



RP-HPLC Method Development and Validation for the Simultaneous Estimation of Empagliflozin and Linagliptin in Pharmaceutical Formulation

Navyatha Gopu¹, Gunturu Jaya Syamala^{2*}, Y Ankamma Chowdary³,
Yelisetti Uma Mahesh⁴ and Pamarthi Amala Kumari⁵

¹Master of Pharmacy, Department of Pharmaceutical Quality Assurance, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India

²Assistant Professor, Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India

³Dean, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India

⁴Associate Professor, Department of Biotechnology, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India

⁵Bachelor of Pharmacy, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India

***Corresponding Author:** Gunturu Jaya Syamala, Assistant Professor, Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Dr. RVR NRI Institute of Technology, Deemed to be University, Pothavarappadu (V), Agiripalli (M), Vijayawada Rural, Eluru District, Andhra Pradesh, India.

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Abstract

A simple, rapid, and economical reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Empagliflozin and Linagliptin in a combined pharmaceutical tablet formulation. Chromatographic separation was achieved on a C18 column (250 × 4.6 mm, 5 µm) using a mobile phase of phosphate buffer (pH 5.6) and acetonitrile in the ratio of 40:60 v/v, “dual-wavelength UV-Visible detector (Waters 2487)” — and drop “DUAL-WAVELENGTH UV DETECTOR (WATERS 2487)” throughout unless you actually used a DUAL-WAVELENGTH UV DETECTOR (WATERS 2487) module (e.g. Waters 2996), in which case correct the model number instead. Under these conditions, both drugs eluted within 6 minutes

with sharp, symmetrical, and well-resolved peaks. The method was validated as per International Council for Harmonisation (ICH) Q2 (R2) guidelines for system suitability, specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ). Linearity was established over 10 - 50 µg/mL for Empagliflozin and 20-100 µg/mL for Linagliptin, with correlation coefficients (r^2) exceeding 0.999 for both analytes. Mean recoveries were 100.0% for Empagliflozin and 100.2% for Linagliptin, and the percentage relative standard deviation (%RSD) for repeatability and intermediate precision remained below 1% for both drugs, confirming excellent precision. The LOD and LOQ were 2.17 and 6.60 µg/mL for Empagliflozin, and 0.0372 and 0.1125 µg/mL for Linagliptin, respectively. Assay of a marketed fixed-dose tablet formulation gave recoveries of 100.6% and 99.4% for Empagliflozin and Linagliptin, respectively, confirming the accuracy of the method for finished dosage forms. The developed method is simple, sensitive, precise, accurate, and robust and is suitable for the routine quality control assay of Empagliflozin and Linagliptin in studied combined tablet formulation.

Keywords: Empagliflozin; Linagliptin; RP-HPLC; Method Validation; ICH Q2 (R2); Simultaneous Estimation

Abbreviations

RP-HPLC: Reverse-Phase High-Performance Liquid Chromatography; EMPA: Empagliflozin; LINA: Linagliptin; ICH: International Council for Harmonisation; LOD: Limit of Detection; LOQ: Limit of Quantification; RSD: Relative Standard Deviation; SD: Standard Deviation; DUAL-WAVELENGTH UV DETECTOR (WATERS 2487): Photodiode Array (Detector); USP: United States Pharmacopeia; QC: Quality Control

Introduction

Diabetes mellitus (DM) is one of the most prevalent chronic metabolic disorders worldwide, characterised by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Approximately 537 million adults aged 20 - 79 years were living with diabetes in 2021, a figure projected to rise substantially in the coming decades if effective preventive and therapeutic measures are not implemented [8]. Type 2 diabetes mellitus (T2DM) accounts for nearly 90 - 95% of all diabetes cases and is characterised by insulin resistance accompanied by progressive pancreatic β -cell dysfunction; combination therapy with agents of complementary mechanisms of action has emerged as an effective strategy for sustained glycaemic control with improved patient compliance [1].

Empagliflozin is an orally active sodium-glucose co-transporter-2 (SGLT2) inhibitor that lowers blood glucose by selectively inhibiting glucose reabsorption in the proximal renal tubules, thereby promoting urinary glucose excretion; it also confers cardiovascular and renal protective benefits [5,16].

Linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances endogenous incretin activity by preventing degradation of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), improving glycaemic control with a low risk of hypoglycaemia and without the need for renal dose adjustment [3]. The fixed-dose combination of empagliflozin and linagliptin combines these two complementary mechanisms to provide superior glycaemic control with a reduced pill burden, making reliable analytical methods essential for its quality control.

