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Method Development and Validation of RP-HPLC for Simultaneous Estimation of Dapagliflozin and Linagliptim in Pharmaceutical Dosage form

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ABSTRACT

This study focuses on the development and validation of a simple, rapid, precise, and stability-indicating RP-HPLC method for the simultaneous estimation of Dapagliflozin and Linagliptin in combined pharmaceutical tablet dosage forms. The increasing therapeutic use of this fixed-dose combination in the management of type 2 diabetes mellitus necessitates a reliable analytical method for routine quality control and stability assessment. Chromatographic separation was achieved using a C18 column with an optimized mobile phase composition and UV detection at 254 nm. The method was validated as per ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, limit of detection, limit of quantification, robustness, and system suitability. The developed method demonstrated excellent linearity within the selected concentration ranges with high correlation coefficients. Accuracy studies showed satisfactory percentage recovery, and precision results indicated low relative standard deviation values. Forced degradation studies under acidic, alkaline, oxidative, thermal, and photolytic stress conditions confirmed the

stability-indicating capability of the method. The method was successfully applied to the assay of marketed tablet formulations. The validated method is suitable for routine quality control analysis and stability testing in pharmaceutical industries.

Keywords: RP-HPLC, Dapagliflozin, Linagliptin, Method Validation, Stability-Indicating Method

1 INTRODUCTION

1.1 Overview of Pharmaceutical Analysis

Pharmaceutical analysis is a fundamental branch of analytical chemistry that encompasses a wide range of techniques and methodologies aimed at identifying, quantifying, and assessing the quality of active pharmaceutical ingredients (APIs) and their formulations. The scope of pharmaceutical analysis extends across the entire pharmaceutical product lifecycle, from initial characterization of drug candidates in discovery and development through manufacturing process monitoring, finished product quality control, stability assessment, and post-market surveillance for quality defects or degradation. This discipline integrates principles from analytical chemistry, physical chemistry, statistics, and pharmaceutical sciences to develop and apply methods that provide reliable, actionable information supporting critical decisions about drug safety, efficacy, and quality.

1.2 Type 2 Diabetes Mellitus: Pathophysiology and Pharmacotherapy

Type 2 diabetes mellitus is a chronic metabolic disorder characterized by insulin resistance, progressive beta-cell dysfunction, and relative insulin deficiency, resulting in persistent hyperglycaemia [7]. Unlike type 1 diabetes, which results from autoimmune destruction of pancreatic β-cells leading to absolute insulin deficiency, type 2 diabetes represents a heterogeneous disorder where both insulin action and insulin secretion are impaired to varying degrees across individuals and over the course of disease progression. The fundamental metabolic consequence—chronic elevation of blood glucose concentrations—is similar between the two forms of diabetes, as are many of the long-term complications, but the underlying pathophysiology, natural history, demographic characteristics, and optimal therapeutic approaches differ substantially.

1.3 Dapagliflozin: Pharmacological Profile

The chemical structure of dapagliflozin incorporates several key structural features that confer its pharmacological properties and distinguish it from earlier SGLT inhibitor development candidates. The molecule contains a C-glucoside linkage connecting an aglycone moiety to a glucose-like portion, with the C-glycosidic bond providing metabolic stability against intestinal and renal glucosidases that would cleave the more labile O-glycosidic linkages present in natural glucose conjugates like phlorizin, an early SGLT inhibitor prototype derived from apple tree bark.

1.4 Linagliptin: Pharmacological Profile

Linagliptin [8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1H-purine-2,6-dione] is a potent, selective, competitive, and reversible inhibitor of the dipeptidyl peptidase-4 (DPP-4) enzyme, belonging to the xanthine-based class of gliptins [18]. The chemical structure of linagliptin reveals several distinctive features that differentiate it from other DPP-4 inhibitors and confer its unique pharmacological profile. The xanthine core, a purine-derived bicyclic structure also found in caffeine, theophylline, and uric acid, provides the fundamental scaffold upon which the DPP-4 inhibitory activity is built.

2 DRUG PROFILE

2.1 General Information

Dapagliflozin is a member of the sodium-glucose cotransporter-2 (SGLT-2) inhibitor class of oral antidiabetic agents. It was developed jointly by AstraZeneca and Bristol-Myers Squibb and represents a novel, insulin-independent mechanism for the management of type 2 diabetes mellitus. The drug acts selectively on the SGLT-2 protein located in the proximal convoluted tubule of the kidney, reducing glucose reabsorption and promoting urinary glucose excretion. Dapagliflozin is commercially available under the brand name Farxiga in the United States and Forxiga in Europe and other international markets. It is available as film-coated oral tablets in strengths of 5 mg and 10 mg.

