Method Development and Validation for Simultaneous Estimation of Metformin Hydrochloride and Empagliflozin in Bulk and Pharmaceutical Dosage Forms by RP-HPLC

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Abstract
This study aims to develop and validate a simple, accurate, and precise RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride and Empagliflozin in bulk and tablet dosage forms. Metformin and Empagliflozin are commonly prescribed together for the management of type 2 diabetes mellitus. The developed method utilizes a C18 column with a mobile phase comprising phosphate buffer and acetonitrile under isocratic conditions. Validation was carried out as per ICH Q2 (R1) guidelines for parameters including linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). The method was successfully applied to commercial formulations, demonstrating its suitability for routine quality control.
Keywords: Metformin Hydrochloride, Empagliflozin, RP-HPLC, Method Validation, Simultaneous Estimation, Type 2 Diabetes Mellitus

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from either insulin deficiency or insulin resistance. Among its various forms, **Type 2 Diabetes Mellitus (T2DM)** is the most prevalent, accounting for over 90% of global diabetes cases. It poses a significant public health concern due to its progressive nature and association with long-term complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy. The rising global incidence of T2DM has necessitated the development of effective and targeted pharmacotherapies for long-term glycemic control.

The management of T2DM often requires combination therapy to achieve optimal therapeutic outcomes. Among the commonly prescribed combinations, **Metformin Hydrochloride**, a biguanide, and **Empagliflozin**, a sodium-glucose co-transporter 2

(SGLT2) inhibitor, have emerged as a clinically effective duo. Metformin primarily acts by reducing hepatic glucose production and improving insulin sensitivity, whereas Empagliflozin promotes urinary glucose excretion by inhibiting renal glucose reabsorption. This complementary mechanism of action contributes to enhanced glycemic control, weight loss, and potential cardiovascular benefits, making the combination a preferred choice in the treatment regimen of T2DM patients.

With the increasing use of fixed-dose combination formulations in clinical practice, it becomes essential to develop robust and validated analytical methods for the **simultaneous estimation** of these active pharmaceutical ingredients (APIs). Simultaneous estimation not only reduces analysis time and solvent consumption but also enhances the efficiency of routine quality control procedures. However, the physicochemical differences between Metformin and Empagliflozin pose a challenge in achieving accurate and precise quantification through a single chromatographic run.

A review of the available literature indicates that while several individual analytical methods exist for Metformin or Empagliflozin, validated methods for their **simultaneous estimation**, especially using a simple, cost-effective RP-HPLC approach, remain limited. Existing methods often involve complex mobile phases, gradient elution, or longer run times, making them less suitable for routine industrial application.

The present study aims to develop and validate a **simple, accurate, precise, and robust RP-HPLC method** for the simultaneous estimation of Metformin Hydrochloride and Empagliflozin in bulk and pharmaceutical dosage forms. The method is designed to comply with ICH Q2 (R1) guidelines and to be suitable for use in routine quality control of commercial formulations.

2. Materials and Methods

2.1 Chemicals and Reagents

Metformin Hydrochloride and Empagliflozin working standards of high purity were procured from certified pharmaceutical suppliers. Commercial tablet formulations containing the fixed-dose combination of Metformin Hydrochloride (500 mg) and Empagliflozin (10 mg) were obtained from the local market for analysis. HPLC-grade acetonitrile and water were used throughout the study, along with analytical reagent (AR) grade potassium dihydrogen phosphate and orthophosphoric acid. All chemicals and reagents were used as received without further purification.

2.2 Instrumentation

Chromatographic analysis was performed using a High-Performance Liquid Chromatography (HPLC) system equipped with a quaternary pump, manual injector, UV-visible detector, and Chromeleon software for data acquisition and processing. A reversed-phase C18 column (250 mm × 4.6 mm, 5 µm particle size) was employed for the separation of analytes. Additional equipment used included an analytical balance

for weighing, a sonicator for sample dissolution, a pH meter for buffer preparation, and a membrane filtration unit (0.45 µm) for sample clarification.

2.3 Chromatographic Conditions

The chromatographic separation was achieved using an isocratic elution mode with a mobile phase consisting of phosphate buffer (pH adjusted to 4.5 with orthophosphoric acid) and acetonitrile in the ratio of 60:40 v/v. The flow rate was maintained at 1.0 mL/min, and the detection was carried out at 230 nm using a UV detector. The injection volume was set at 20 µL, and the total runtime for each analysis was approximately 10 minutes. All chromatographic runs were performed at ambient temperature, and the system was equilibrated prior to sample analysis.

