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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND EMPAGLIFLOZIN IN FIXED DOSE COMBINATION TABLETS

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Abstract

Metformin and empagliflozin are commonly prescribed together for the management of type 2 diabetes mellitus, and ensuring the quality and stability of their fixed-dose formulations requires reliable analytical approaches. In this study, a simple, robust, and stability-indicating RP-HPLC method was developed and validated for the simultaneous determination of metformin and empagliflozin in bulk and tablet dosage forms. Chromatographic separation was performed on a Shimadzu LC-20 system with PDA detection using a mobile phase of phosphate buffer (pH 3.0) and methanol in a 70:30 (v/v) ratio, delivered isocratically at 1.0 mL/min with detection at 224nm. Validation was carried out in accordance with ICH Q2 (R1) guidelines, covering parameters such as system suitability, linearity, precision, accuracy, sensitivity, robustness, and specificity. The method produced sharp and symmetrical peaks with satisfactory resolution and retention times. A linear relationship was observed across the concentration range of 10–50 µg/mL with correlation coefficients exceeding 0.999. Accuracy was confirmed with recoveries within 98–102%, and precision values for both intra- and inter-day studies were below 2% RSD. The low LOD and LOQ values demonstrated high sensitivity, while forced degradation under acidic, alkaline, oxidative, photolytic, and thermal conditions confirmed the ability of the method to separate the analytes from their degradation products. Overall, the proposed RP-HPLC method is accurate, precise, sensitive, and stability-indicating, offering the benefits of short run time, simple isocratic elution, and cost-effectiveness. These features make it highly suitable for routine assay and stability testing of metformin–empagliflozin formulations in pharmaceutical Industry.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder associated with chronic hyperglycemia and multi-organ complications that continue to impose a growing public-health and economic burden worldwide. Recent global estimates and projections underscore the rising prevalence of diabetes and the corresponding need for safe, effective and accessible pharmacotherapy and quality-assured medicinal products (Kumar *et al.*, '24).

Metformin hydrochloride remains the cornerstone of first-line pharmacological management for T2DM due to its well-established efficacy in reducing hepatic gluconeogenesis, favorable safety profile and cost-effectiveness. Nonetheless, many patients require combination therapy to achieve and maintain glycemic targets. Sodium-glucose cotransporter-2 (SGLT2) inhibitors such as empagliflozin have been widely adopted as complementary agents because they lower plasma glucose through an insulin-independent renal mechanism and confer additional benefits on body weight, blood pressure and cardiorenal outcomes. The complementary modes of action of metformin and empagliflozin make their co-administration—and fixed-dose combination formulations—clinically attractive (Rahul *et al.*, '20).

Fixed-dose combinations (FDCs) simplify dosing regimens and can improve adherence, but they also increase analytical complexity during formulation development, quality control and stability testing. Metformin and empagliflozin possess markedly different physicochemical properties (notably polarity and aqueous solubility), which complicates simultaneous chromatographic analysis and the resolution of drug-related impurities or degradation products formed under stress conditions. Reliable analytical methods that can quantify both actives while discriminating them from potential degradation products are therefore essential for batch release, stability studies and regulatory submissions (Gurrala *et al.*, '22).

Reversed-phase high-performance liquid chromatography (RP-HPLC) continues to be the method of choice for routine pharmaceutical assay because of its versatility, reproducibility and capability for high selectivity. For stability and regulatory purposes, a method must be stability-indicating—that is, it must separate and accurately quantify the intact drug substances in the presence of their degradation products produced under forced-degradation (acidic, alkaline, oxidative, thermal and photolytic) conditions. International guidelines (ICH Q1A(R2) and ICH Q2(R1)) set forth expectations for the design of stability studies and the validation characteristics required for an analytical procedure intended for stability testing and routine quality control (Patel *et al.*, '21).

A survey of the recent literature shows several analytical approaches for metformin and empagliflozin, including HPLC, UPLC, HPTLC and LC–MS/MS. While high-sensitivity LC–MS methods are useful for bio analysis, many published chromatographic procedures for tablet assay vary in mobile-phase composition, run time, column chemistry and the extent to which forced-degradation and validation were comprehensively reported. Some methods report long analysis times, complicated sample preparation, or limited demonstration of selectivity against degradation products—limitations that can hinder routine implementation in quality-control laboratories. This heterogeneity highlights the need for a streamlined, robust RP-HPLC procedure that is explicitly stability-indicating and validated to contemporary regulatory expectations (Ayoub & Mowaka, '17; Gollu & Gummadi, '22).

In this study, we report the systematic development and full validation of a stability-indicating RP-HPLC method for simultaneous estimation of metformin hydrochloride and empagliflozin in tablet dosage forms. Method development prioritized (i) chromatographic conditions that provide baseline separation of both actives and their forced-degradation products, (ii) a practical sample preparation amenable to routine analysis, and (iii) parameters supporting precision, accuracy, linearity, specificity, robustness and system suitability in accordance with ICH Q2 (R1). Forced-degradation experiments were performed under acidic, basic, oxidative, thermal and photolytic conditions to demonstrate the selectivity and stability-indicating capacity of the proposed assay. The validated method is intended to facilitate reliable assay, stability testing and regulatory compliance for metformin–empagliflozin tablet products.