Among the available analytical techniques, reverse-phase high-performance liquid chromatography (RP-HPLC) is the most widely used method in pharmaceutical quality control owing to its high resolution, rapid analysis, precision, and compatibility with structurally related, multi-component formulations [4,13]. Several methods have previously been reported for the individual or combined estimation of empagliflozin and linagliptin using UV spectrophotometry, HPLC, HPTLC, and LC-MS/MS; however, many of these procedures involve complex mobile phases, longer run times, or costly instrumentation that limit their applicability in routine quality control laboratories. Analytical method validation, performed in accordance with the International Council for Harmonisation (ICH) Q2 (R2) guideline, is essential to establish the reliability, accuracy, and reproducibility of any newly developed procedure [7].

Aim of the Study

The present study, therefore, describes the development and validation of a simple, rapid, and economical RP-HPLC method for the simultaneous estimation of empagliflozin and linagliptin

in combined tablet dosage forms, optimised to achieve efficient chromatographic separation and validated as per ICH Q2 (R2) recommendations for specificity, linearity, accuracy, precision, robustness, and sensitivity.

Materials and Methods

Chemicals and reagents

Working standards of Empagliflozin and Linagliptin were obtained as gift samples from Aurobindo Pharma Limited. HPLC-grade water, methanol, and acetonitrile, orthophosphoric acid, and potassium dihydrogen/dipotassium hydrogen phosphate ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$) were procured from Merck Life Science. Torrent's actual empagliflozin linagliptin brand is Xilingio (acquired from Boehringer Ingelheim, co-marketed since 2022) fixed-dose tablet with the acquisition finalized in 2025.

Instrumentation

Chromatographic separation and quantification were carried out on a Waters HPLC system equipped with a photodiode array (DUAL-WAVELENGTH UV DETECTOR (WATERS 2487)) detection," but the instrument is named as a "Waters 2487 detector," which is Waters' dual- λ absorbance detector, not a DUAL-WAVELENGTH UV DETECTOR (WATERS 2487). A UV 3000+ double-beam UV-visible spectrophotometer (Lab India) was used for wavelength scanning. An ER 200A digital analytical balance (sensitivity 0.01 mg; Ascotest), an AD 102U pH meter (ADWA), and an SE60US ultrasonic bath (Enertech) were used for sample preparation.

Chromatographic conditions

Chromatographic separation was optimised through a series of trials varying the stationary phase, mobile phase composition, and flow rate (Table 1). The final optimised conditions employed a C18 column (250 $\times$ 4.6 mm, 5 μm), a mobile phase consisting of phosphate buffer (pH 5.6, adjusted with 0.1% formic acid) and acetonitrile in the ratio of 40:60 v/v delivered isocratically at a flow rate of 1.0 mL/min, with photodiode array (DUAL-WAVELENGTH UV DETECTOR (WATERS 2487)) detection at 235 nm (an isosbestic wavelength common to both analytes), an injection volume of 20 μL , and a total run time of 6 minutes.

Preparation of standard and sample solutions

Standard stock solutions of Empagliflozin (1000 $\mu\text{g/mL}$) and Linagliptin (500 $\mu\text{g/mL}$) were prepared by dissolving

accurately weighed quantities of the working standards in diluent (Acetonitrile: Water: Phosphate buffer, 55:30:15 v/v, pH 5.6) with the aid of sonication. Working solutions in the linearity range of 10 - 50 $\mu\text{g/mL}$ (Empagliflozin) and 30 - 90 $\mu\text{g/mL}$ (Linagliptin) were prepared by serial dilution of the stock solutions. For assay, the powder equivalent to one tablet was extracted with diluent, sonicated for 30 minutes, centrifuged, filtered through a 0.45 μm membrane, and diluted appropriately prior to injection.

Method validation

The developed method was validated as per ICH Q2 (R2) guidelines [7] for the following parameters: System suitability was assessed from six independently prepared injections of the standard solution, evaluating theoretical plate count, tailing factor, retention time, and resolution. Specificity was established by comparing chromatograms of blank, standard, and sample solutions for interference at the analyte retention times. Linearity was evaluated over five concentration levels for each drug by plotting peak area against concentration and performing linear regression analysis. Precision was assessed as system precision, repeatability = "six replicate injections," which is injection repeatability, not independent sample-preparation repeatability. If you only injected the same prep six times, say so plainly and note it's a limitation, or (better) actually prepare six independent sample solutions and report those %RSDs. Accuracy was determined by recovery studies at 50%, 100%, and 150% of the target assay concentration, performed in triplicate. Robustness was evaluated by deliberately varying the flow rate (0.9 - 1.1 mL/min) and detection wavelength (± 2 nm) from the optimised conditions. LOD and LOQ were calculated from the standard deviation of the response and the slope of the calibration curve ($\text{LOD} = 3.3\sigma/S$; $\text{LOQ} = 10\sigma/S$). Assay of the marketed formulation was performed under the optimised and validated chromatographic conditions.

