QUALITY BY DESIGN FRAMEWORK FOR THE OPTIMIZATION OF RP-HPLC SEPARATION OF METFORMIN HYDROCHLORIDE AND EMPAGLIFLOZIN

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ABSTRACT:
Background: Type 2 diabetes mellitus is a chronic metabolic condition affecting millions, often requiring combination therapies for effective glycemic control. The fixed-dose combination of Metformin hydrochloride (a biguanide) and Empagliflozin (an SGLT-2 inhibitor) is widely used due to their synergistic mechanisms that reduce glucose production, enhance insulin sensitivity, and promote urinary glucose excretion. Ensuring the quality and therapeutic efficacy of this combination requires analytical methods that are not only accurate but inherently robust.
Objective: The primary objective of this study was to develop and validate a selective, accurate, and precise RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride and Empagliflozin using a Quality by Design (QbD) approach.
Methodology: A QbD-based framework was employed, beginning with the definition of the Analytical Target Profile (ATP). Critical Method Parameters (CMPs), such as mobile phase composition and flow rate, were evaluated for their impact on Critical Quality Attributes (CQAs), including retention time, plate count and tailing factor. The column used for analysis was Inertsil C18, a strong peak shape chosen by ODS. It has been found that ambient temperature is well-suited to the nature of the drug solution. Due to the good peak area, adequate retention time, and good resolution, the flow rate was set to 1.0 mL /min. Different mobile phase ratios were tested; a methanol:Buffer (80:20) ratio was selected due to symmetrical peaks and good resolution.
Results: The method demonstrated excellent linearity for Metformin (range of 40-200 µg/mL) and Empagliflozin (range of 1-5 µg/mL), with correlation coefficients (R^2) exceeding 0.99. Accuracy was confirmed through recovery studies, yielding results between 99% and 101%. Precision studies showed a relative standard deviation (% RSD) of less than 2.0%, indicating high reproducibility.
Conclusion: The developed QbD-based RP-HPLC method is simple, cost-effective, and highly robust for the simultaneous determination of Metformin and Empagliflozin. This validated approach complies with ICH guidelines and is suitable for routine quality control analysis in pharmaceutical settings, offering a higher degree of method understanding and reliability than traditional trial-and-error development.
Keywords: Metformin, Empagliflozin, RP-HPLC, QbD, Validation, ICH Guidelines, Simultaneous Estimation.
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INTRODUCTION:

Quality by Design (QbD) is defined by the sources as a systematic, science-based approach to development that starts with predefined objectives and emphasizes a deep understanding of products and processes to ensure control based on sound science and quality risk management.(1,2) In analytical chemistry, this is referred to as Analytical Quality by Design (AQbD), a pre-validation methodology that establishes a comprehensive framework of method variables to incorporate robustness during the development phase rather than testing for quality after the method is finished.(3,4) The AQbD process typically begins with the definition of an Analytical Target Profile (ATP), specifying the measurement goals and performance requirements such as accuracy, precision, and range. This is followed by technique selection and risk assessment often using Ishikawa (fishbone) diagrams or Failure Mode Effect Analysis (FMEA) to identify Critical Method Parameters (CMPs) that significantly impact Critical Quality Attributes (CQAs). Design of Experiments (DoE) statistical tools, such as Box-Behnken, Central Composite, and Fractional Factorial designs, are then utilized to optimize these parameters and define the Design Space or Method Operable Design Region (MODR). Operating within this multidimensional space ensures method robustness and provides regulatory

flexibility, as performance criteria are prioritized over strict instrument settings.(5,6)

RP-HPLC method development and validation are critical in pharmaceutical analysis because they ensure the reliability, accuracy, and suitability of analytical techniques used for drug identification and quantification.(7) Since RP-HPLC offers high sensitivity, selectivity, and efficiency, it has become a cornerstone for separating complex mixtures and identifying drugs and their impurities in various matrices, including bulk substances, formulations, and biological fluids.(8) Method development is essential to improve analytical outcomes by reducing testing durations and increasing throughput, ease of use, and precision, which ultimately lead to long-term cost savings and reduced operator errors. Furthermore, developing sensitive approaches allows for the early detection of contaminants in upstream processes where remedial interventions are more economical. Validation is equally vital, as it provides credible evidence that a method consistently meets its intended purpose and complies with strict regulatory requirements from bodies such as the ICH and USFDA. Rigorous validation of parameters like specificity, linearity, and robustness ensures that the method is reliable for routine quality control, stability testing, and ensuring the safety and efficacy of pharmaceutical products.(9)