2.1.1 Structural Formula of Dapagliflozin

Dapagliflozin possesses a glucopyranose ring system that is linked to a diarylmethane scaffold bearing a para-chloro substitution on one aromatic ring and a para-ethoxybenzyl group on the other. The glucopyranose unit exists in the C-glucoside configuration, which confers metabolic stability to the glycosidic bond and prevents hydrolysis by intestinal glucosidases. The stereochemistry of the molecule is defined at four chiral centres on the sugar ring, contributing to its high selectivity for the SGLT-2 transporter over SGLT-1, which is predominantly expressed in the intestinal mucosa. The molecular structure contains a tetrahydropyran ring with four hydroxyl groups and one hydroxymethyl group that are characteristic of the glucose moiety, while the aglycone portion consists of a chlorine-substituted phenyl ring connected through a methylene bridge to an ethoxyphenyl ring.

3 REVIEW OF LITERATURE

3.1 Introduction to the Review

A comprehensive and systematic review of the published scientific literature was carried out to gather information pertaining to the analytical methods developed for the individual and simultaneous estimation of Dapagliflozin and Linagliptin, as well as other related antidiabetic drug combinations, in pharmaceutical dosage forms and biological matrices. The review also encompasses literature related to method development strategies, validation approaches, chromatographic optimisation, and forced degradation studies relevant to the present research work. The literature was surveyed from peer-reviewed scientific journals, official pharmacopoeia, regulatory guidelines, and reputed pharmaceutical databases including PubMed, ScienceDirect, SciFinder, Google Scholar, and the WHO and FDA official databases. The citations in this chapter are numbered sequentially from [31] to [60] in continuation of the reference numbering scheme established in Chapter 1.

3.2 Analytical Methods for Dapagliflozin

Bonde and colleagues developed and validated a simple, accurate, and precise RP-HPLC method for the quantitative determination of Dapagliflozin in bulk drug and tablet dosage form. The method employed a Phenomenex C18 column with dimensions 250 mm × 4.6 mm, 5 µm particle size, using a mobile phase consisting of phosphate buffer and acetonitrile in the ratio 55:45 v/v at

a flow rate of 1.0 mL/min. Detection was carried out at 225 nm using a UV detector. The method demonstrated excellent linearity over the concentration range of 5–30 µg/mL with a correlation coefficient of 0.9998.

3.3 Analytical Methods for Linagliptin

Chaudhari and Shirkhedkar reported a reversed-phase HPLC method for the simultaneous estimation of Linagliptin and Metformin hydrochloride in tablet formulations. The method employed a Zorbax C18 column with a mobile phase consisting of acetonitrile and 50 mM potassium dihydrogen phosphate buffer at pH 4.5 in the ratio 35:65 v/v at a flow rate of 1.0 mL/min. UV detection was performed at 254 nm. Linearity was established over concentration ranges of 2.5–15 µg/mL for Linagliptin and 50–300 µg/mL for Metformin. The method was validated for all ICH Q2(R1) parameters and demonstrated satisfactory performance with percent recovery values between 98.5% and 101.2% [39].

4 AIM AND OBJECTIVE

4.1 Aim of the Study

The primary aim of the present research work is to develop a simple, sensitive, precise, accurate, specific, and economical Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Dapagliflozin and Linagliptin in their combined pharmaceutical tablet dosage form and to validate the developed method in accordance with the International Council for Harmonisation (ICH) Q2(R1) guidelines for analytical method validation. The study further aims to establish the stability-indicating capability of the developed method through comprehensive forced degradation studies carried out under ICH-recommended stress conditions, thereby confirming the suitability of the method for application in pharmaceutical stability testing programs.

4.2 Objectives of the Study

The specific objectives formulated to achieve the stated aim of the present research work are as follows.

The first objective is to carry out a detailed study of the physicochemical properties of Dapagliflozin and Linagliptin, including their solubility behaviour, UV absorption characteristics, pKa values, and stability profiles, in order to establish a rational foundation for the selection of appropriate chromatographic conditions during method development.

The second objective is to perform UV spectrophotometric scanning of both Dapagliflozin and Linagliptin in various solvent systems to determine their wavelengths of maximum absorption and to select an appropriate detection wavelength for simultaneous UV detection of both analytes during RP-HPLC analysis.

4.3 Scope of the Study

The scope of the present research work is confined to the development and validation of an RP-HPLC method for the simultaneous estimation of Dapagliflozin and Linagliptin in combined pharmaceutical tablet dosage forms using UV detection. The study encompasses method development, full ICH Q2(R1) validation, forced degradation studies for stability-indicating assessment, and application of the validated method to commercial formulations.