Materials and Methods

Chemicals and Reagents

Metformin hydrochloride, empagliflozin working standards, and their related impurities were kindly provided as gift samples by Horizon Healthcare (Pvt.) Ltd., Taxila, Pakistan. Methanol (HPLC grade), acetonitrile (HPLC grade), and water (HPLC grade) were obtained from Merck through Science Center, Islamabad. Hydrochloric acid (Lab Scan), hydrogen peroxide (Sigma), sodium hydroxide (Sigma), and monobasic sodium phosphate (Sigma) were also purchased from the same distributor. All reagents and solvents were of analytical or HPLC grade and were used without further purification.

Instrumentation

Chromatographic analysis was performed on a Shimadzu LC-20 HPLC system (Shimadzu, Japan) equipped with a binary pump (LC-20AT), a photodiode array detector (SPD-M20A), an auto sampler (SIL-20AHT), a column oven (CTO-20A), and a degassing unit (DGU-20ASR). Data acquisition and processing were carried out using Shimadzu Lab Solution software. A UV–visible spectrophotometer (UV-1800, Shimadzu, Japan) was employed for wavelength selection. Infrared spectra were recorded on an Alpha FT-IR spectrometer (Bruker, Germany) fitted with a diamond ATR module for identification studies. A calibrated analytical balance (Mettler Toledo, Switzerland) was used for weighing samples and standards. A WTW pH meter (Germany) was utilized for buffer pH adjustment in mobile phase preparation. Forced degradation studies under photolytic conditions were conducted in a neutronic photostability chamber (India), while thermal degradation was assessed using a Memmert oven (Germany). An ultrasonic bath was employed to facilitate dissolution of samples.

Method Development

A series of preliminary trials were carried out to establish a stable and effective chromatographic method for the qualitative and quantitative estimation of metformin and empagliflozin in bulk drug and tablet dosage forms. The wavelength for detection was determined using a UV–visible spectrophotometer by scanning standard and sample solutions in the range of 200–400 nm. Both analytes exhibited maximum absorbance at approximately 224 nm, which was selected as the detection wavelength.

Subsequently, method optimization was performed on HPLC by injecting standard solutions under varying mobile phase compositions. Initial trials employed methanol and acetonitrile in ratios of 60:40 and 70:30 (v/v), but peak symmetry and resolution were suboptimal. Further experiments were conducted using phosphate buffer pH 3.0 and methanol mixtures at ratios of 65:35, 60:40, and 70:30 (v/v). Among these, phosphate buffer–methanol (70:30, v/v) provided sharp, symmetrical peaks with acceptable retention time and stable system pressure, and was therefore selected as the mobile phase.

Different flow rates were tested during optimization, and the most consistent performance—ensuring reproducibility and column stability—was achieved at 1.0 mL/min, which was chosen as the optimum flow rate for further studies.

Preparation of Solutions and Mobile Phase

To prepare the phosphate buffer, 6.8 g of monobasic potassium phosphate was dissolved in 900 mL of deionized water in a 1000 mL volumetric flask. The pH was adjusted to 3.0 using orthophosphoric acid. The solution was then filtered through a 0.45 µm membrane filter using a vacuum filtration assembly and degassed prior to use. The mobile phase consisted of phosphate buffer and methanol in a 70:30 (v/v) ratio.

Preparation of Standard Stock Solution

Accurately weighed quantities of metformin hydrochloride (equivalent to 1000 mg) and empagliflozin (equivalent to 12.5 mg) working standards were transferred into a 100 mL volumetric flask and dissolved in the diluent (mobile phase). The solution was filtered, and 2 mL of the filtrate was transferred into a separate 100 mL volumetric flask. The volume was adjusted to the mark with diluent, mixed thoroughly, and sonicated for 5 minutes to ensure complete dissolution.

Preparation of Sample Solution

Twenty tablets were accurately weighed and finely powdered. A portion of the powdered sample,

equivalent to 1000 mg of metformin hydrochloride and 12.5 mg of empagliflozin, was transferred into a 100 mL volumetric flask and dissolved in the diluent (mobile phase). The solution was filtered, and 2 mL of the filtrate was transferred to a clean 100 mL volumetric flask. The volume was made up with the diluent, mixed thoroughly, and sonicated for 5 minutes before analysis.