Metformin HCl is widely recognized as the preferred initial pharmacologic monotherapy for Type 2 diabetes mellitus (T2DM), primarily exerting its antihyperglycemic effects by suppressing hepatic gluconeogenesis. While it is generally well-tolerated without causing weight gain or significant hypoglycemia, common side effects involve gastrointestinal intolerance, and it carries a boxed warning for the rare risk of metformin-associated lactic acidosis, which contraindicates its use in patients with an eGFR below 45 mL/min/1.73 m^2. In contrast, Empagliflozin is a sodium-glucose cotransporter-2 (SGLT2) inhibitor that operates through an insulin-independent mechanism by inhibiting glucose reabsorption in the kidneys and increasing urinary glucose excretion. In addition to improving glycemic control, Empagliflozin is indicated to reduce the risk of major adverse cardiovascular events and slow the progression of kidney disease in adults with T2DM and established cardiovascular disease, though it is associated with an increased incidence of genital mycotic and urinary tract infections. When used together, often as a single-pill fixed-dose combination, these agents provide complementary modes of action that effectively lower glycated hemoglobin while offering the added benefits of modest reductions in body weight and blood pressure.(10–12)

The increasing use of multi-drug therapies to achieve synergistic therapeutic effects against conditions such as oral hypoglycaemic, inflammation, and infections has created a critical demand for robust analytical techniques. While individual methods for various active ingredients may exist, there is often a distinct lack of established techniques for their simultaneous quantification in combined dosage forms or complex delivery systems. Simultaneous RP-HPLC estimation significantly enhances analytical efficiency by allowing multiple drugs to elute in a single run, thereby reducing analysis time and costs while eliminating the need for tedious sample pretreatments. Therefore, optimized simultaneous methods are necessary to address specific analytical challenges, such as resolving compounds with disparate solubility profiles or stabilizing sensitive drugs through strategic adjustments to the mobile phase.(13–15)

Analytical methods for estimating Metformin and Empagliflozin encompass various UV spectrophotometric approaches, including simultaneous equation (Vierodt's), absorbance ratio (Q-analysis), area under curve (AUC), and first derivative (zero-crossing) techniques.(16–18) Chromatographic methods such as RP-HPLC are frequently utilized for bulk drug and tablet analysis, typically employing C18 columns with detection wavelengths set at 227 nm or 260 nm.(19–21) For biological matrices like human plasma, highly sensitive LC-MS/MS(22) and UPLC-MS/MS methods(23) are employed, often featuring specialized extraction techniques like solid-phase extraction (SPE) or freezing lipid precipitation to minimize matrix effects during pharmacokinetic studies. Furthermore, stability-indicating methods using RP-HPLC and UPLC-DAD have been developed to ensure accurate quantification in the presence of degradation products. Compared with previously reported analytical methods for empagliflozin and metformin HCl, the present RP-HPLC method is advantageous because of its simplicity, practicality, and suitability for routine quality control. Its PDA detection, simple mobile-phase composition, and efficient separation support accurate and reproducible simultaneous estimation.

MATERIALS AND METHODS:

Chemicals and Reagents:

HPLC-grade methanol, acetonitrile, and water, and analytical reagent (AR) grade potassium dihydrogen phosphate and orthophosphoric acid were used in the current RPHPLC method. All the chemicals and reagents were of AR or HPLC quality.

Instrumentation:

The chromatographic analysis was carried out using a Waters Model 2690/5 Compact HPLC system equipped with an Inertsil C18 ODS column with PDA (model 2996) detector. An electronic analytical balance and a sonicator were used to

weigh and degas the mobile phase and sample solutions, respectively. All chromatographic data were acquired and processed using the instrument's data acquisition software.

Analytical Target Profile (ATP)

It was established to develop a robust, reliable, and efficient RP-HPLC method for the simultaneous estimation of Metformin Hydrochloride and Empagliflozin in pharmaceutical dosage form. The method was designed to achieve complete separation of both analytes with a retention time of less than 10 min, resolution greater than 2.0, tailing factor less than 2.0, and theoretical plate count greater than 2000, thereby ensuring accurate, precise, and reproducible chromatographic performance suitable for routine quality control analysis.

Preparation of the standard solution:

Separately weighed 10 mg of metformin HCl and Empagliflozin and dissolved in 10 ml of mobile phase in individual volumetric flask to make 1000 PPM of each drug concentration. Drug solutions were sonicated for 20 minutes and further dilution were prepared as per the requirement of the study by diluting with mobile phase.

HPLC experimental conditions:

Several trials were taken for RPHPLC system for the simultaneous estimation of both the drugs. The mobile phase such as Acetonitrile:Methanol (80:20), Water and methanol (40:60), Acetonitrile:Methanol (50:50) were run to separate the both the drugs. The drug were separated but trails was fail due to peak merging and other several issues in the separation which are not under consideration of analytical target profile. Therefore for the perfect separation of both analyte methanol: buffer (pH 3.4). The method was evaluated and optimized based on the results of preliminary experiments, with emphasis on critical parameters; a 3^2 full factorial experimental design was applied to investigate the influence of mobile phase composition and flow rate on retention time, theoretical plate count and tailing factor.