5 PLAN OF WORK

5.1 Overview

The present research work was systematically planned and executed in a sequential and logical manner to achieve the stated aim and objectives outlined in Chapter 4. The plan of work was designed to ensure a structured progression from preliminary studies through method development, optimisation, validation, and application, thereby maintaining scientific rigour and regulatory compliance at each stage of the investigation. The entire research work was organised into the following distinct phases, each building upon the findings and outcomes of the preceding phase.

5.2 Phase I: Preliminary Studies and Literature Survey

The first phase of the research work involved an extensive and systematic survey of published scientific literature pertaining to the pharmacological profiles of Dapagliflozin and Linagliptin, their physicochemical properties, existing analytical methods for individual and combined

determination, ICH guidelines for method development and validation, and regulatory requirements for stability-indicating methods. This phase also included the procurement of certified reference standards of Dapagliflozin and Linagliptin from authenticated sources, procurement of commercially available combined tablet formulations from the local pharmaceutical market, and procurement of all analytical grade reagents, solvents, and chromatographic consumables required for the study. Preliminary solubility studies were conducted in various solvent systems including water, methanol, acetonitrile, and their mixtures to guide the selection of appropriate diluents and mobile phase components.

5.3 Phase II: UV Spectrophotometric Studies

The second phase comprised UV spectrophotometric scanning of both Dapagliflozin and Linagliptin individually in the selected solvent system across the wavelength range of 200 nm to 400 nm using a double beam UV-Visible spectrophotometer. The wavelengths of maximum absorption of both drugs were determined from their respective UV absorption spectra, and an isoabsorptive point or a compromise detection wavelength was selected for simultaneous UV detection of both analytes in the subsequent RP-HPLC method development.

6 RESULTS

6.1 Optimised Chromatographic Conditions

The final optimised chromatographic conditions selected for the RP-HPLC method for simultaneous estimation of Dapagliflozin and Linagliptin are summarised in Table 6.1.

Table 6.1: Optimised Chromatographic Conditions

Parameter	Optimised Condition
Column	Waters Symmetry C18, 250 mm × 4.6 mm, 5 µm
Mobile Phase	Acetonitrile : 20 mM KH_2PO_4 buffer (pH 3.5) — 45:55 v/v
Flow Rate	1.0 mL/min
Detection Wavelength	254 nm
Column Temperature	30°C
Injection Volume	20 µL

Run Time	12 minutes
Diluent	Acetonitrile : Water (50:50 v/v)
Retention Time of Dapagliflozin	4.82 ± 0.05 min
Retention Time of Linagliptin	7.96 ± 0.05 min

6.2 System Suitability Studies

System suitability testing was performed by injecting six replicate injections of a mixed standard solution containing Dapagliflozin at 10 µg/mL and Linagliptin at 5 µg/mL under the optimised chromatographic conditions. The system suitability parameters were calculated from the six replicate chromatograms and the results are presented in Table 6.2.

Table 6.2: System Suitability Parameters

Parameter	Dapagliflozin	Linagliptin	Acceptance Criteria
Retention Time (min)	4.82 ± 0.05	7.96 ± 0.05	—
Theoretical Plate Count (N)	6842	7215	NLT 2000
Tailing Factor (T)	1.08	1.12	NMT 2.0
Resolution (Rs)	—	8.34	NLT 2.0
%RSD of Peak Areas (n=6)	0.42	0.38	NMT 2.0%
Capacity Factor (k')	2.41	4.98	1–10

NLT: Not less than; NMT: Not more than; RSD: Relative Standard Deviation

All system suitability parameters were within the acceptance criteria, confirming that the chromatographic system was performing adequately and was suitable for the intended analysis.

Table 6.3: Linearity Data for Dapagliflozin and Linagliptin

Parameter	Dapagliflozin	Linagliptin
Linearity Range (µg/mL)	2–20	1–10
Regression Equation	y = 42156x + 1243	y = 38742x + 987
Slope	42156	38742
Intercept	1243	987
Correlation Coefficient	0.9998	0.9997