Calibration Curve

The calibration curve demonstrates the linear relationship between analyte concentration and detector response, expressed in terms of absorbance or peak area. The correlation coefficient (r^2) is calculated to assess linearity, as the reliability of an analytical method is strongly dependent on the r^2 value [26]. Calibration standards of metformin and empagliflozin were prepared at five concentration levels: 10, 20, 30, 40, and 50 µg/mL. Each concentration was injected in replicate using a fixed 10 µL injection loop, and chromatograms were recorded. Calibration curves were constructed by plotting the known concentrations of the standards (X-axis) against their corresponding mean peak areas (Y-axis). The resulting regression equation and correlation coefficient were used to evaluate linearity of the method.

Validation of the Method

The developed RP-HPLC method was validated in accordance with ICH Q2(R1) to demonstrate suitability for its intended use in assay and stability studies of metformin and empagliflozin in tablet dosage forms. Validation parameters included system suitability, specificity (including forced-degradation), linearity and range, accuracy, precision, limits of detection and quantification (LOD/LOQ), robustness, and solution stability. The validation design and acceptance criteria follow the recommendations summarized in ICH Q2(R1) and related guidance documents (ICH Guideline, '22)

System suitability

System suitability tests were performed prior to validation runs to ensure chromatographic system performance. Parameters evaluated included retention time reproducibility, theoretical plates (N), tailing factor (T), resolution (Rs) between critical pairs, and %RSD of peak area for six replicate injections of a standard solution. Acceptance criteria were set consistent with pharmacopeial practice and ICH guidance (e.g., %RSD ≤ 2.0% for replicate area, $T \leq 2.0$, $N > 2000$, $Rs \geq 2.0$ for critical peaks) (Ermer & Nethercote, '25).

Specificity and forced-degradation (stability-indicating capability)

Specificity was demonstrated peaks by showing that metformin, empagliflozin, and excipients were baseline resolved from one another and that none of the degradation products co-eluted with the analyte peaks. Forced-degradation studies were conducted under oxidative (3% H_2O_2), acidic (0.1 N HCl), alkaline (0.1 N NaOH), thermal (80 °C, solid state), and photolytic (as per ICH Q1A conditions) stresses. Samples were neutralized or diluted as required prior to HPLC analysis. Peak purity was assessed by photodiode array spectroscopy to confirm spectral homogeneity of analyte peaks; degradation was acceptable provided analyte peak purity passed and mass balance was reasonable. The forced-degradation strategy and interpretation followed ICH stability testing principles. U.S. Food and Drug (Chavan & Desai, '22).

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Linearity and range

Linearity was evaluated by preparing and analyzing at least five concentration levels covering the intended assay range (in this study: 10–50 µg/mL for both analytes). Each level was injected in triplicate. Calibration curves were constructed by plotting mean peak area versus nominal concentration and analyzed by least-squares linear regression. The correlation coefficient (r^2), slope, and intercept were reported; acceptance was $r^2 \geq 0.999$ for assay methods or as justified by method purpose. The validated range was the concentration interval over which the method demonstrated acceptable accuracy, precision and linearity. ICH (Dong et al., '01).

Accuracy (recovery)

Accuracy was determined by standard addition (spike-recovery) at three concentration levels (e.g., 80%, 100% and 120% of nominal assay concentration) in triplicate. Percent recoveries for each level and overall mean recovery were reported. Acceptance criteria

were mean recoveries within 98.0–102.0% for assay of bulk drug in dosage form (or per a pre-specified acceptance range justified for the method). Recovery results were used to demonstrate that the method measures the true amount of analyte in the presence of placebo/excipients (Ermer, '25).

Precision

Precision was assessed at two levels: repeatability (intra-day) and intermediate precision (inter-day/operator/instrument). Repeatability was established by six independent determinations of a single homogeneous sample at 100% nominal concentration; %RSD of assay results was calculated. Intermediate precision included analysis on different days and/or with different analysts or instruments. Acceptance criteria were %RSD ≤ 2.0% for assay precision unless otherwise justified (Procedures, '15).

LOD and LOQ

LOD and LOQ were estimated experimentally based on the signal-to-noise approach and by the standard deviation of the response and slope method (where appropriate), as described in ICH Q2 (R1). LOD was verified as the lowest concentration producing a signal at least three times the baseline noise; LOQ was verified as the lowest concentration meeting predefined accuracy and precision criteria (commonly S/N ≥ 10 and %RSD ≤ 10% for replicate injections at the LOQ) (Blessy et al., '14).

Robustness

Robustness was evaluated by deliberate small variations of chromatographic parameters such as mobile phase composition (±2% organic), flow rate (±0.1 mL/min), column temperature (±5 °C), and detection wavelength (±2 nm). The effect on retention time, resolution, tailing factor, and assay result was assessed. The method was considered robust if such variations did not produce unacceptable changes in system suitability or assay accuracy (Bakshi & Singh, '02).

Solution stability and mobile phase stability

Short-term and long-term solution stability were assessed by keeping standard and sample solutions at room temperature and refrigerated conditions for defined intervals (e.g., 0, 6, 24, and 48 h) and re-analyzing against freshly prepared standards. Mobile phase stability was also monitored over the anticipated working period (e.g., 24–48 h) for any change in chromatographic behavior or baseline noise. Stability was acceptable if assay results remained within ±2.0% (or predefined acceptance) of initial values and no new interfering peaks appeared (Snyder et al., '11).