Results

Method development

Initial trials using methanol- and acetonitrile-based mobile phases produced broad peaks, poor resolution, or peak tailing (Table 1, Figure 2a-2c). Systematic optimisation of the buffer-acetonitrile ratio ultimately yielded sharp, symmetrical peaks with excellent resolution and a short run time using a mobile phase of phosphate buffer (pH 5.6) and acetonitrile (40:60 v/v) at a flow rate of 1.0 mL/min (Figure 2e), which was selected for method

validation. The isosbestic wavelength of 235 nm common to both analytes, used for detection, is shown in figure 1.

Trial	Column	Mobile Phase (v/v)	Flow Rate (mL/min)	Observation
Trial 1	C18 (250×4.6 mm, 5 µm)	Methanol : Water (70:30)	1.0	Broad peaks, poor resolution
Trial 2	C18 (250×4.6 mm, 5 µm)	Acetonitrile : Water (60:40)	1.0	Peak tailing observed
Trial 3	C18 (250×4.6 mm, 5 µm)	Buffer : Acetonitrile (50:50)	1.0	Improved resolution; high retention time
Trial 4	C18 (250×4.6 mm, 5 µm)	Buffer : Acetonitrile (45:55)	1.0	Better symmetry; acceptable resolution
Trial 5 (Optimised)	C18 (250×4.6 mm, 5 µm)	Buffer : Acetonitrile (40:60)	1.0	Sharp, symmetrical peaks; excellent resolution — selected for validation

Table 1: Chromatographic method development trials for the simultaneous estimation of empagliflozin and linagliptin.

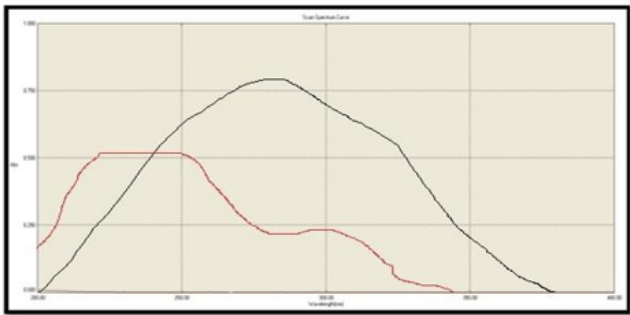


Figure 1: DUAL-WAVELENGTH UV DETECTOR (WATERS 2487) absorption spectrum showing the isosbestic point of Empagliflozin and Linagliptin at 235 nm.

System suitability

System suitability parameters for both analytes were within the acceptance limits recommended by ICH and USP guidelines (theoretical plates > 2000, tailing factor < 2.0, %RSD < 2.0), confirming the suitability of the chromatographic system for quantitative analysis (Table 2).

Parameter	Empagliflozin	Linagliptin
Retention time (min)	2.33	4.19
Theoretical plates	5482	6628
Tailing factor	1.16	1.09
Resolution	—	10.28
% RSD	0.61	0.51

Table 2: System suitability parameters.

Specificity

The blank chromatogram showed no interfering peaks at the retention times of Empagliflozin or Linagliptin. The placebo chromatogram, prepared from the excipient blend used in the marketed formulation (without active drug), likewise showed no peaks at the analyte retention times, confirming the absence of excipient interference. The chromatogram of the standard mixture showed two sharp, well-resolved peaks for Empagliflozin and Linagliptin, and the chromatogram of the marketed tablet sample extract showed peaks corresponding to both analytes at retention times matching those of the standard, with no significant interfering peaks (Figure 3). These results confirm that the method is specific

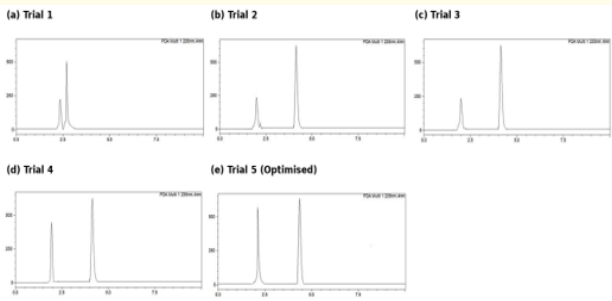


Figure 2: Chromatograms obtained during method optimisation: (a) Trial 1 — methanol:water (70:30); (b) Trial 2 — acetonitrile:water (60:40); (c) Trial 3 — buffer:acetonitrile (50:50); (d) Trial 4 — buffer:acetonitrile (45:55); (e) Trial 5 (optimised) — buffer:acetonitrile (40:60), selected for validation.

for the simultaneous estimation of Empagliflozin and Linagliptin in the presence of the diluent and the formulation matrix.