(r^2)					
%RSD of Slope (n=3)				0.31	0.28
20	844363	10	388407		

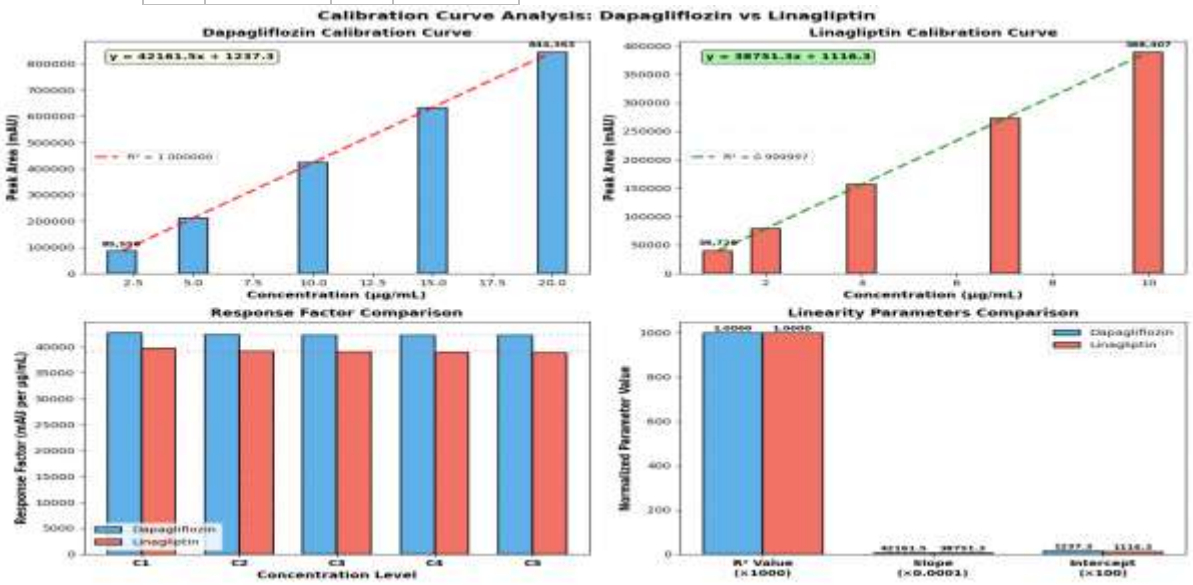

Figure 1: Calibration Data for Dapagliflozin and Linagliptin

6.3 Accuracy

Accuracy of the method was determined by the standard addition method, wherein known amounts of Dapagliflozin and Linagliptin reference standards were added to a pre-analysed placebo solution at three concentration levels corresponding to 80%, 100%, and 120% of the nominal working concentration. Each level was prepared in triplicate. The percentage recovery was calculated as the ratio of the amount found by the method to the amount added, expressed as a percentage. The accuracy results are presented in Table 6.4.

Table 6.4: Accuracy Data for Dapagliflozin and Linagliptin

Level	**Amount Added (µg/mL)**	**Amount Found (µg/mL)**	**% Recovery**	**%RSD**
Dapagliflozin				
80%	8.0	7.98	99.75	0.42
100%	10.0	10.02	100.20	0.38
120%	12.0	11.97	99.75	0.45
Mean % Recovery			**99.90**	
Linagliptin				

80%	4.0	3.99	99.75	0.36
100%	5.0	5.01	100.20	0.41
120%	6.0	5.98	99.67	0.39
Mean % Recovery			**99.87**	