Factorial design

A 3^2 full factorial design was used to improve the RP-HPLC method for simultaneous estimation of metformin HCl and Empagliflozin considering two independent variables such as concentration of methanol in mobile phase and flow rate. The methanol concentration was increased to three different values 60%, 70% and 80% (v/v with phosphate buffer) and the flow rate was examined at 0.8, 1.0 and 1.2 mL/min (table 1). This design produced nine experimental runs which provided a detailed investigation of the effect of methanol concentration and flow rate adjustments on significant chromatographic responses such as retention duration, theoretical plate count, and tailing factor. The factorial design methodology allowed to establish the best chromatographic conditions and to understand the interaction between the two variables, which guarantees a robust and dependable technique in the spirit of QbD. Surface plots and ANOVA were used to analyze the experimental data so as to visualize the interaction effects of methanol concentration and flow rate as well as to evaluate the statistical significance of each factor on the chromatographic results.

Table 1: Factors for independent variables

Factor	Name	Level	Low Level	Medium Level	High Level
A	Mobile phase composition (% of Methanol)	3	60	70	80
B	Flow Rate	3	0.8	1.0	1.2

Evaluation of Experimental Results:

In the view of ATP and factors selected for experimental design, the efficacy and performance of the developed method were evaluated by considering the retention time, theoretical plates and tailing factor as the main response parameters by assessing the experimental findings. The elution properties and separation of metformin HCl and Empagliflozin were observed by retention time to ensure that both analytes were well resolved within a suitable run time. The effectiveness of the columns was denoted by the theoretical plates; the more the theoretical plates, the better the separation performance and the lower the band broadening. The peak symmetry was assessed with the aid of the tailing factor. Values close to 1.0 indicate sharp, symmetric peaks, which are necessary for accurate quantification. The results were analyzed using factorial trials with ANOVA and surface plots to examine the effects of methanol concentration and flow rate.

Method Validation

System Suitability

System Suitability parameters are measured to ensure the entire analytical system is performing correctly before or during analysis. Key parameters evaluated include the retention time, theoretical plate count (a measure of column efficiency) and tailing factor (a measure of peak symmetry). Acceptable criteria, such as a tailing factor of less than 2 and high theoretical plate counts, must be met for the results to be valid.

Specificity:

The specificity was assessed according to the ICH guidelines by injecting blank and standard under the optimized RP-HPLC conditions. The

chromatograms were evaluated for interferences during the retention time of Metformin Hydrochloride and Empagliflozin.

Linearity and Range:

Linearity is established by creating calibration curves that demonstrate a direct proportionality between the concentration of the analytes and the peak area. For standard RP-HPLC methods, concentration ranges are often reported as 40–200 µg/mL for Metformine HCl and 1–5 µg/mL for EMPA. The correlation coefficient (*R*2) was greater than 0.999 for both drugs which confirm a strong linear relationship.

Accuracy (Recovery Studies)

Accuracy measures how close the experimental results are to the true or accepted reference value, often determined through recovery studies using the standard addition method. The studies were performed at three different concentration levels *viz.* 50%, 100%, and 150% of the target concentration.

Precision

Precision evaluates the degree of reproducibility between a series of measurements. It was categorized into Repeatability, Intra-day precision), which tests samples multiple times on the same day, and Intermediate Precision (Inter-day precision), which tests samples across different days, analysts, or instruments. Results are expressed as % %RSD. For a method to be considered precise, the %RSD values are expected to be less than 2%.

Sensitivity (LOD and LOQ):

The sensitivity of the analytical method was quantified by the Limit of Detection (LOD) and the Limit of Quantitation (LOQ).

Specificity and Selectivity

Specificity is the ability of the method to measure the analyte unequivocally in the presence excipients. This is often evaluated by comparing chromatograms of blank samples, placebo solutions, and standard solutions to ensure there is no interference at the retention times of MET and EMPA.

Robustness and Ruggedness

Robustness tests the method's capacity to remain unaffected by small but deliberate variations in experimental parameters. These variations may include changes in wavelength, pH, flow rate, column temperature, or mobile phase composition. Ruggedness evaluates the reproducibility of results under different conditions, such as using different instruments, different analysts, or different laboratories. In both cases, the resulting %RSD should remain within acceptable limits (typically < 2%) to prove method stability.