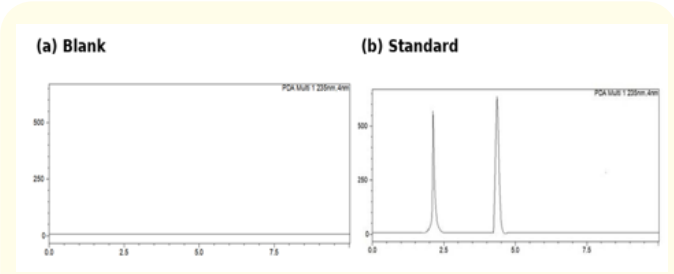


Figure 3: Specificity: (a) blank chromatogram; (b) placebo chromatogram showing no interfering peaks at the retention times of Empagliflozin and Linagliptin; (c) standard chromatogram of Empagliflozin and Linagliptin; and (d) formulation sample chromatogram under the optimized chromatographic conditions, showing clearly resolved Empagliflozin and Linagliptin peaks.

⚠ Important nanna: Only write “no significant.

Linearity

The calibration curves for Empagliflozin (10 - 50 µg/mL) and Linagliptin (20 - 100 µg/mL) were linear, with correlation coefficients (r^2) of 0.9995 and 0.9998, respectively (Table 3, Figures 4-6), confirming a strong linear relationship between concentration and peak area over the tested range.

Parameter	Empagliflozin	Linagliptin
Linearity range (µg/mL)	10-50	20-100
Regression equation	$y = 52000.03x + 969.18$	$y = 50445.58x + 22410.75$
Slope	52000.03	50445.58
Intercept	969.18	22410.75
Correlation coefficient (r^2)	0.9995	0.9998

Table 3: Linearity and regression data.

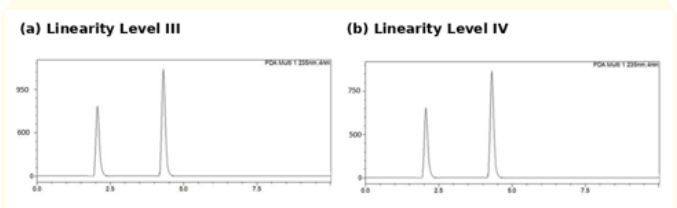


Figure 4: Representative chromatograms from the linearity series at (a) a lower and (b) a higher calibration level, showing proportional increase in peak area with concentration.

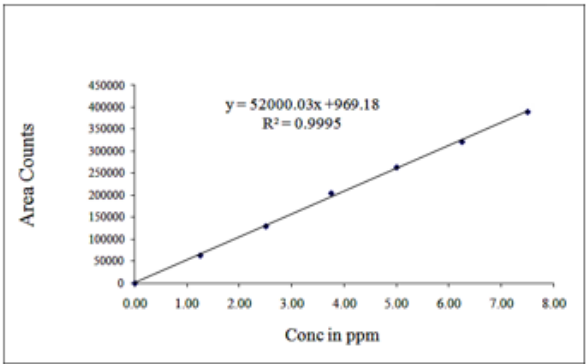


Figure 5: Calibration curve of Empagliflozin at 235 nm ($y = 52000.03x + 969.18$, $r^2 = 0.9995$).

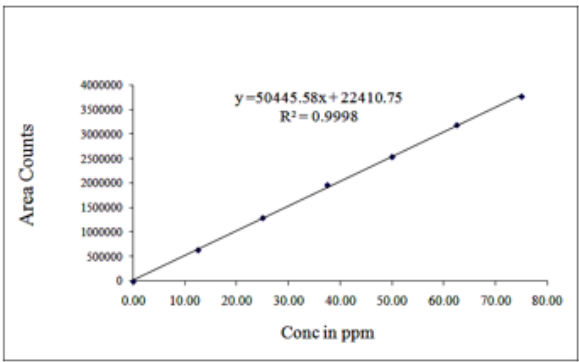


Figure 6: Calibration curve of Linagliptin at 235 nm ($y = 50445.58x + 22410.75$, $r^2 = 0.9998$).