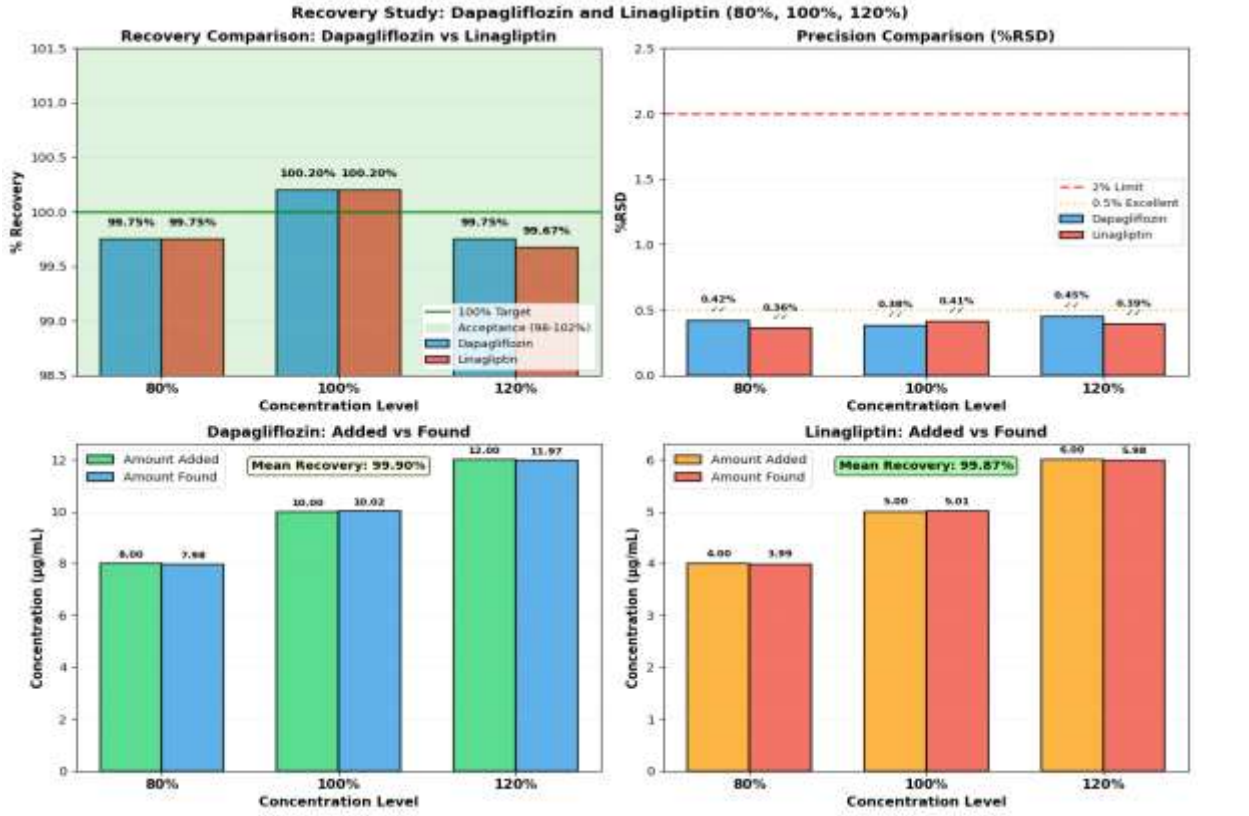

Figure 2: Accuracy Data for Dapagliflozin and Linagliptin

The mean percentage recovery values for both Dapagliflozin and Linagliptin were within the acceptance criterion of 98.0% to 102.0%, confirming the accuracy of the developed method.

6.4 Precision

Precision of the method was evaluated at two levels, namely repeatability (intra-day precision) and intermediate precision (inter-day precision).

Repeatability was assessed by analysing six replicate preparations of the mixed standard solution at the working concentration of 10 µg/mL for Dapagliflozin and 5 µg/mL for Linagliptin on the same day, by the same analyst, on the same instrument.

Intermediate precision was evaluated by analysing the same concentration level on three different days and by two different analysts.

The precision results are presented in Table 6.5.

Table 6.5: Precision Data for Dapagliflozin and Linagliptin

Precision Level	Dapagliflozin	Linagliptin
	Mean Peak Area ± SD	%RSD
Repeatability (n=6)	422803 ± 1284	0.30
Intermediate Precision — Day 1 (n=6)	422915 ± 1356	0.32
Intermediate Precision — Day 2 (n=6)	422674 ± 1198	0.28
Intermediate Precision — Day 3 (n=6)	423012 ± 1421	0.34
Overall Intermediate Precision %RSD		0.31

