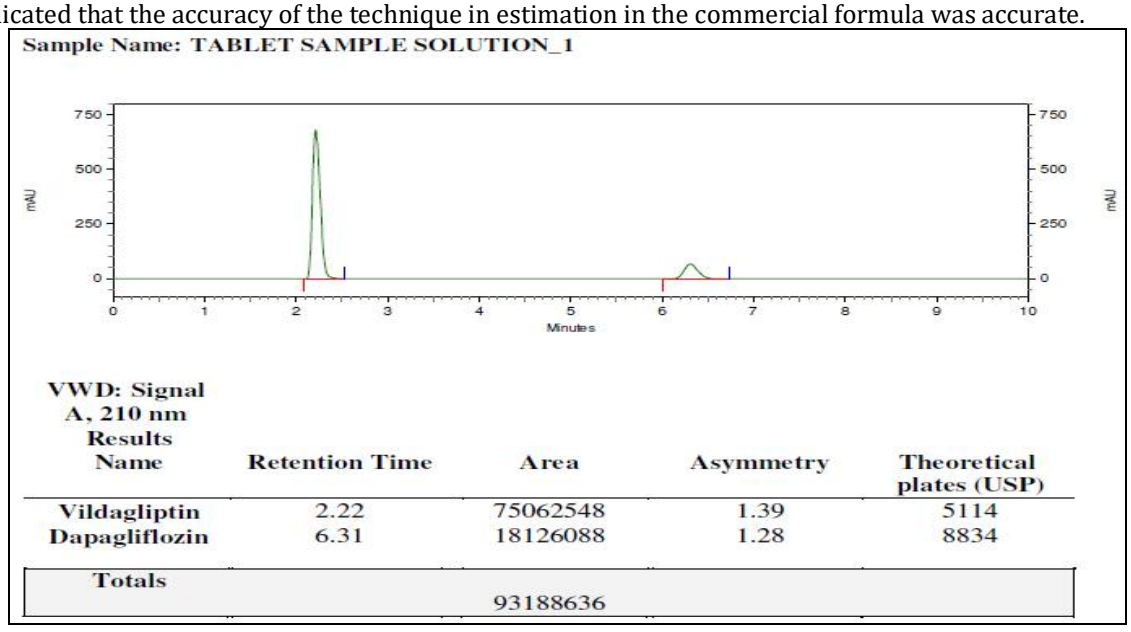
VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)	Resolution (USP)
Vildagliptin	2.21	75459253	1.40	5179	0.00
Dapagliflozin	6.31	18610253	1.29	8812	18.35
Totals		94069506			

Fig. No. 5: Typical chromatogram Standard solution 1 of system suitability solution.

Market Tablet Dosage Form Analysis (Jalra-DP Tablet)

Jalra-DP Tablet was assayed by applying the developed RP–HPLC method. Average tablet weight was 382.6 mg. Assay values were 98.66% and 97.99% for Vildagliptin and Dapagliflozin, respectively. As can be seen from Figure No. , both the drugs were also resolved properly with retention times 2.22 min (Vildagliptin) and 6.31 min (Dapagliflozin). All the values within the acceptability range of 90–110% indicated that the accuracy of the technique in estimation in the commercial formula was accurate.



VWD: Signal A, 210 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical plates (USP)
Vildagliptin	2.22	75062548	1.39	5114
Dapagliflozin	6.31	18126088	1.28	8834
Totals		93188636		

Fig. No. 6: Typical chromatogram Jalra-DP Tablet sample.

a) Solution Stability

An assessment of stability was done for the standard and sample test solutions of Vildagliptin and Dapagliflozin under standard laboratory conditions. The solutions were scanned at zero time, 12 hours, and 24 hours. % absolute differences in peak areas of standard solutions in 24 hours were 0.67% for Vildagliptin and 0.76% for Dapagliflozin. As shown in Figure for test solutions, these values were 0.84% and 0.98%, respectively. All values were below acceptance value of NMT 2.0%, indicating that test and standard solutions were stable for a minimum time of 24 hours at laboratory conditions.

b) Specificity

RP–HPLC method specificity defined was validated by a placebo and blank injection. No peaks at the Vildagliptin (2.22 min) and Dapagliflozin (6.31 min) retention times were observed on blank as well as on placebo chromatograms. Figure No.7 and clearly show that there was no interference, i.e., the method is specific for both analytes and is acceptable.