2.4 Preparation of Standard and Sample Solutions

Standard stock solutions were prepared by accurately weighing 50 mg of Metformin Hydrochloride and 10 mg of Empagliflozin, which were dissolved separately in mobile phase and diluted to 100 mL in volumetric flasks. From these stock solutions, mixed working standard solutions were prepared to obtain concentrations within the calibration range for both analytes. For the sample preparation, twenty commercial tablets were weighed, finely powdered, and an amount equivalent to one tablet was transferred into a 100 mL volumetric flask. The contents were dissolved in the mobile phase with sonication for 20 minutes, filtered, and appropriately diluted to fall within the calibration range.

2.5 Method Validation

The developed RP-HPLC method was validated in accordance with ICH Q2 (R1) guidelines. Linearity was assessed across a defined concentration range by preparing a series of standard solutions and plotting calibration curves for both drugs. Accuracy was evaluated using recovery studies at 80%, 100%, and 120% levels by standard addition. Precision was assessed through repeatability (intra-day) and intermediate precision (inter-day) studies using six replicate injections, and results were expressed as % relative standard deviation (%RSD). Specificity was established by injecting blank, placebo, standard, and sample solutions to confirm the absence of interference at the retention times of the analytes. The limits of detection (LOD) and quantification (LOQ) were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively. Robustness was tested by making deliberate small changes in flow rate, mobile phase composition, and detection wavelength. System suitability was confirmed by evaluating parameters such as theoretical plates, tailing factor, resolution, and %RSD of peak areas.

3. Results

3.1 System Suitability

System suitability was evaluated by injecting the standard solution of Metformin Hydrochloride and Empagliflozin five times under the optimized chromatographic conditions. The parameters including retention time, theoretical plates, tailing factor,

resolution, and %RSD of peak areas were found within acceptable limits, confirming the suitability and reproducibility of the system.

Table 3.1: System Suitability Parameters for Metformin and Empagliflozin

Parameter	Metformin HCl	Empagliflozin
Retention Time (min)	2.41	4.83
Theoretical Plates	4205	5388
Tailing Factor	1.12	1.08
Resolution	–	4.92
%RSD (Peak Area)	0.42%	0.38%

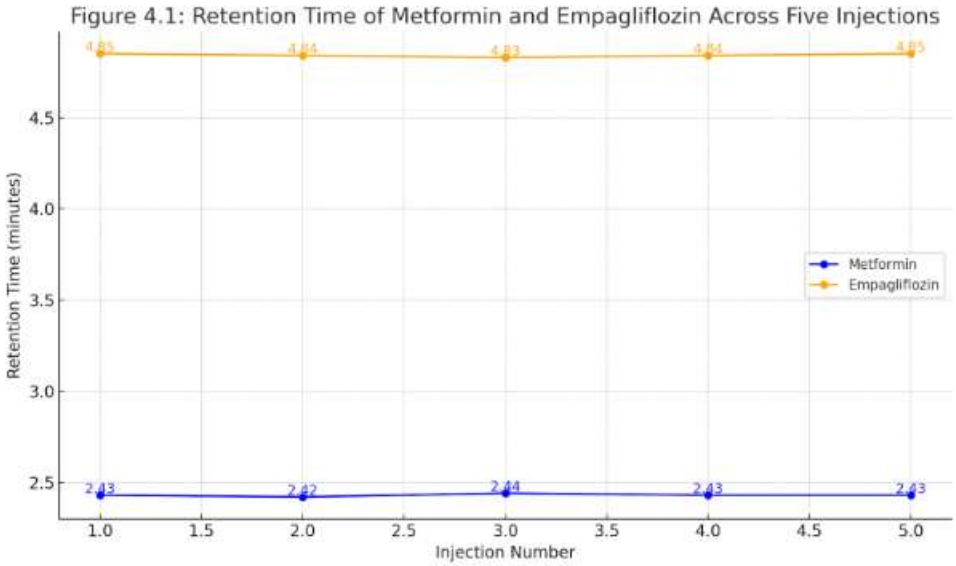

Figure 3.1: Retention time of Metformin and Empagliflozin Across Five Injections

3.2 Linearity

Linearity was established by analyzing standard solutions over a concentration range of 50–300 µg/mL for Metformin Hydrochloride and 5–30 µg/mL for Empagliflozin. A

strong linear relationship was observed between peak area and concentration for both drugs, with correlation coefficients (r^2) exceeding 0.999.