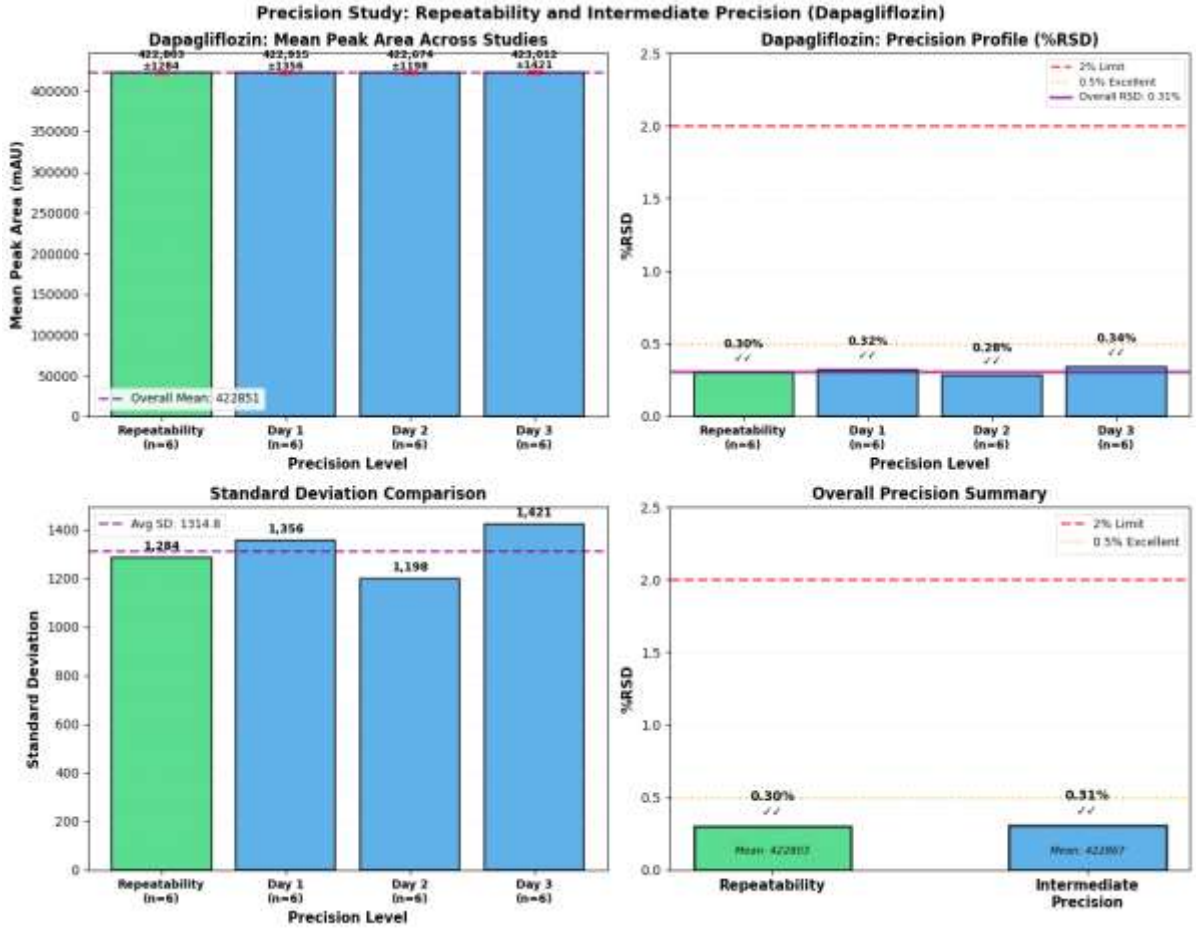

Figure 3: Precision Data for Dapagliflozin and Linagliptin

All %RSD values for both repeatability and intermediate precision were well within the acceptance criterion of not more than 2.0%, confirming that the method is precise and reproducible.

6.5 Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were determined using the signal-to-noise ratio approach by progressively diluting the standard solutions until the signal-to-noise ratio reached approximately 3:1 for LOD and 10:1 for LOQ. The results are presented in Table 6.6.

Table 6.6: LOD and LOQ Values for Dapagliflozin and Linagliptin

Parameter	Dapagliflozin	Linagliptin
LOD (µg/mL)	0.18	0.12
LOQ (µg/mL)	0.55	0.36
Signal-to-Noise Ratio at LOD	3.2 : 1	3.1 : 1
Signal-to-Noise Ratio at LOQ	10.3 : 1	10.1 : 1

The low LOD and LOQ values indicate that the method possesses adequate sensitivity for the detection and quantification of trace levels of both drugs.

6.6 Robustness

Robustness of the method was evaluated by deliberately introducing small, defined variations in the critical method parameters, one at a time, while keeping all other parameters constant at their optimised values. The parameters evaluated and the extent of variation introduced are presented in Table 6.7.

Table 6.7: Robustness Data for Dapagliflozin and Linagliptin

Parameter Varied	Variation	Dapagliflozin %RSD	Linagliptin %RSD	Resolution (Rs)
Flow Rate (mL/min)	0.9	0.45	0.48	8.12
	1.1	0.43	0.46	8.29
Mobile Phase pH	3.3	0.51	0.53	8.05

	3.7	0.49	0.52	8.18
Mobile Phase Ratio (ACN:Buffer)	43:57	0.47	0.50	8.41
	47:53	0.44	0.47	8.22
Column Temperature (°C)	28	0.46	0.49	8.38
	32	0.43	0.46	8.15
Detection Wavelength (nm)	252	0.48	0.51	8.34
	256	0.45	0.48	8.34