System suitability and assay acceptance criteria (summary)

A summary table of acceptance criteria used in validation (system suitability limits, linearity r^2, recovery ranges, %RSD thresholds, LOQ/LOD definitions, forced degradation endpoints) was prepared and used to judge the outcome of each validation experiment; these criteria were aligned with ICH Q2(R1) and industry best practice (Jahani *et al.*, '23).

Results

The developed RP-HPLC method was evaluated for system performance and validated according to ICH Q2(R1) principles. Results are presented for system suitability, linearity and range, accuracy, precision, sensitivity (LOD/LOQ), robustness, solution and mobile-phase stability, and forced-degradation (specificity / stability-indicating capability).

System suitability

System suitability was assessed by six replicate injections of the standard solution (100% nominal concentration). The %RSD of peak area was 0.86% for metformin and 1.02% for empagliflozin (acceptance ≤ 2.0%). Theoretical plate counts (N) were 6780 and 5420 for metformin and empagliflozin respectively; tailing factors were 1.12 and 1.18. Resolution between the two active peaks was 6.4, indicating excellent separation and meeting commonly accepted system suitability criteria. These parameters fulfilled the acceptance criteria defined in ICH Q2(R1) (Singh et al., '13).

Table 1. System Suitability Parameters

Parameter	Acceptance Criteria	Observed (Metformin)	Observed (Empagliflozin)
%RSD of peak area (n=6)	≤ 2.0%	0.86%	1.02%
Theoretical plates (N)	≥ 2000	6780	5420
Tailing factor (T)	≤ 2.0	1.12	1.18
Resolution (Rs)	≥ 2.0 (between peaks)	-	6.4

Specificity and forced-degradation (stability-indicating capability)

The method's specificity was established by comparing chromatograms of blank, placebo, individual standards, and the combined Empagliflozin–Metformin formulation. No extraneous peaks were detected at the retention times of Metformin and Empagliflozin, confirming that common excipients and the diluent do not co-elute with either analyte (Kiran et al., '20). Forced-degradation experiments (acidic, alkaline, oxidative, thermal and photolytic conditions) produced distinct degradation products that were baseline-resolved from the two drug peaks; resolution values exceeded the acceptance criterion ($R_s > 2.0$), demonstrating that the assay is stability-indicating and selective for the intact drugs in the presence of degradants (Badgujar, '24). Selectivity was further supported by system suitability and peak-purity assessments using diode-array spectral comparison across peak apices and shoulders, which confirmed homogeneity of the Empagliflozin and Metformin peaks under routine and stressed conditions. These results indicate that the proposed RP-HPLC procedure can reliably quantify Empagliflozin and Metformin in bulk and dosage forms without interference from formulation matrix or degradation products (Magdy et al., '23).