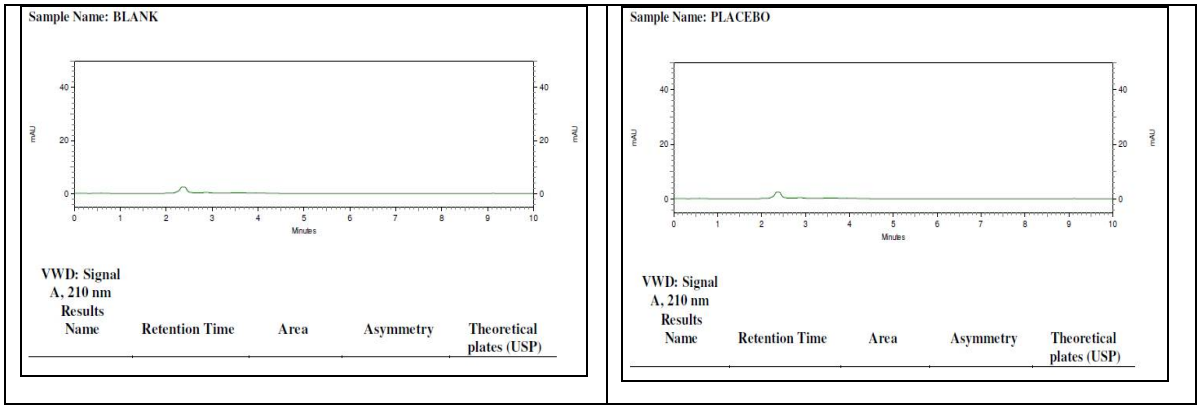


Fig. No. 7: Typical chromatogram of Placebo solution and Bland Solution.

c) Linearity and Range

Linearity of RP–HPLC method reported was validated by the analysis of five concentrations between 50% and 150% target concentration of both Vildagliptin (50–150 µg/mL) and Dapagliflozin (5–15 µg/mL). Each one was run in triplicate, and mean peak area and %RSD were computed. The two drugs' calibration plots were a linear perfect correlation with R^2 values of 0.99999 for Vildagliptin and 0.99996 for Dapagliflozin, which were far superior to the NLT specified 0.98 acceptance limits. %RSD of area at every level was less than 2.0%, validating the precision of the method. Linear regression equations were:

Vildagliptin: Y = 755665.94 X – 227373.80

Dapagliflozin: Y = 1849290.56 X – 52221.20

The approach exhibited linear response across the provided concentration range for both Dapagliflozin and Vildagliptin and all the linearity validation requirements were fulfilled. These findings verify that the technique is linear over the range given for both analytes.