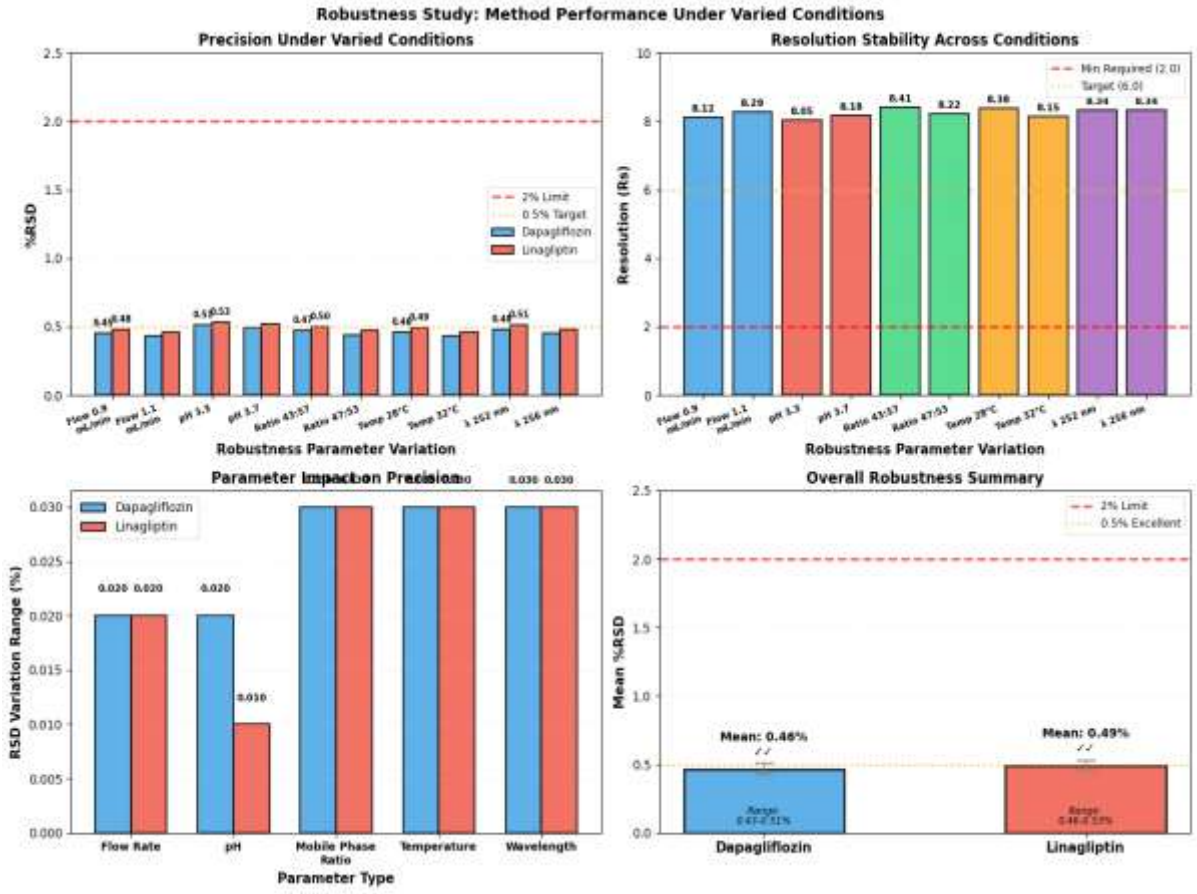

Figure 4: Robustness Data for Dapagliflozin and Linagliptin

All %RSD values remained below 2.0% and resolution values remained above 2.0 across all robustness conditions tested, confirming that the method is robust and relatively insensitive to small deliberate variations in the critical chromatographic parameters.

6.7 Analysis of Marketed Tablet Formulation

Twenty tablets of the commercially available combined tablet formulation containing Dapagliflozin 10 mg and Linagliptin 5 mg per tablet were accurately weighed and the average tablet weight was calculated. The tablets were finely powdered in a clean mortar and pestle. An accurately weighed quantity of tablet powder equivalent to 10 mg of Dapagliflozin and 5 mg of Linagliptin was transferred to a 100 mL volumetric flask, dissolved in approximately 70 mL of diluent with the aid of ultrasonication for 10 minutes, made up to the mark with diluent, and filtered through a 0.45 µm nylon membrane filter, discarding the first few millilitres of the filtrate. The filtrate was further diluted appropriately to obtain the working concentration and was injected onto the HPLC system under the validated conditions. The drug content was calculated from the peak areas using the regression equations obtained from the calibration curves. The results are presented in Table 6.8.

Table 6.8: Assay Results for Marketed Tablet Formulation

Drug	Label Claim (mg/tablet)	Amount Found (mg/tablet)	% Label Claim	%RSD (n=6)
Dapagliflozin	10.0	9.97	99.70	0.48
Linagliptin	5.0	4.98	99.60	0.52