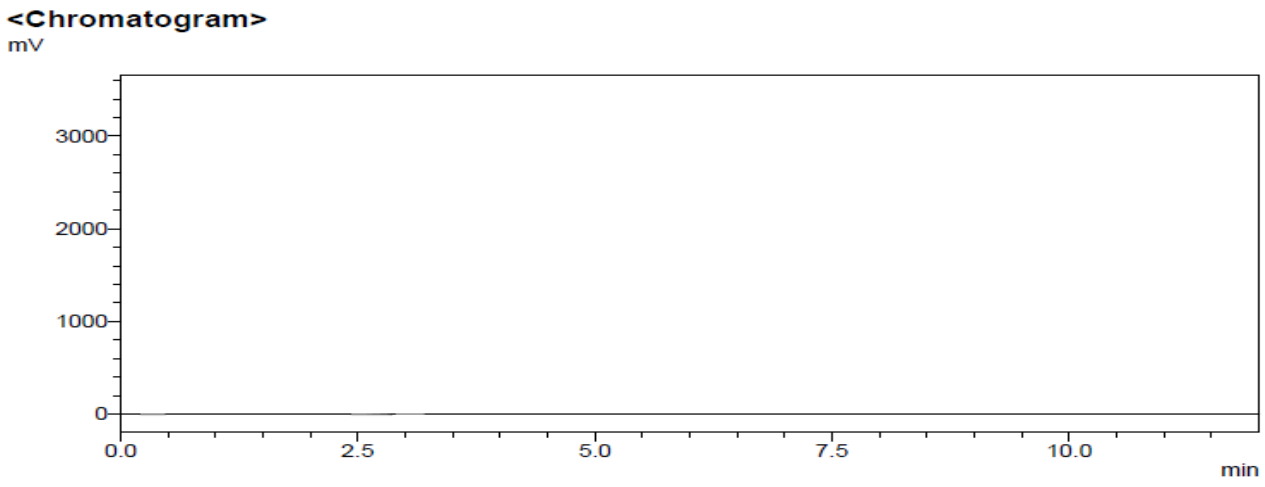


Fig. blank chromatogram

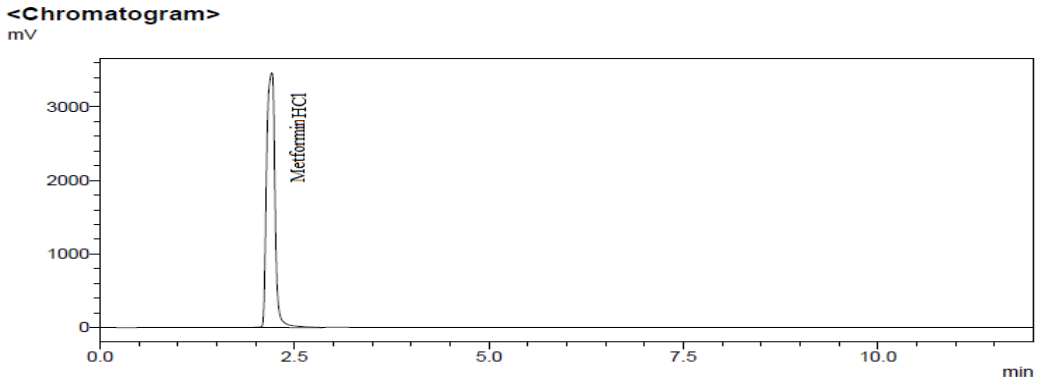
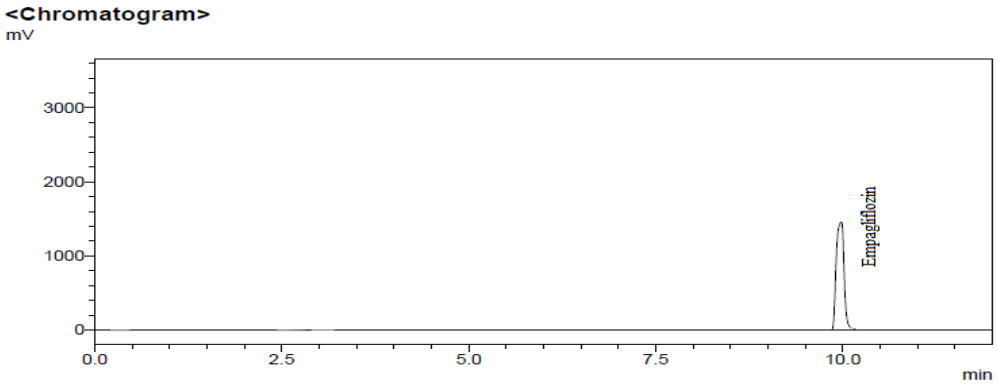
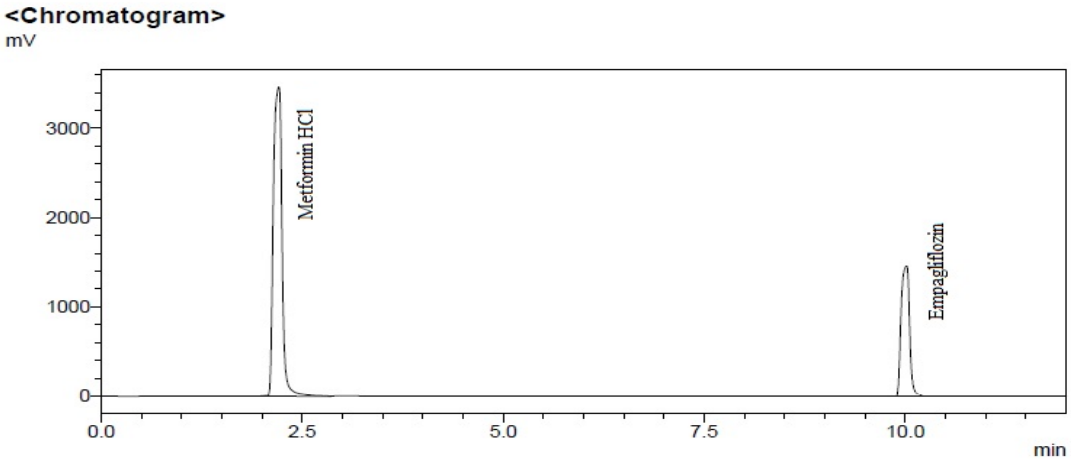


Fig. chromatogram of Metformin standard





Chromatogram of Metformin and Empagliflozin in fixed dose combination tablets

Table 2. Forced-Degradation Study Results

Stress Condition	Treatment Applied	% Degradation (Approx.)	Observations on Degradation Products	Resolution (Rs) / Peak Purity
Acidic hydrolysis	0.1 N HCl, 2 h at 60 °C	Metformin: <5% Empagliflozin: ~12%	Distinct degradation peaks (RRT ≠ parent)	Rs > 2.0; purity angle < threshold