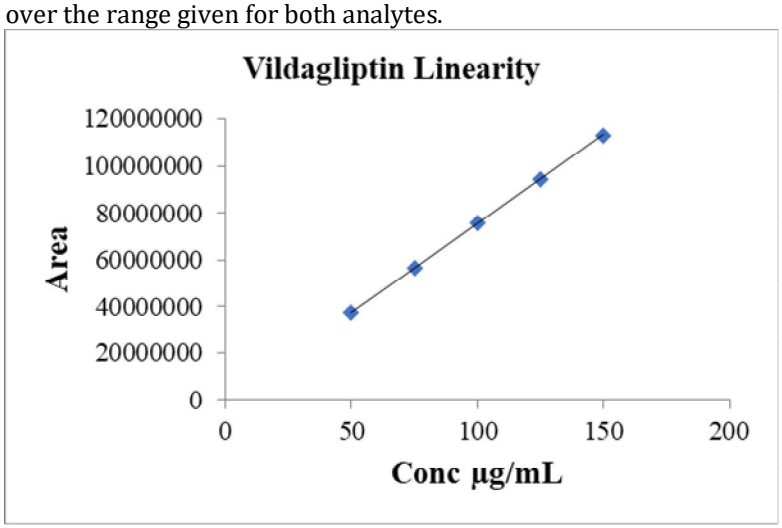


Fig. No. 8: Linearity Cure of Vildagliptin

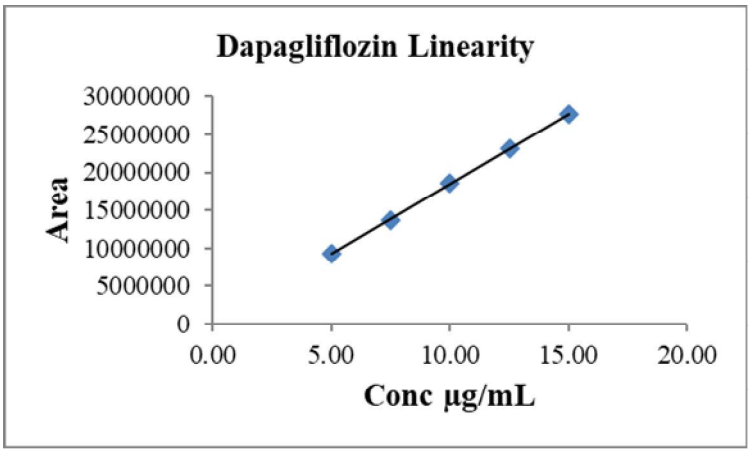


Fig. No. 9: Calibration curve of Dapagliflozin

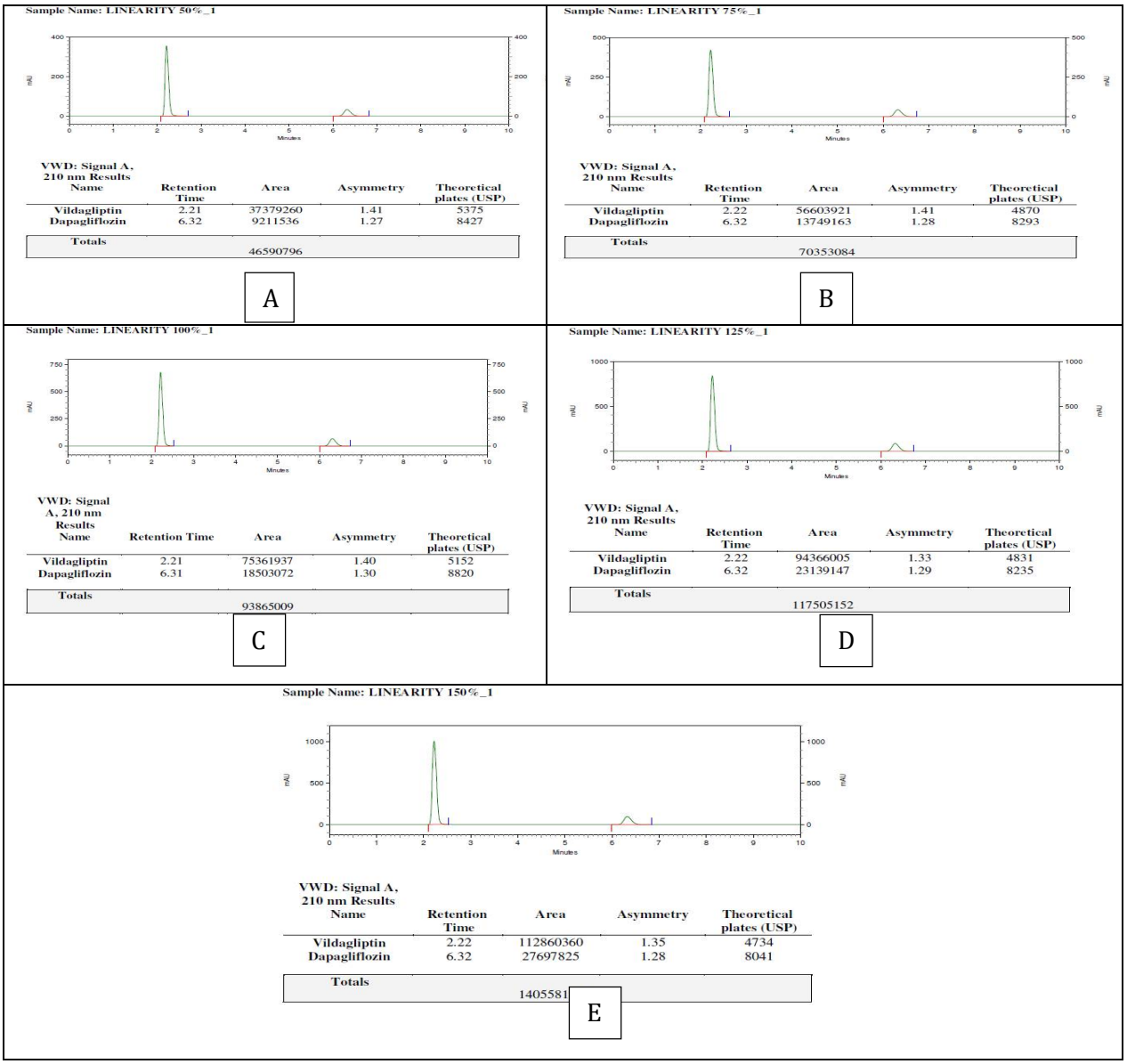


Fig. No. 10: Typical chromatograms at linearity levels 50%, 75%, 100%, 125%, and 150%

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

Sensitivity of the RP-HPLC method developed was confirmed by calculating the detection and quantification limits (LOD and LOQ) based on residual standard deviation (σ) and slope of the calibration curve. For Vildagliptin, LOD was 0.440 µg/mL and LOQ was 1.333 µg/mL were determined by the method. For Dapagliflozin, LOD was 0.116 µg/mL and LOQ was 0.350 µg/mL. These findings show that the procedure is very sensitive and enables one to make a valid detection and quantitative determination of both Vildagliptin and Dapagliflozin even at ultra-low concentration levels.

e) Accuracy (Recovery):

Precise and accurate results of RP–HPLC were established through recovery studies by spiking known standards of Vildagliptin and Dapagliflozin in the sample matrix and determination.

The total recovery of Vildagliptin was 99.38% with % RSD of 0.748, and for Dapagliflozin was 99.37% with a %RSD of 0.695. It indicates the method is very good in precision and accuracy and well within the recovery range of 98–102%.

Table No. 3: Result and statistical data of Accuracy of Vildagliptin and Dapagliflozin

Parameter	Vildagliptin		Dapagliflozin	
	Amount Added (µg/mL)	Mean % Recovery	Amount Added (µg/mL)	Mean % Recovery
50% Level	50.20	98.99	5.04	99.06
	50.10		5.20	
	50.20		5.28	
100% Level	100.10	99.30	10.33	99.59
	100.20		10.16	
	100.00		10.08	
150% Level	150.00	99.86	15.12	99.45
	150.20		15.04	
	150.10		15.20	