RESULTS AND DISCUSSION:

Factorial design

The current RP-HPLC method for simultaneous quantification of Metformin Hydrochloride and Empagliflozin was optimized using the 3^2 full factorial design (table 2) as a part of AQbD methodology. The design included two independent variables; methanol concentration (Factor A) and flow rate (Factor B). Each had three levels, low, medium and high. Methanol concentration was examined at 60%, 70% and 80% while flow rate was explored at 0.8, 1.0 and 1.2 mL/min which give 9 experimental runs. The impacts of these important technique parameters on three chromatographic responses, namely retention time (RT), theoretical plate count (PC) and tailing factor (TF) of both drugs have been studied. The best chromatographic condition for shortest retention time, maximum theoretical plate count and acceptable tailing factor were determined using analysis of variance (ANOVA) and response surface methods, thereby providing a robust, reliable and efficient analytical approach.

Table 2: Optimization of parameters of Metformin Hydrochloride and Empagliflozin by using 3^2 full factorial designs

	Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4	Response 5	Response 6
Run	A: Methanol Conc	B: Flow Rate	RT (MET)	PC (MET)	TF (MET)	RT (Empa)	PC (Empa)	TF (Empa)
1	60	1	4.5	8450	1.03	7.8	11320	1.02
2	80	1.2	3.05	8800	1.02	5.38	11850	1.01
3	70	0.8	4.8	8600	1.04	8.1	11500	1.02
4	60	1.2	3.75	8350	1.03	6.5	11200	1.02
5	70	1	4.1	8550	1.02	7.1	11450	1.01
6	80	1	3.662	8670	1.021	6.455	11635	1.015
7	70	1.2	3.4	8700	1.02	5.95	11600	1.01
8	60	0.8	5.6	8400	1.05	9.2	11150	1.03
9	80	0.8	4.6	8900	1.03	7.95	1190	1.02

							0	

Optimized Condition Obtained:

The optimum chromatographic conditions for the simultaneous estimation of Metformin Hydrochloride and Empagliflozin were established via 3² full factorial design and are shown in Table 3. Optimization was performed to maximize the theoretical plate count, minimize the tailing factor and get suitable retention durations for both analytes, all of which was assessed using a desirability function. Optimum conditions were 80% methanol and 1.0 mL/min flow rate, which gave the retention period of 3.739 min for Metformin Hydrochloride and 6.540 min for Empagliflozin. Theoretical plates were 8797.222 and 11797.500 for Metformin Hydrochloride and Empagliflozin respectively which shows great efficiency of the column. The tailing factors obtained was 1.022 for Metformin Hydrochloride and 1.014 for Empagliflozin, indicating symmetrical peak morphologies which are important for reliable measurement. The desirability score of 1.000 shows that these circumstances give the best tradeoff between all response variables.

Figure 2 is the contour plot of desirability of effect of different combination of methanol concentration and flow rate on the overall performance of the method. The darkest region of the plot represents the greatest desirability and is the ideal zone. The QbD based structural optimization strategy ensures the robustness, reliability and regulatory compliance of the proposed RP-HPLC technique.

Table 3: Obtained solution for optimized trail of Metformin Hydrochloride and Empagliflozin by using 3² full factorial design

Number	Methanol Conc	Flow Rate	RT (MET)	PC (MET)	TF (MET)	RT (Empa)	PC (Empa)	TF (Empa)	Desirability	
1	*80.000*	*1.000*	3.7739	8797.2222	1.0222	6.5400	11797.5000	1.0144	1.000	Selected

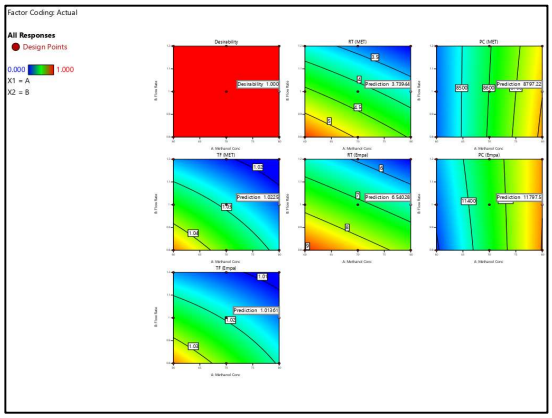

Figure 2: Contour plot of desirability for obtaining optimized trail

The effect of Retention Time of Metformin Hydrochloride:

The 2FI model for the RT of Metformin Hydrochloride was highly significant ($F = 96.02$, $p < 0.0001$). This indicates a good agreement of the experimental data with the predicted model. The RT was strongly affected by methanol concentration (A) ($F = 62.65$, $p = 0.0005$) and flow rate (B) ($F = 224.10$, $p < 0.0001$) where flow rate was the most effective factor. The RT decreased with increasing concentration of methanol from 60% to 80% and flow rate from 0.8 to 1.2 mL/min owing to the higher elution strength of the mobile phase and quicker migration of analytes across the column. The interaction between methanol concentration and flow rate (AB) was not significant ($p = 0.3037$), which means that both parameters impacted the retention time separately. The 3D response surface plot revealed a virtually flat falling surface thus verifying the linear impact of the two main technique parameters and very little interaction. The results show that quick analysis with good chromatographic performance may be achieved by proper tuning of methanol concentration and flow rate.