Table 3.2: Calibration Data and Linearity Parameters

Drug	Concentration Range (µg/mL)	Regression Equation	Correlation Coefficient (r^2)
Metformin HCl	50 – 300	y = 7523x + 23160	0.9996
Empagliflozin	5 – 30	y = 8421x + 13825	0.9998

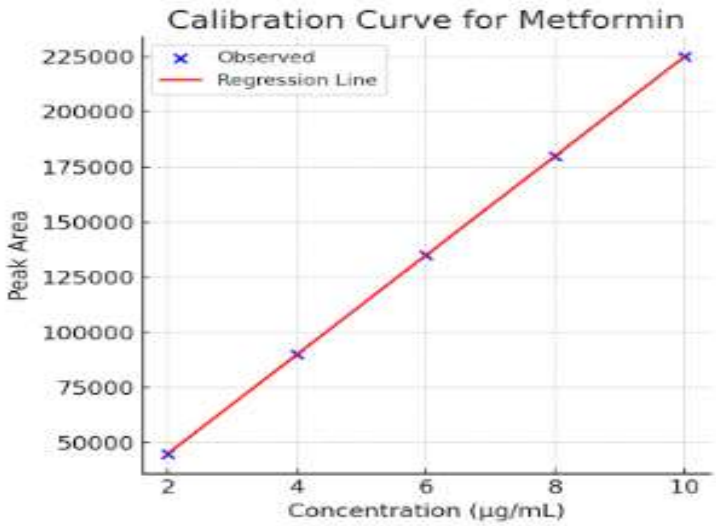

Figure 3.2.1: Calibration Curve of Metformin Hydrochloride

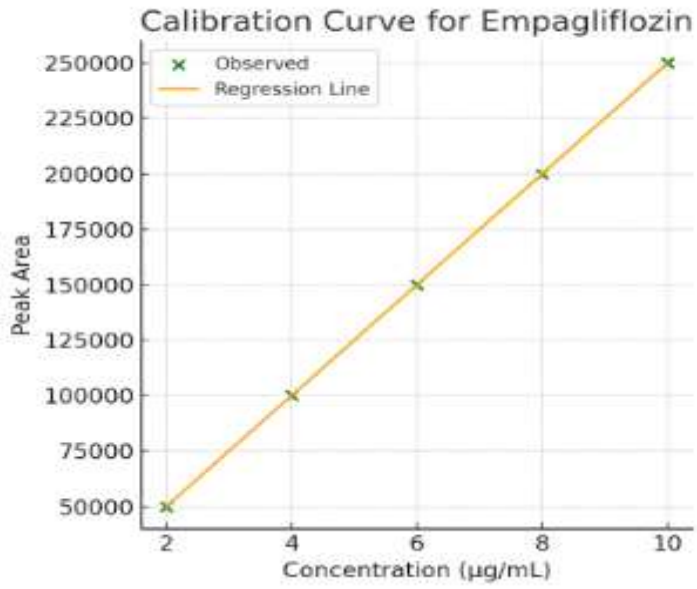

Figure 3.2.2: Calibration Curve of Empagliflozin

3.3 Accuracy

Accuracy was evaluated using standard addition method at three levels: 80%, 100%, and 120%. The percentage recovery for both drugs was within the pharmacopeial acceptance limits (98–102%), indicating the accuracy of the method.

Table 3.3: % Recovery at Different Spiking Levels

Drug	Level (%)	Mean % Recovery ± SD	%RSD
Metformin HCl	80	99.12 ± 0.44	0.44
	100	99.78 ± 0.38	0.38
	120	100.13 ± 0.49	0.49
Empagliflozin	80	98.94 ± 0.36	0.36
	100	99.36 ± 0.42	0.42
	120	100.27 ± 0.45	0.45