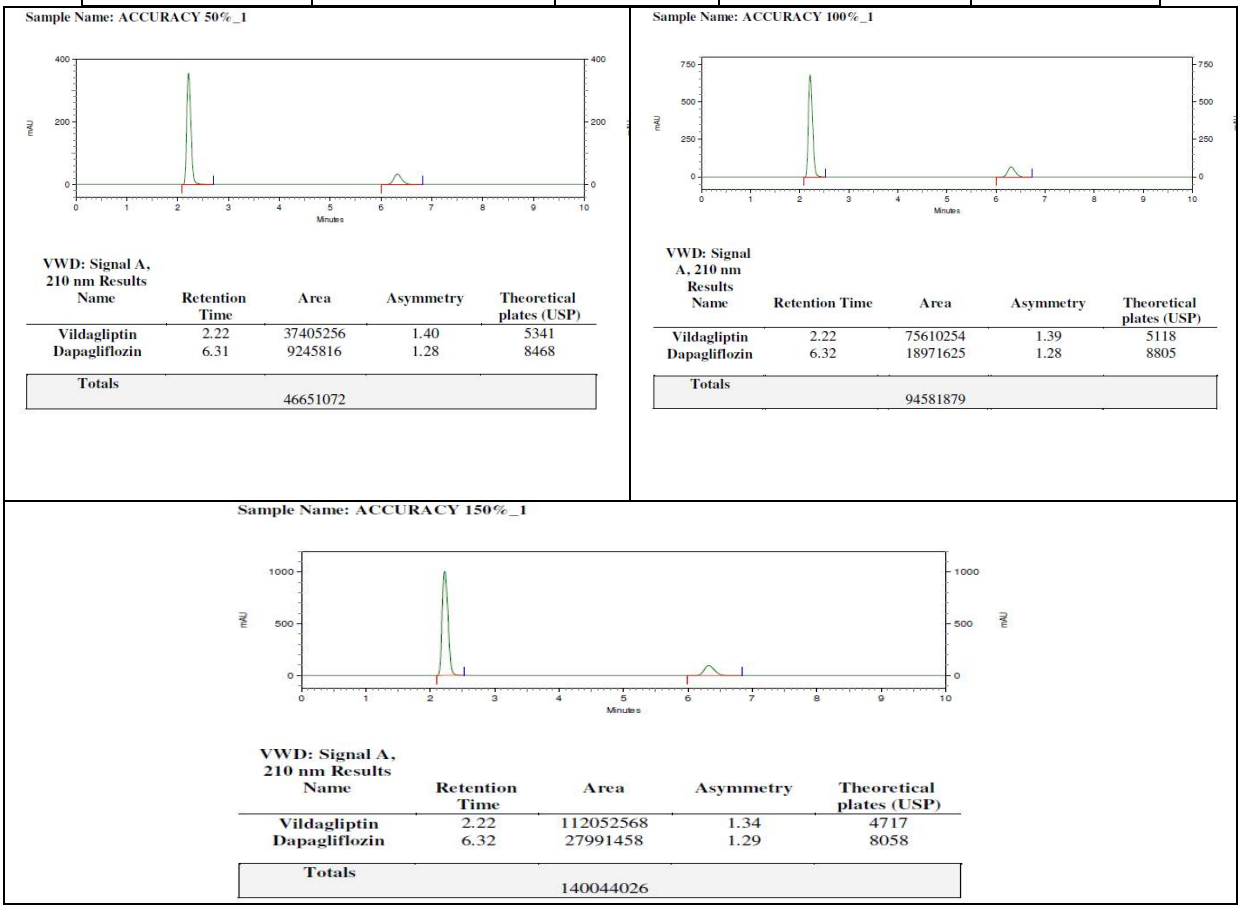


Fig. No. 11: Typical chromatograms at linearity levels 50%, 100%, 150%

f) Precision

The accuracy of the RP–HPLC method described was validated by estimating six replicate test samples with similar conditions (repeatability) and on another day by a different analyst (intermediate precision). The % assay of each sample of all six samples ranged between 90–110% range of acceptability, while %RSD of precision, intermediate precision, and total (12 samples) ranged in the tolerance limit of NMT 2.0%. These results demonstrate the method to be precise and repeatable for the co-estimation of Dapagliflozin and Vildagliptin.

Table No. 4: Result for Vildagliptin of Intra- day and Inter- Day Precision of test sample assay

Precision Type	Mean (% Assay)	Standard Deviation (SD)	%RSD
Repeatability (Intra-day)	99.17	1.1808	1.191
Intermediate Precision	98.65	1.0783	1.093
Overall Precision	98.912	1.1112	1.123

Table No. 5: Result for Dapagliflozin of Intra- day and Inter- Day Precision of test sample assay

Precision Type	Mean (% Assay)	Standard Deviation (SD)	%RSD
Repeatability (Intra-day)	99.19	1.5455	1.558
Intermediate Precision	99.23	1.446	1.457
Overall Precision	99.21	1.427	1.439