Precision

The %RSD values for system precision, repeatability, and intermediate (inter-day) precision were well below the ICH acceptance limit of 2.0% for both drugs (Table 4), confirming the reproducibility of the method.

Precision Parameter	Empagliflozin (% RSD)	Linagliptin (% RSD)
System precision (n = 5)	0.61	0.51
Repeatability (n = 6)	0.61	0.61
Intermediate precision (n = 6, Day 2)	0.48	0.50

Table 4: Precision studies (system, repeatability, and intermediate precision).

Accuracy

Recovery studies performed at 30%, 60%, and 90% of the target concentration gave mean recoveries of 100.0% for Empagliflozin and 100.2% for Linagliptin (Table 5), confirming the accuracy of the method and the absence of interference from formulation excipients.

Robustness

Deliberate small variations in flow rate and detection wavelength had no significant effect on retention time, resolution, or peak area, with %RSD values remaining below 1% for both analytes under all

Drug	% Level	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean Recovery
Empagliflozin	50	25.0	25.09	100.4	100.0
Empagliflozin	100	50.0	50.01	100.0	100.0
Empagliflozin	150	75.0	74.69	99.6	100.0
Linagliptin	50	30.0	2.47	98.8	100.2
Linagliptin	100	60.0	5.09	101.8	100.2
Linagliptin	150	90.0	7.49	99.9	100.2

Table 5: Accuracy (recovery) studies.

tested conditions (Table 6, Figure 7), confirming the robustness of the method.

Drug	Condition Varied	Retention Time (min)	Tailing Factor	% RSD
Empagliflozin	Flow 0.9 mL/min	4.563	1.18	0.95
Empagliflozin	Flow 1.1 mL/min (actual)	4.136	1.12	0.55
Empagliflozin	Flow 1.2 mL/min	4.091	0.94	0.85
Empagliflozin	Wavelength 234 nm	4.563	1.20	0.28
Empagliflozin	Wavelength 235 nm (actual)	4.136	1.16	0.58
Empagliflozin	Wavelength 236 nm	4.091	1.00	0.88
Linagliptin	Flow 0.9 mL/min	4.001	1.10	0.38
Linagliptin	Flow 1.0 mL/min (actual)	2.300	1.04	0.17
Linagliptin	Flow 1.1 mL/min	3.600	1.02	0.28
Linagliptin	Wavelength 233 nm	3.316	1.08	0.49
Linagliptin	Wavelength 235 nm (actual)	2.331	1.02	0.44
Linagliptin	Wavelength 237 nm	2.350	0.97	0.54

Table 6: Robustness studies (deliberate variation in flow rate and detection wavelength).

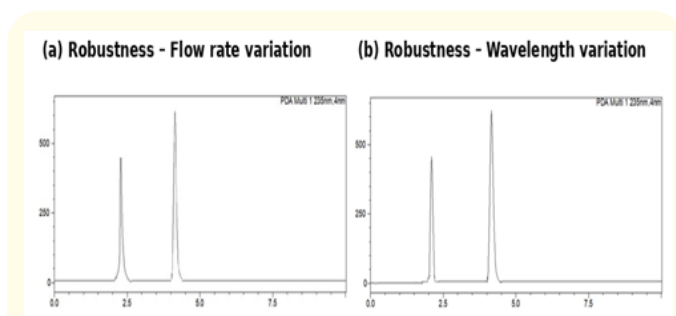


Figure 7: Representative robustness chromatograms: (a) deliberate variation in flow rate; (b) deliberate variation in detection wavelength. Both conditions produced well-resolved peaks comparable to the optimised method.

Sensitivity (LOD and LOQ)

The LOD and LOQ were 2.17 and 6.60 $\mu\text{g/mL}$ for Empagliflozin, and 0.0372 and 0.1125 $\mu\text{g/mL}$ for Linagliptin, respectively (Table 7), indicating that the method is sufficiently sensitive for trace-level detection and quantification of both analytes.

Drug	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
Empagliflozin	2.17	6.60
Linagliptin	0.0372	0.1125

Table 7: Sensitivity parameters (LOD and LOQ).