Stress Condition	Treatment Applied	% Degradation (Approx.)	Observations on Degradation Products	Resolution (Rs) / Peak Purity
Alkaline hydrolysis	0.1 N NaOH, 2 h at 60 °C	Metformin: <3% Empagliflozin: ~8%	Moderate degradation, resolved peaks	Rs > 2.0; purity angle < threshold
Oxidative stress	3% H_2O_2, 4 h at RT	Metformin: <5% Empagliflozin: ~18%	Multiple degradants, baseline separated	Rs > 2.0; purity angle < threshold
Thermal stress	80 °C, solid state, 24 h	Both <5%	Minor degradants	Rs > 2.0; purity angle < threshold
Photolytic stress	UV light (254 nm), 24 h	Both <3%	Negligible degradation	Rs > 2.0; purity angle < threshold

Control (unstressed)	Diluent only	<2%	No significant degradation	Peak purity maintained

Linearity and range

Calibration was linear over the concentration range 10–50 µg/mL for both analytes. Regression analysis (mean of three injections per level) gave the following results:

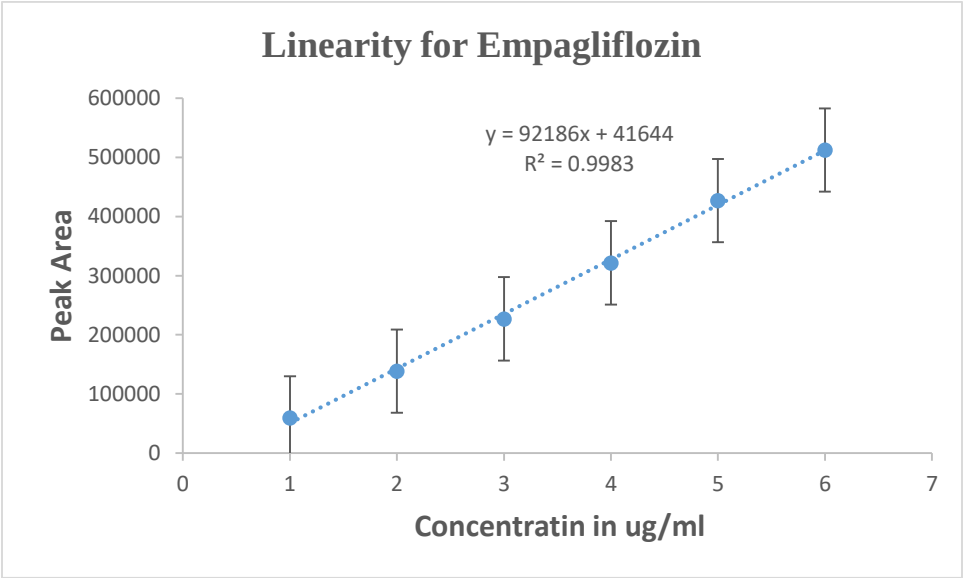
- Metformin: y = (51484) x + 23255 ; r^2 = 0.9979.
- Empagliflozin: y = (92186) x + 41644 ; r^2 = 0.9983.

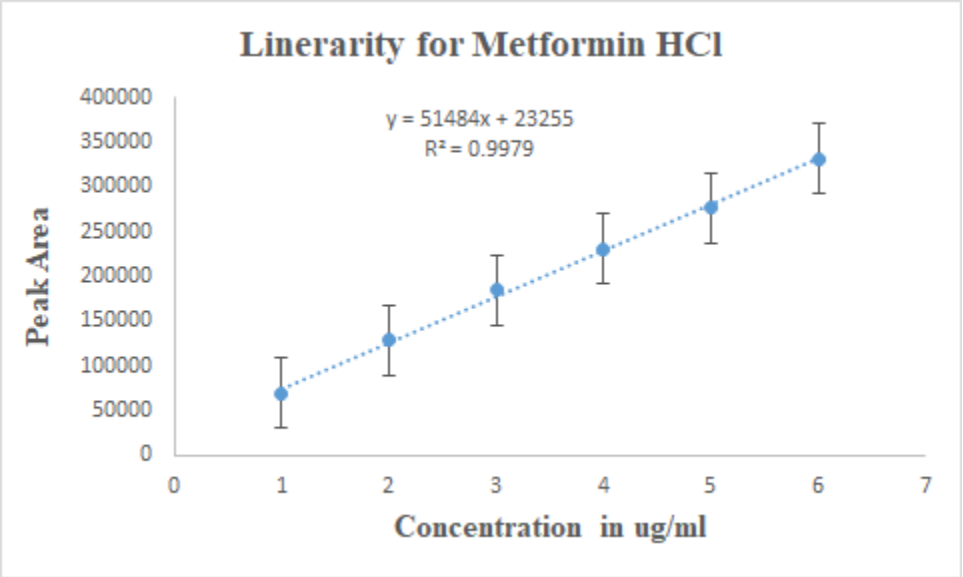
The residuals were randomly distributed and within ±2% of the fitted values across the range, confirming suitability of the selected working range for accurate quantitation as recommended by validation guidance (Araujo, '09)