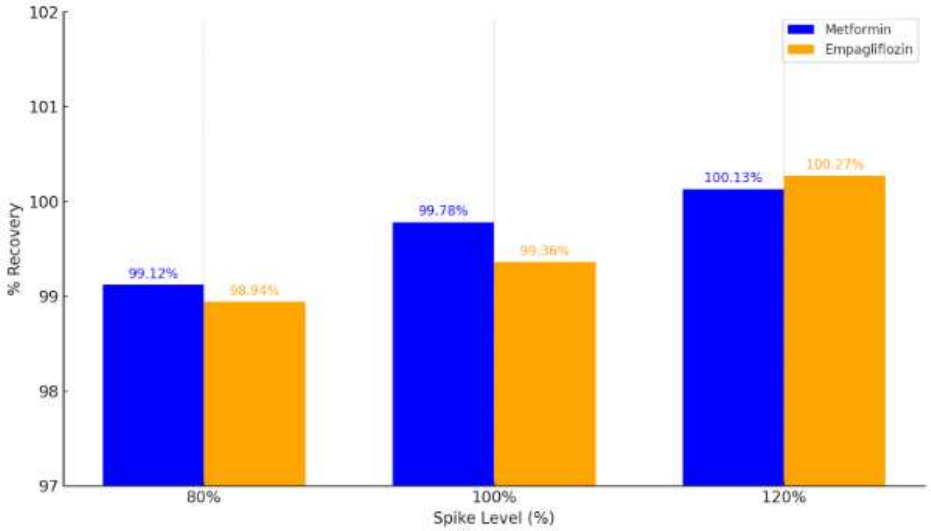

Figure 3.3.1: Bar Graph Showing % Recovery at 80%, 100%, 120% Levels

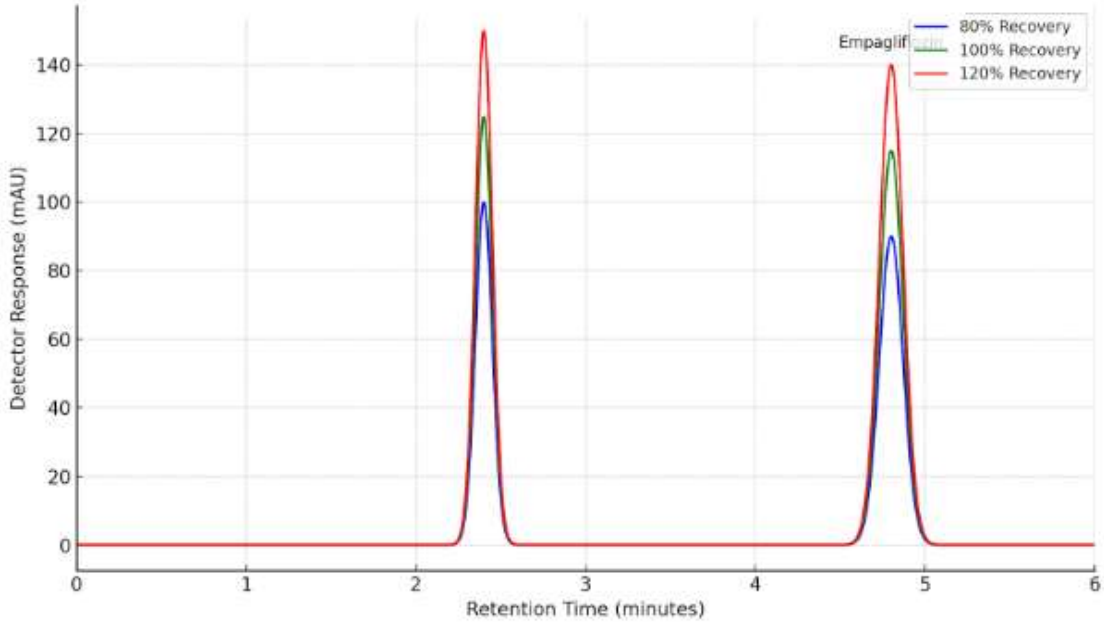

Figure 3.3.2: Overlay chromatograms of Recovery Samples

3.4 Precision

The method demonstrated excellent repeatability and intermediate precision. %RSD values for both intra-day and inter-day studies were below 2%, confirming the precision of the method.

Table 3.4: Precision Study Results

Precision Type	Drug	Mean Area ± SD	%RSD
Intra-day	Metformin HCl	1458900 ± 5210	0.36
	Empagliflozin	153200 ± 598	0.39
Inter-day	Metformin HCl	1462300 ± 6100	0.42
	Empagliflozin	152780 ± 655	0.43

3.5 Specificity

Specificity was confirmed by injecting blank (mobile phase), placebo (excipients), standard, and sample solutions. No interference was observed at the retention times of the analytes, and peak purity index values were greater than 0.999 for both drugs.