Assay of marketed formulation

Assay of the marketed fixed-dose combination tablet gave percentage recoveries of 100.6% for Empagliflozin and 99.4% for Linagliptin (Table 8), both within the acceptable range of 98–102%, confirming the applicability of the method to the analysis of finished dosage forms. A consolidated summary of all validation parameters against ICH acceptance criteria is presented in table 9.

Drug	Label Claim (mg)	Amount Found ($\mu\text{g/mL}$)	% Recovery
Empagliflozin	10	10.12	100.6
Linagliptin	5	4.97	99.4

Table 8: Assay of the marketed formulation (Xilingio-2).

Validation Parameter	ICH Acceptance Criteria	Observed Result	Compliance
System suitability	%RSD < 2.0	Within limits	Passed
Specificity	No interference at analyte RT	No interference observed	Passed
Linearity (r^2)	≥ 0.999	0.9995 - 0.9998	Passed
Accuracy	Recovery 98-102%	99.6 - 101.8%	Passed
Repeatability	%RSD < 2.0	0.52 - 0.87%	Passed
Intermediate precision	%RSD < 2.0	0.48 - 0.50%	Passed
Robustness	%RSD < 2.0 after deliberate changes	Within limits	Passed
LOD	As calculated	2.17 / 0.0372 $\mu\text{g/mL}$	Acceptable
LOQ	As calculated	6.60 / 0.1125 $\mu\text{g/mL}$	Acceptable
Assay	98-102%	99.4 - 100.6%	Passed

Table 9: Summary of validation parameters against ICH Q2 (R2) acceptance criteria.

Discussion

Several RP-HPLC methods have previously been reported for the estimation of empagliflozin and linagliptin, individually or in combination with other antidiabetic agents (Table 10). Patel, *et al.* [10] developed a stability-indicating RP-HPLC method using a phenyl column with a 10-minute run time; Marie and Salim [9] applied an analytical quality-by-design (AQbD) approach with a 12-minute run time for a ternary combination with metformin;

Ghawate, *et al.* [6] and the method reported in the Pharmaceutical and Chemical Journal [15] both employed C18 columns with 8-minute run times. In comparison, the presently developed method achieves complete separation of both analytes within 6 minutes using a simple isocratic mobile phase and single-wavelength DUAL-WAVELENGTH UV DETECTOR (WATERS 2487) detection at 235 nm, offering a shorter analysis time, simpler mobile phase composition, and comparable or superior chromatographic efficiency relative to these reported procedures.

Method (Author, Year)	Mobile Phase	Column	Wavelength (nm)	Run Time (min)
Patel., <i>et al.</i> [10], 2022	Perchloric acid : CAN	Phenyl	226	10
Marie and Salim [9], 2022	Buffer : CAN	C18	248	12
Ghawate., <i>et al.</i> [6], 2023	Buffer : CAN	C18	230	8
<i>Pharm. & Chem. Journal</i> [15], 2024	Buffer : CAN	C18	230	8
Present method	Phosphate buffer : ACN (40:60)	C18 (250×4.6 mm, 5 µm)	235	6

Table 10: Comparison of the developed RP-HPLC method with previously reported methods.

All validation parameters — system suitability, specificity, linearity, accuracy, precision, robustness, and sensitivity — met the acceptance criteria specified in the ICH Q2 (R2) guideline [7], confirming that the method is reliable and reproducible for routine use. The excellent linearity ($r^2 > 0.999$), low %RSD values ($< 1\%$) for precision and robustness, and recoveries close to 100% for both bulk drug and the marketed formulation collectively demonstrate that the method is free from significant systematic or random error. The short run time and simple mobile phase composition also make the method economical in terms of solvent consumption and analysis time, both of which are important considerations for high-throughput quality control laboratories. The method may additionally serve as a useful reference procedure for future biological-matrix and design-of-experiments-based optimisation studies of empagliflozin and linagliptin combinations, such as those described by Thallapalli and Manda [14], Satapathy, *et al.* [12], and Patel., *et al.* [11].

Conclusion

A simple, rapid, sensitive, accurate, precise, robust, and specific RP-HPLC method was successfully developed and validated for the simultaneous estimation of Empagliflozin and Linagliptin in bulk drug and combined pharmaceutical tablet dosage forms using a dual-wavelength UV visible detector. All validation parameters complied with ICH Q2 (R2) acceptance criteria, and the method accurately quantified both drugs in a marketed formulation. This removes the unsupported “regulatory evaluation” and “active pharmaceutical ingredients” claims (since your specificity data — even after adding the placebo/sample chromatograms — only demonstrates suitability for this one tablet formulation, not bulk API), while still giving credit for what the validation actually supports.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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