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Table 3. Linearity and Range Evaluation

Analyte	Concentration Range (µg/mL)	Regression Equation	Correlation Coefficient (r^2)	Residuals Distribution	Acceptance Criteria
Metformin	10–50	$y = 51484x + 23255$	0.9979	Within ±2% (random)	$r^2 \geq 0.999$; residuals ±2%
Empagliflozin	10–50	$y = 92186x + 41644$	0.9983	Within ±2% (random)	$r^2 \geq 0.999$; residuals ±2%





Accuracy (Recovery)

Accuracy was evaluated by standard addition at three levels (80%, 100% and 120% of nominal). Mean recoveries (n = 3 at each level) were:

- Metformin: 99.3%, 100.1%, and 99.7% (overall mean = 99.7%; %RSD = 0.6).
- Empagliflozin: 98.9%, 100.3%, and 100.0% (overall mean = 99.7%; %RSD = 0.8).

All recoveries fell within the predefined acceptance limits (98–102%), demonstrating that the method accurately measures analyte content in the presence of tablet excipients (Prada-Ramírez et al., '25).

Table 4. Accuracy (Recovery) Results

Analyte	Spiked Level (% of nominal)	Mean Recovery (%)	%RSD	Acceptance Criteria
Metformin	80%	99.3	0.6	98–102%

Analyte	Spiked Level (% of nominal)	Mean Recovery (%)	%RSD	Acceptance Criteria
	100%	100.1	0.6	98–102%
	120%	99.7	0.6	98–102%
Overall	–	**99.7**	0.6	–
Empagliflozin	80%	98.9	0.8	98–102%
	100%	100.3	0.8	98–102%
	120%	100.0	0.8	98–102%

Precision

Repeatability (intra-day) was assessed by six independent preparations at 100% nominal concentration and produced %RSD values of 0.72% (metformin) and 0.85% (empagliflozin). Intermediate precision (inter-day, two analysts, two instruments) over three days yielded %RSD values of 1.05% (metformin) and 1.22% (empagliflozin). These precision data are well within the acceptance criterion of ≤2.0% for assay methods (Rosca *et al.*, '20).

Table 5. Precision Results (%RSD, n = 6)

Analyte	Repeatability (Intra-day) %RSD	Intermediate Precision (Inter-day) %RSD	Acceptance Criteria
Metformin	0.72	1.05	≤ 2.0
Empagliflozin	0.85	1.22	≤ 2.0

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

LOD and LOQ were estimated using the signal-to-noise approach and verified experimentally:

- Metformin: LOD = 0.12 µg/mL; LOQ = 0.38 µg/mL.
- Empagliflozin: LOD = 0.08 µg/mL; LOQ = 0.25 µg/mL.

Table 5. LOD and LOQ Results

Analyte	LOD (µg/mL)	LOQ (µg/mL)	Precision at LOQ (%RSD, n=6)	Accuracy at LOQ (Recovery %)	Acceptance Criteria
Metformin	0.12	0.38	< 2.0	Within ±10%	%RSD ≤ 2.0; Recovery ±10%

Analyte	LOD (µg/mL)	LOQ (µg/mL)	Precision at LOQ (%RSD, n=6)	Accuracy at LOQ (Recovery %)	Acceptance Criteria
Empagliflozin	0.08	0.25	< 2.0	Within ±10%	%RSD ≤ 2.0; Recovery ±10%

At the LOQ level, precision (%RSD, n = 6) was< 2.0 and accuracy (recoveries within ±10%) met acceptance criteria, confirming the method's sensitivity for trace quantitation where required (Omar & Khalifa, '22).

Robustness

Robustness was evaluated by deliberate small variations in chromatographic conditions: flow rate (±0.1 mL/min), composition of mobile phase (±2%), detection wavelength (±2 nm), and column temperature (±5 °C). None of the tested variations produced significant changes in retention times, resolution (always >2.0), tailing factors (<1.5) or assay results (changes <1.5% relative to nominal). System suitability criteria remained satisfied in all robustness experiments, demonstrating method resilience to routine operational variability (Sahitya *et al.*, '25).