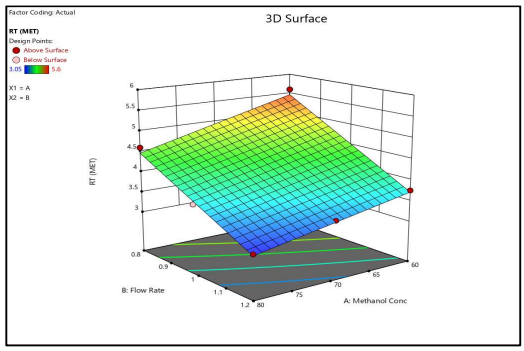

Figure 3: The effect of Retention Time of Metformin Hydrochloride

The effect of Theoretical plate of Metformin Hydrochloride:

The 2FI model for theoretical plates (PC) of Metformin Hydrochloride was significant (F = 8.85, p = 0.0192) indicating that the chromatographic parameters considered substantially affected the column efficiency. Methanol concentration (A) showed a significant positive influence (F = 26.43, p = 0.0036) whereas flow rate (B) (F = 0.0483, p = 0.8348) and the interaction term (AB) (F = 0.0724, p = 0.7986) were not significant. The 3D response surface and contour plots indicated that the theoretical plate count gradually increased from ~8350 to 8900 with increasing the methanol concentration from 60% to 80%, which meant higher chromatographic performance and enhanced column efficiency. The almost vertical contour lines and flat response surface further revealed that methanol concentration was the primary factor influencing theoretical plates and that flow rate change had little impact in the examined range. In general, the improved methanol concentration increased the number of theoretical plates and the positive effect on the chromatographic efficiency was not appreciably affected by the flow rate.

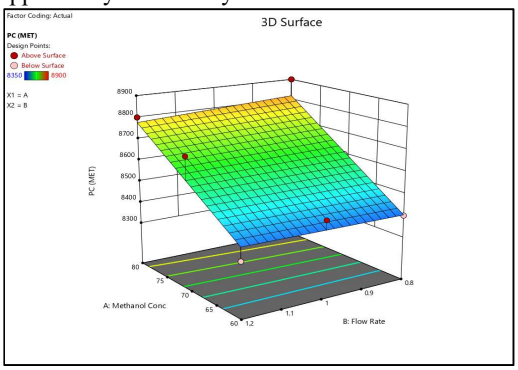

Figure 4: The effect of Theoretical plate of Metformin Hydrochloride

The effect of Tailing Factor of Metformin Hydrochloride:

The 2FI model for the tailing factor (TF) of Metformin Hydrochloride was significant (F = 6.55, p = 0.0349) showing that the developed chromatographic parameters had considerable effect on the peak symmetry. The tailing factor was significantly affected by methanol concentration (A) (F=7.17, p=0.0440) and flow rate (B) (F=11.78, p=0.0186), with flow rate having a considerably high impact. The interaction between methanol concentration and flow rate (AB) was not significant (F = 0.7069, p = 0.4388) indicating the parameters influenced the tailing factor separately. The 3D response surface and contour plots indicated that the tailing factor gradually decreased from around 1.05 to 1.02 with the rising methanol concentration (60–80%) and flow rate (0.8–1.2 mL/min), which confirmed that the peak symmetry was enhanced under the optimum circumstances.

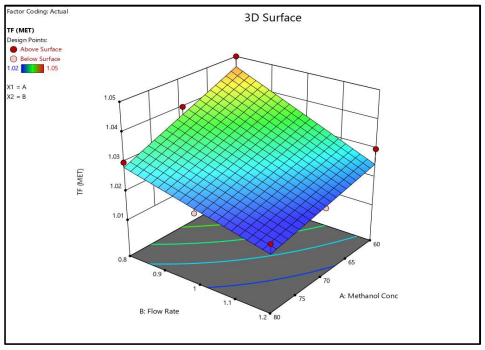

Figure 5: The effect of Tailing Factor of Metformin Hydrochloride

The effect of Retention Time of Empagliflozin:

The 2FI model for RT of Empagliflozin was very significant (F=114.49, p <0.0001), demonstrating a considerable effect of the specified chromatographic variables on the retention time. (A) Methanol concentration (F = 68.82, p = 0.0004) and (B) flow rate (F = 274.52, p < 0.0001) were significant model factors, with flow rate having the highest influence on retention time. Increasing the methanol concentration from 60 to 80% and the flow rate from 0.8 to 1.2 mL/min significantly decreased the retention time because of higher elution strength of the mobile phase and quicker migration of the analyte through the column. The interaction (AB) of methanol concentration and flow rate was not significant (F = 0.1264, p = 0.7367), which indicated that each factor had an independent effect on the retention time. The 3D response surface (figure 6) demonstrated a smooth, almost flat drop in retention time from about 9.2 min to 5.38 min, demonstrating the linear effects of the two crucial technique parameters with little interaction. These results indicate that methanol concentration and flow rate modification may allow fast elution of Empagliflozin with satisfactory chromatographic performance.