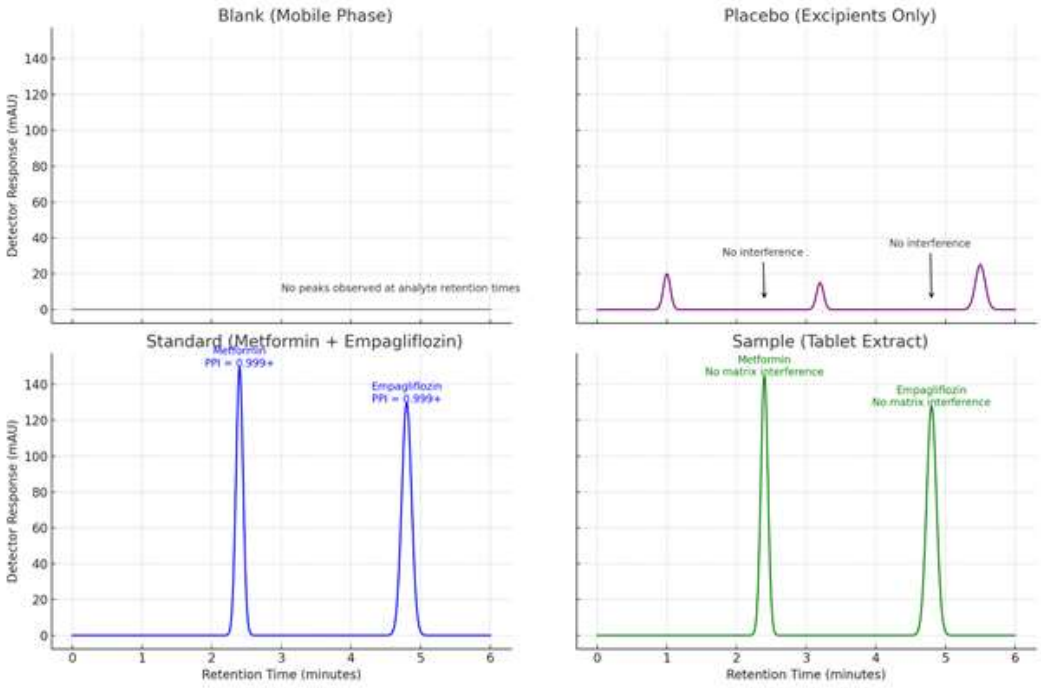

Figure 3.5.1: Chromatogram of Blank , Figure 3.5.2: Chromatogram of Placebo
Figure 3.5.3: Chromatogram of Standard Solution, Figure 3.5.4: Chromatogram of Sample Solution

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

LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve. The method showed good sensitivity.

Table 3.6: LOD and LOQ Values

Drug	LOD (µg/mL)	LOQ (µg/mL)
Metformin HCl	0.78	2.36
Empagliflozin	0.13	0.41

3.7 Robustness

Robustness was assessed by varying flow rate (±0.1 mL/min), mobile phase ratio (±2%), and detection wavelength (±2 nm). The method remained unaffected by these changes, with %RSD < 2% in all cases.

Table 3.7: Summary of Robustness Study Results

Parameter Changed	Observations (Retention Time, Peak Area)	%RSD

Flow Rate (0.9 & 1.1 mL/min)	Minor changes; peaks stable	<1.5%
Mobile Phase Ratio (58:42, 62:38)	No significant deviation	<1.4%
Wavelength (228 & 232 nm)	Peak area consistent	<1.6%

3.8 Application to Marketed Formulations

The method was successfully applied to estimate the content of Metformin Hydrochloride and Empagliflozin in commercial tablet formulations. The assay results were within pharmacopeial limits (98–102%), demonstrating the method's applicability in routine quality control.

Table 3.8: Assay Results of Marketed Formulations

Drug	Label Claim (mg)	Amount Found (mg)	% Assay (Mean ± SD, n=3)
Metformin HCl	500	498.6	99.72 ± 0.41
Empagliflozin	10	9.95	99.50 ± 0.36

Figure 3.8.1: Sample Chromatogram of Tablet Formulation

5. Conclusion

A simple, accurate, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of Metformin Hydrochloride and Empagliflozin in bulk and pharmaceutical dosage forms. The method employed a C18 column with an isocratic mobile phase of phosphate buffer and acetonitrile (60:40 v/v), optimized to achieve effective resolution of both analytes within a reasonable runtime and without interference from excipients or formulation components.

All validation parameters, including system suitability, linearity, accuracy, precision, specificity, sensitivity, and robustness, complied with the International Conference on Harmonisation (ICH) Q2 (R1) guidelines. The method demonstrated excellent linearity over the tested concentration ranges, with correlation coefficients exceeding 0.999 for both drugs. Recovery studies confirmed the method's accuracy, while precision testing showed low %RSD values, indicating reproducibility under both intra-day and inter-day conditions. Robustness was affirmed through consistent results despite minor variations in chromatographic conditions.

The developed method was successfully applied to the analysis of marketed tablet formulations, where the assay values for both Metformin and Empagliflozin were within pharmacopeial limits. These findings confirm that the method is suitable for routine quality control and batch release testing in pharmaceutical industries.

In conclusion, the validated RP-HPLC method provides a cost-effective, sensitive, and reliable analytical tool for the simultaneous estimation of Metformin Hydrochloride and Empagliflozin, supporting the growing need for quality assurance in fixed-dose combination therapies used for the treatment of Type 2 Diabetes Mellitus.

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