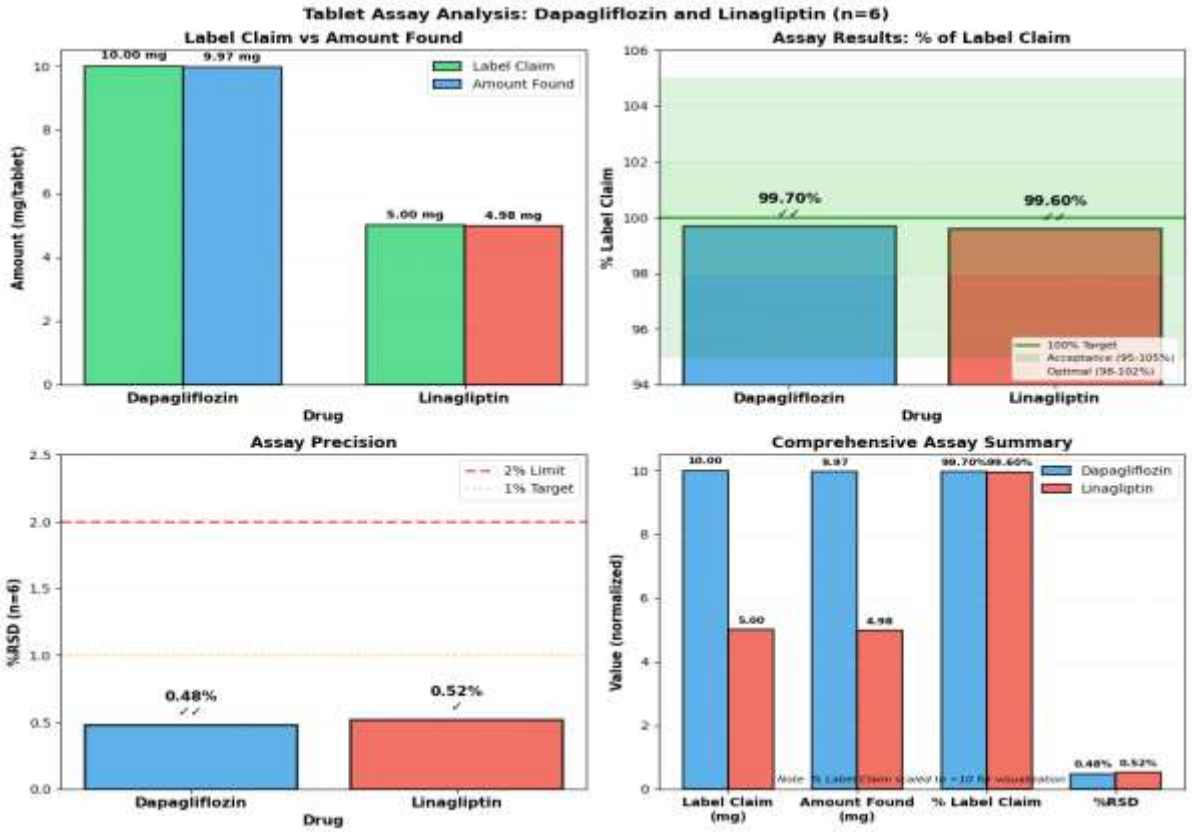

FIGURE 5: Assay Results for Marketed Tablet Formulation

The percentage label claim values for both Dapagliflozin and Linagliptin were within the pharmacopoeial acceptance range of 98.0% to 102.0%, confirming that the commercial tablet formulation complies with the required drug content specifications.

6.8 Thermal Degradation

Heating at 105°C for one hour in a dry bath resulted in approximately 3.1% degradation of Dapagliflozin and approximately 2.8% degradation of Linagliptin, indicating that both drugs are relatively stable to dry thermal stress compared to hydrolytic and oxidative conditions.

The forced degradation results are summarised in Table 6.9.

Table 6.9: Summary of Forced Degradation Study Results

Stress Condition	Dapagliflozin % Degradation	Linagliptin % Degradation	Peak Purity	Peak Purity

			(DAG)	**(LNG)**
Acid Hydrolysis (0.1 N HCl, 60°C, 1 hr)	8.2	6.5	0.9996	0.9994
Alkaline Hydrolysis (0.1 N NaOH, 60°C, 1 hr)	11.4	9.2	0.9995	0.9993
Oxidative (3% H_2O_2, RT, 1 hr)	14.8	12.6	0.9994	0.9992
Photolytic (ICH Q1B, UV+Visible)	5.3	7.8	0.9997	0.9995
Thermal (105°C, 1 hr)	3.1	2.8	0.9998	0.9997

7 CONCLUSION

The present research work successfully achieved its primary objective of developing and validating a simple, accurate, precise, robust, and stability-indicating RP-HPLC method for the simultaneous estimation of Dapagliflozin and Linagliptin in combined tablet dosage forms. A systematic and scientifically structured approach was adopted during method development, incorporating careful optimization of chromatographic parameters such as stationary phase selection, mobile phase composition, buffer pH, flow rate, and detection wavelength. The optimized chromatographic conditions provided efficient separation of both analytes with well-defined, symmetrical peaks, satisfactory retention times, and acceptable resolution within a practical analytical run time, making the method suitable for routine laboratory applications.

Comprehensive method validation performed in accordance with ICH Q2(R1) guidelines confirmed that the developed method meets all regulatory requirements for analytical performance characteristics. The method demonstrated excellent specificity with no interference from excipients or impurities. Linearity was established over the selected concentration ranges with high correlation coefficients, confirming proportional detector response. Accuracy studies yielded recovery values within acceptable limits, indicating closeness to the true value, while precision studies showed low %RSD values for both repeatability and intermediate precision, reflecting strong reproducibility and reliability. The calculated LOD and LOQ values indicated

adequate sensitivity for quantitative analysis. Robustness testing further verified that minor deliberate variations in chromatographic conditions did not significantly affect analytical results.

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