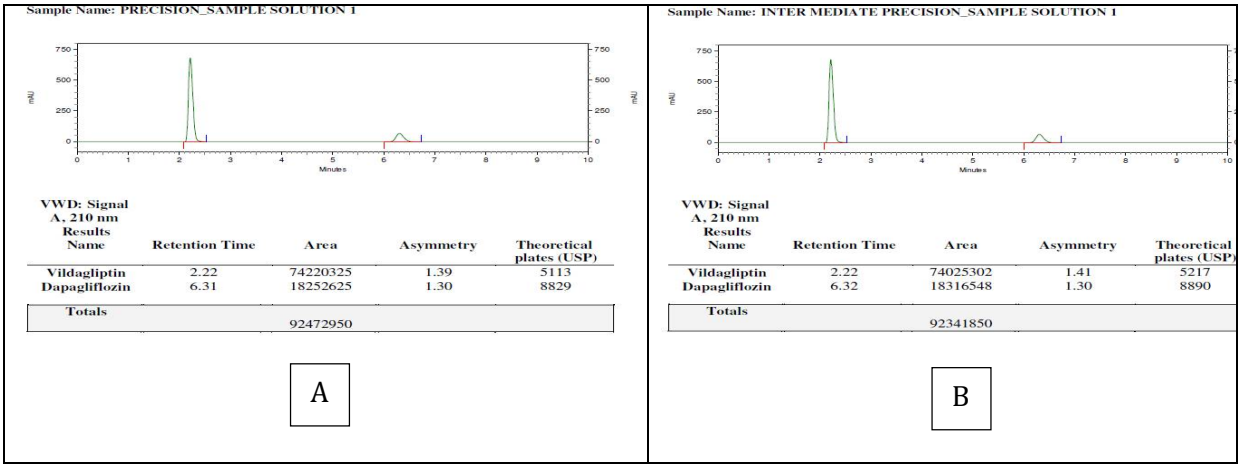


Fig. No. 12: A) Typical chromatogram of Repeatability precision (Sample 1).
B) Typical chromatogram of Inter-day precision (Sample 1).

g) Robustness

The stability of the RP–HPLC method attained was tested under various conditions of wavelength (±3 nm), flow rate (± 10%), and column oven temperature (±2°C) by introducing a small deliberate change with no retention time (R.T.), peak area, asymmetry, or theoretical plates alteration for Vildagliptin and Dapagliflozin. System suitability parameters were at acceptable levels, indicating that the method is reliable and stable when subjected to minimal small deliberate alterations.

Table No. 6: Robustness Data for Vildagliptin

Change in Parameter	R.T.	Standard Area	Asymmetry	Theoretical Plates
Wavelength +3 nm (213 nm)	2.23	77632025	1.38	4681
Wavelength -3 nm (207 nm)	2.22	68251365	1.39	4862
Flow Rate +10% (1.1 mL/min)	2.04	69815628	1.42	4389
Flow Rate -10% (0.9 mL/min)	2.46	81042058	1.40	4725
Column Temp +2°C (42°C)	2.23	75325691	1.37	4993
Column Temp -2°C (38°C)	2.22	75520145	1.39	5246

Table No.7: Robustness Data for Dapagliflozin

Change in Parameter	R.T.	Standard Area	Asymmetry	Theoretical Plates
Wavelength +3 nm (213 nm)	6.32	16308093	1.28	7912
Wavelength -3 nm (207 nm)	6.33	15956930	1.29	7847
Flow Rate +10% (1.1 mL/min)	5.78	16923562	1.30	6652
Flow Rate -10% (0.9 mL/min)	7.07	19248136	1.28	8464
Column Temp +2°C (42°C)	6.32	18202513	1.31	8996
Column Temp -2°C (38°C)	6.32	18169036	1.28	8625

CONCLUSION

The current study readily developed a low-cost, precise, and reproducible RP-HPLC method for the determination of Vildagliptin and Dapagliflozin in combination as bulk drugs and drug formulations. The method used acetonitrile and 0.1% trifluoroacetic acid in water in the ratio of 50:50 v/v as mobile phase and detected at 210 nm through a UV detector. The method developed was of high resolution and cleared the critical validation parameters—like system suitability, specificity, linearity, accuracy, precision, and robustness—according to ICH guidelines. Validation results were seen within acceptable ranges, where correlation coefficients (R^2) were close to 1, percent recovery between 98-102%, and relative standard deviation (%RSD) less than 2.0%. These findings are a proof that the method is valid and reliable for quality control analysis on a regular basis. In addition, the method was efficiently applied to analyze commercially available products such as Jalra-DP tablets and provided correct assay value for the active ingredients. Stability and specificity studies also further evidenced the robustness and reliability of the method and its suitability to be employed in routine pharmaceutical quality control laboratories.

Conflict of Interest:

No potential disputes of interest have been identified by the author.

Funding Source:

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Ethical Statement:

There was no human subjects or animals utilized in this study. Standard laboratory protocols and regulatory requirements were followed in the execution of all analytical tests. The building and confirmation of an analytical technique for a medicinal substance was the only goal of the investigation. For this kind of investigation, ethical approval was not required.

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