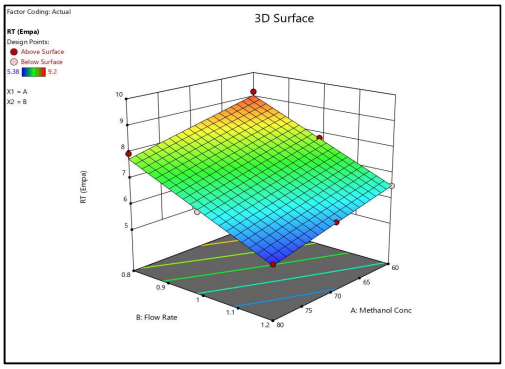

Figure 6: The effect of Retention Time of Empagliflozin

The effect of Theoretical plate of Empagliflozin:

The 2FI model for theoretical plates (PC) of Empagliflozin was significant (F = 13.18, p = 0.0083), which is an indication of the considerable effect of the chosen chromatographic parameters on column efficiency. Out of all studied parameters, methanol concentration (A) exhibited a significant positive influence (F = 39.20, p = 0.0015) whereas flow rate (B) (F = 0.1333, p = 0.7300) and interaction term (AB) (F = 0.1999, p = 0.6735) were not significant. The 3D response surface and contour plots showed that the rise of methanol concentration from 60% to 80% steadily raised the theoretical plate count from around 11150 to 11900, which indicated the improved column efficiency and chromatographic performance. In general, the adjustment of methanol concentration enhanced the theoretical plate count, leading to greater column efficiency and chromatographic separation of Empagliflozin.

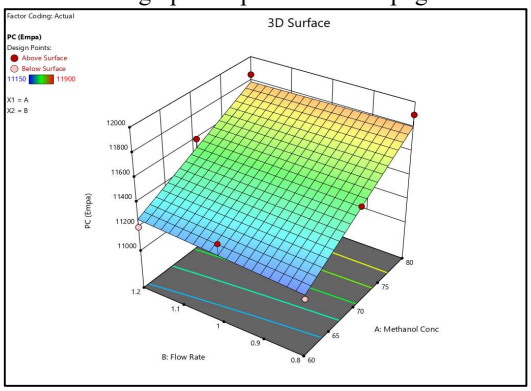

Figure 7: The effect of Theoretical plate of Empagliflozin

The effect of Tailing Factor of Empagliflozin:

The 2FI model for the tailing factor (TF) of Empagliflozin was significant (F = 6.10, p = 0.0399) and confirmed the effect of the chromatographic variables on the peak symmetry. The all significant factor among those tested was flow rate (B) (F=11.81, p=0.0185) while methanol concentration (A) revealed a modest but statistically non-significant influence (F=5.79, p=0.0612). The interaction of methanol concentration and flow rate (AB) was likewise not significant (F = 0.7087, p = 0.4383), demonstrating that two parameters had separate effects. 3D response surface (figure 8) revealed that the tailing factor reduced gradually from around 1.04 to 1.01 with the rising flow rate (0.8–1.2 mL/min) and methanol concentration had only a little affected.

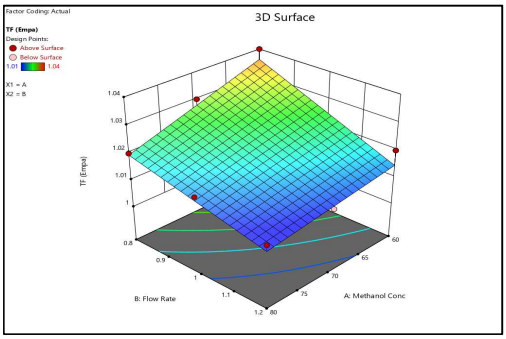

Figure 8: The effect of Tailing Factor of Empagliflozin

Analytical Method Validation

System Suitability

The performance of the chromatographic system was evaluated through system suitability parameters to ensure the reliability of the analytical procedure. Optimized conditions yielded well resolved peaks with distinct retention times. Retention times of 3.664 minutes for Metformin Hydrochloride and 6.459 minutes for Empagliflozin was achieved in the current developed method. Chromatographic performance was confirmed by high theoretical plate counts, often exceeding 8000 for Metformin Hydrochloride and 11000 for Empagliflozin, and tailing factors maintained between 1.01 and 1.03, well within acceptable analytical limits.