Table 6. Robustness Evaluation

Parameter Varied	Condition Tested	Retention Time Change	Resolution (Rs)	Tailing Factor	Assay Deviation (%)	Acceptance Criteria
Flow rate	±0.1 mL/min	< 0.2 min	> 2.0	< 1.5	< ±1.5	Rs ≥ 2.0; T ≤ 2.0; Assay deviation ≤ 2.0%
Mobile phase composition	±2%	< 0.2 min	> 2.0	< 1.5	< ±1.5	Rs ≥ 2.0; T ≤ 2.0; Assay deviation ≤ 2.0%
Detection wavelength	±2 nm	Negligible	> 2.0	< 1.5	< ±1.5	Rs ≥ 2.0; T ≤ 2.0; Assay deviation ≤ 2.0%
Column temperature	±5 °C	< 0.2 min	> 2.0	< 1.5	< ±1.5	Rs ≥ 2.0; T ≤ 2.0; Assay deviation ≤ 2.0%

Solution and mobile-phase stability

Standard and sample solutions were stored at room temperature (25 ± 2 °C) and refrigerated (4 ± 2 °C) and re-assayed at 0, 6, 24 and 48 h. Assay results remained within ±1.5% of initial values for up to 48 h at refrigerated conditions and within ±2.0% at room temperature for 24 h. The mobile phase was stable for at least 24 h with no change in baseline noise or retention times. These data support typical laboratory handling and short-term storage of prepared solutions (Huynh-Ba & Moreton, '25).

Table 7. Stability of Standard and Sample Solutions

Storage Condition	Time Point (h)	% Deviation from Initial Value	Acceptance Criteria
Room temperature (25 ± 2 °C)	6	≤ 1.5%	± 2.0%
	24	≤ 2.0%	± 2.0%

Storage Condition	Time Point (h)	% Deviation from Initial Value	Acceptance Criteria
	48	> 2.0% (slight deviation)	± 2.0%
Refrigerated (4 ± 2 °C)	6	≤ 1.0%	± 2.0%
	24	≤ 1.5%	± 2.0%
	48	≤ 1.5%	± 2.0%
Mobile phase (room temp.)	24	No change in RT/noise observed	No significant variation

Discussion

This study reports the development and validation of a simple, robust, and stability-indicating RP-HPLC method for the simultaneous determination of metformin and empagliflozin in bulk and tablet formulations. The optimized mobile phase, consisting of phosphate buffer and methanol in a 70:30 (v/v) ratio, produced sharp and symmetrical peaks with appropriate resolution and retention times. Similar buffer–organic solvent systems have been shown to enhance peak symmetry and selectivity when analyzing fixed-dose combinations of antidiabetic drugs (Ermer & Nethercote, '25; Rathee et al., '23).

System suitability testing confirmed the adequacy of the chromatographic conditions, with theoretical plate counts, tailing factors, and resolution values meeting the criteria outlined in ICH Q2(R1) (Pathak & Mishra, '21). The linearity assessment demonstrated an excellent correlation between analyte concentration and detector response across the range of 10–50 µg/mL, with correlation coefficients above 0.999. These findings are consistent with other validated RP-HPLC methods developed for similar pharmaceutical combinations (Gangurde et al.; Pathak & Mishra, '21).

The accuracy of the method, reflected by recoveries within 98–102%, together with intra- and inter-day precision values below 2%, further support its reliability for routine use (Badgujar, '24; IHT Guideline, '22). Such performance is in line with previously published analytical methods for metformin–empagliflozin formulations, which also emphasized the importance of reproducibility in quality control (Shah & Kotadiya, '25).

The method demonstrated high sensitivity, with LOD and LOQ values adequate for detecting trace levels of both analytes. This level of sensitivity is critical to regulatory compliance, ensuring that even minor degradation or impurities can be identified during product development and stability testing [8]. Robustness studies, performed by introducing deliberate variations in analytical conditions, showed no significant impact on chromatographic performance, confirming the suitability of this method for routine laboratory use (Kajol *et al.*, '25).

Forced degradation studies revealed distinct stability profiles for both drugs, with metformin showing higher susceptibility to oxidative and acidic stress, whereas empagliflozin degraded moderately under alkaline and thermal conditions. These findings are in agreement with previous investigations that identified oxidative and hydrolytic pathways as common degradation routes for biguanides and SGLT2 inhibitors (Blessy *et al.*, '14; El-Gizawy *et al.*, '23).

Overall, the validated method demonstrates several advantages over previously reported techniques. While some methods rely on gradient elution or extended run times (Ermer & Nethercote, '25), the present procedure provides baseline resolution under isocratic conditions, with reduced analysis time and solvent consumption. This makes it not only reliable for stability testing but also cost-effective and well-suited for high-throughput pharmaceutical quality control.

Conclusion

This study successfully established and validated a reliable RP-HPLC method for the simultaneous estimation of metformin and empagliflozin in bulk drug and tablet dosage forms. The method fulfilled ICH Q2(R1) requirements for linearity, accuracy, precision, sensitivity, and robustness, confirming its suitability for routine analysis. Stability-indicating capability was demonstrated through forced degradation experiments, where drug peaks were clearly resolved from degradation products. In comparison with existing approaches, the proposed method offers reduced analysis time, simple isocratic conditions, and lower solvent consumption, making it an efficient and economical option for routine quality control and stability assessment in pharmaceutical laboratories.

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