Table 4: System suitability for Metformin Hydrochloride and Empagliflozin

	Metformin Hydrochloride				Empagliflozin			
Injection	**RT**	**Peak Area**	**Plate count**	**Tailing**	**RT**	**Peak Area**	**Plate count**	**Tailing**
Mean	3.664	3085125	8623	1.01096	6.459	10968500	11851.6	1.01034
SD	0.001871	1770.545	------	------	0.004	585.3203	------	------
%RSD	0.05106	0.05739	------	------	0.06192	0.05336	------	------

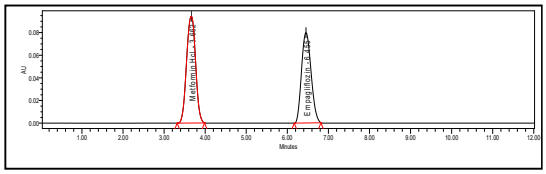

Figure 9: Suitability of the HPLC System Standard Chromatogram

Specificity:

The specificity studies revealed that the chromatograms of the samples of Metformin Hydrochloride and Empagliflozin showed a unique and favorable chromatogram. Conversely, the blank (diluent) showed no response or interference. This confirms the specificity of the approach (figure 10 and 11 for standard and blank respectively).

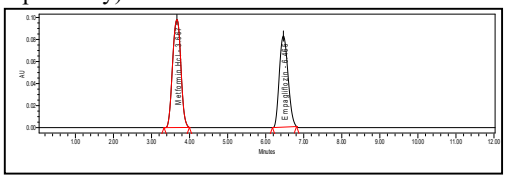

Figure 10: Chromatogram for Standard.

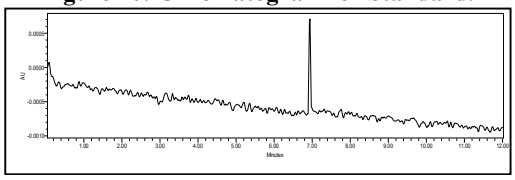

Figure 11: Chromatogram for blank injection

Linearity and Range

Linearity was established by analyzing a series of concentrations for both drugs, showing an excellent correlation between peak area and concentration. For Empagliflozin, linearity ranges were reported between 1–6 µg/mL with R^2 was 0.9993. Metformin Hydrochloride exhibited linearity over wider ranges, such as 40–240 µg/mL with R^2 was 0.9995. These results indicate that the methods follow Beer's Law precisely over the selected concentration intervals.

Table 5: Linearity results for Metformin Hydrochloride and Empagliflozin

Metformin Hydrochloride		**Empagliflozin**	
Concentration (ppm)	**Area (Linagliptin)**	**Concentration**	**Área**
0	0	0	0
40	1437638	1	56963
80	2236342	2	82693
120	3080291	3	10953
160	3822602	4	13635
200	4592637	5	15976
240	5291546	6	18634
Slope	76581	Slope	26475
y-Intercept	-30826	y-Intercept	24061
Correlation	0.999	Correlation	0.999

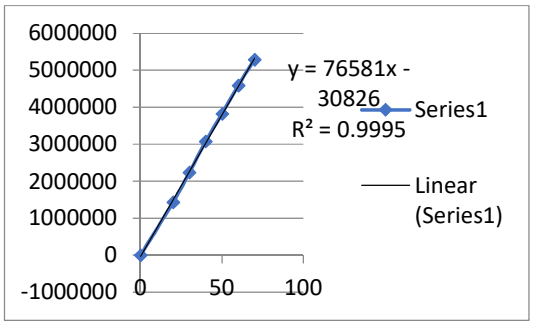

Figure 12: Linearity graph of Metformin Hydrochloride

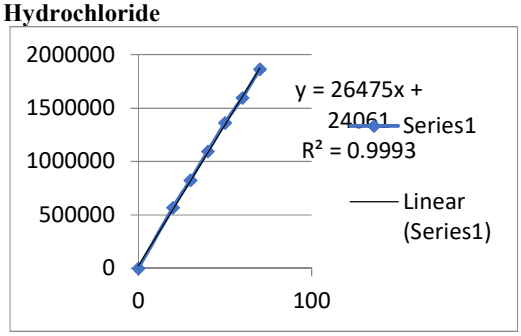

Figure 13: Linearity graph of Empagliflozin

Precision

The precision of the developed methods was verified through repeatability and intermediate precision studies. In all cases, the %RSD remained below 2%, demonstrating high reproducibility, confirming the methods are precise even when performed on different days.

Table 7: Repeatability data for Metformin Hydrochloride and Empagliflozin

Sr. No	**Metformin**		**Empagliflozin**	
	Area	**%Assa**	**Area**	**%Assa**
1.	308056	101.57	109632	101.25
2.	308456	101.70	109546	101.17
3.	308347	101.67	109347	100.98
4.	308375	101.68	109437	101.07
5.	308045	101.57	109235	100.88
Mean	308256	101.638	109439	101.07
SD	1917.17	0.06300	1569.56	0.14713
%RS	0.06219	0.06199	0.14341	0.14558

Table 8: Intermediated precision study for Metformin Hydrochloride

Analyst	**1**		**2**	
Sr. No	**AUC**	**Assay (%)**	**AUC**	**Assay (%)**
1.	3080634	101.57	3080564	101.57
2.	3080752	101.58	3080227	101.70
3.	3080185	101.56	3080342	101.67

4.	3080664	101.58	3080761	101.68
5.	3080741	101.57	3080784	101.57
Mean	3080595.2	101.572	3080536	101.638
SD	234.70556	0.008367	248.0067	0.063008
%RSD	0.00761884	0.008237	0.008051	0.061992

Table 9: Intermediated precision study for Empagliflozin

Analyst	**1**		**2**	
Sr. No	**AUC**	**Assay (%)**	**AUC**	**Assay (%)**
1.	1095660	101.19	1093487	100.98
2.	1094891	101.12	1091785	100.82
3.	1093378	100.97	1093621	100.10
4.	1092346	100.88	1095462	101.17
5.	1091178	100.77	1093237	100.96
Mean	1093170	100.955	1093141	100.80
SD	1812.814	0.171202	1493.042	0.370459
%RSD	0.165831	0.169582	0.136583	0.367519

Accuracy (Recovery Studies)

Method accuracy was assessed using the standard addition method at three different concentration levels (typically 50%, 100%, and 150%). The mean percentage recovery for both drugs was consistently within the acceptable range of 98% to 102%. Reported recovery values for Empagliflozin ranged from 99.07% to 100.33%, while Metformin Hydrochloride showed recoveries between 99.11% and 100.70%. These results verify that the methods are accurate and free from interference by formulation excipients.

Table 6: Accuracy study for Metformin Hydrochloride and Empagliflozin

Metformin Hydrochloride			
Concentration % of the spiked level	**% Recovery**	**Statistical Analysis of % Recovery**	
50 %	100.01	**Mean**	99.96
	99.92	**%RSD**	0.654
	99.90		
100 %	99.91	**Mean**	99.90
	99.94	**%RSD**	0.768
	99.12		
150 %	99.98	**Mean**	100.08
	100.05	**%RSD**	0.543
	100.03		
Empagliflozin			
50 %	99.92	**Mean**	99.99
	99.86	**%RSD**	0.964
	100.01		
100 %	99.96	**Mean**	99.98
	100.06	**%RSD**	0.784
	100.03		
150 %	99.93	**Mean**	99.96
	99.94	**%RSD**	0.876
	99.93		

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

The sensitivity of the methods was determined by calculating the LOD and LOQ based on the standard deviation of the response and the slope of the calibration curve. For Empagliflozin, LOD values were found as low as 0.073 µg/mL with LOQ at 0.22 µg/mL and for Metformin HCl LOD of 0.08 µg/mL and LOQ of 0.23 µg/mL.

CONCLUSION:

The research successfully established a simple, precise, and highly robust RP-HPLC method for the simultaneous estimation of Metformin hydrochloride and Empagliflozin in bulk and tablet dosage forms using a QbD framework. By defining the ATP and evaluating CMPs, the study ensured that chromatographic performance was inherently built into the method rather than merely tested after development. The method was comprehensively validated in accordance with ICH Q2 guidelines. Statistical analysis confirmed excellent linearity over the studied concentration ranges, with correlation coefficients (R^2) typically exceeding 0.99. Accuracy was verified through recovery studies, which yielded results between 98% and 102%, confirming the absence of interference from common pharmaceutical excipients. Precision was demonstrated by % RSD values of less than 2.0% indicating a high degree of repeatability and reproducibility. A central achievement of the QbD approach was the verification of method robustness. Furthermore, the separation of the two drugs distantly polar in nature was efficiently achieved on a C18 column with a short run time, making the method both time-saving and cost-effective. In summary, this QbD-based RP-HPLC method is selective, sensitive, and reliable. It is well-suited for routine quality control analysis in the pharmaceutical industry, offering a superior analytical tool for ensuring the therapeutic efficacy and quality of Metformin and Empagliflozin combination therapies.

CONFLICT OF INTEREST:

The authors declare that the there is no conflict of interest.

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