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QbD-Driven Analytical Method Development and Validation for Linagliptin and Empagliflozin by RP-HPLC

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ABSTRACT

The current investigation focused on the development and validation of an analytical method driven by Quality by Design (QbD) for the simultaneous analysis of Linagliptin and Empagliflozin utilizing RP-HPLC. A 2FI factorial design was utilized to identify and optimize key method variables, specifically the composition of the mobile phase (% methanol) and the flow rate. Methanol and acetonitrile were used as the mobile phase in an isocratic chromatographic separation process on a C18 column. For both medications, the wavelength of detection was set at 289 nm. Specificity, linearity, precision, accuracy, robustness, LOD, and LOQ were among the parameters that were examined during the validation process in accordance with the ICH Q2(R1) guidelines. The developed technique exhibited distinct, well-defined peaks for Linagliptin and Empagliflozin, with retention times within acceptable parameters. Linearity was confirmed across a concentration range of 20-80ug/ml for Empagliflozin and Linagliptin analysis, with correlation coefficients (R^2) of 0.999 for both compounds, demonstrating an outstanding linear response. The method showed high accuracy, with recovery rates ranging from 98–102%, and precision with %RSD values below 2%. The LOD and LOQ values verified the method's sensitivity. ANOVA analysis indicated that the mobile phase and flow rate significantly impacted retention time, theoretical plates, and tailing factors. No interference was detected in blank chromatograms, ensuring specificity. The method proved robust against minor deliberate variations in chromatographic conditions. The research effectively developed a QbD-based RP-HPLC technique that is straightforward, accurate, precise, and dependable for the concurrent estimation of Linagliptin and Empagliflozin. This method is appropriate for regular quality control assessments and stability investigations of pharmaceutical products.

Keywords: *RP-HPLC, QbD, Diabetes mellitus, Linagliptin, Empagliflozin, Analytical Method.*

1. INTRODUCTION

Any sample that can dissolve in a solvent can have its constituent compounds separated, identified, and quantified using the chromatography process[1]. QbD, is the process of achieving a specific, predictable quality with predefined, desired specifications. An extremely helpful aspect of QbD is the comprehension of factors and the effects of their interactions through a desired set of experiments[2]. Linagliptin is a medication taken by mouth that inhibits dipeptidyl peptidase 4 (DPP)-2. It was initially approved in the US, Europe, Japan, and several other regions in 2011 for enhancing blood sugar regulation in adults diagnosed with type 2 diabetes mellitus[3]. Like other DPP-4 inhibitors, linagliptin is a low-risk oral antidiabetic medication that does not cause weight gain and has a minimal risk of hypoglycemia[4]. In 2014, the oral hypoglycemic medication empagliflozin was approved and put into clinical use. It is classified as an inhibitor of sodium-glucose cotransporter 2 (SGLT2), which was developed to treat type 2 diabetes mellitus (T2DM). SGLT2 is a new target for T2DM treatment since it is the primary transporter responsible for glucose reabsorption from glomerular filtrate[5].

A literature survey revealed various methods has been developed for the determination of Empagliflozin including HPLC[6], UV-Spectrophotometry, UHPLC-QTOF-MS, HPLC-UV and Quadrupole Time-of-Flight Mass Spectrometry, and Linagliptin including HPLC, UV-Spectrophotometry, HPTLC and combination of Linagliptin and Empagliflozin by HPLC[7]. Although these techniques illustrate the viability of estimating each drug both separately and in combination, most have been created using traditional trial-and-error methods which may not include systematic risk evaluations studies. In the current

regulatory landscape, there is an increasing focus on analytical techniques that are not just accurate and precise, but also robust, reproducible, and capable of delivering consistent performance across varying conditions. This highlights the significance of applying the QbD approach. Consequently, even with existing methods for Linagliptin and Empagliflozin, pursuing the development of a QbD-based RP-HPLC method is warranted, as it provides a more reliable, robust, and lifecycle-manageable analytical tool that aids in effective product development, quality control, and regulatory compliance.

Chemicals and Reagents:

All chemicals and solvents utilized in this research were of analytical grade and met the necessary standards for HPLC and analytical applications. The dependability, accuracy, and precision of HPLC analysis heavily rely on the purity and quality of the reagents, solvents, and filtration materials employed during both sample preparation and analysis. To maintain data integrity and reproducibility, only high-purity solvents and reagents were used throughout the method development and validation stages.

2. METHODS

The chromatographic assessment was conducted using a Waters Model 2690/5 series HPLC system, which featured a Rheodyne manual injector (model 7725i) with a fixed loop volume of 20 µL. The setup included a quaternary gradient pump and a programmable UV/VIS detector (SPD-10AVP) for detection purposes.[8-12] Data collection and analysis were performed using Empower software, version 2. The chromatographic separation was accomplished utilizing an Inertsil C18 ODS column with dimensions of 250 mm × 4.6 mm and a particle size of 5 µm, ensuring effective resolution and retention for the analytes.

Preparation of the standard solution:

Accurately, 10 mg each of Linagliptin and Empagliflozin was measured separately and placed into two distinct 10 mL volumetric flasks. Approximately 7 mL of the mobile phase was added to both flasks, and the mixtures were subjected to sonication for 20 minutes to ensure thorough dissolution. The volume was then adjusted to the mark with the same mobile phase to create 1000 ppm (1000 µg/mL) standard stock solutions for each drug.[13-15]

HPLC experimental conditions:

A series of trials for HPLC method development were conducted utilizing a Waters Model 2690/5 series HPLC system along with a programmable variable wavelength UV/VIS detector (SPD-10AVP). Separation was achieved with an Inertsil ODS C18 column (250 × 4.6 mm, 5 µm particle size) at ambient temperature, using a flow rate of 1.0 mL/min and setting the detection wavelength at 289 nm. Several trials were performed using mobile phases of Water: Acetonitrile (90:10 v/v) and Water: Methanol (45:55 v/v). However, the peaks were partially merged and considered unsatisfactory, and the peak shapes remained suboptimal with additional enhancement needed for separation and retention times. Following the outcomes of these initial trials, the method was systematically evaluated and refined focusing on critical parameters; a 3^2 full factorial experimental design was utilized to investigate the impact of mobile phase composition (Methanol: Acetonitrile) and flow rate on retention time, theoretical plate count, and tailing factor.[16-18]

HPLC method development by QbD approach

The HPLC method was established utilizing a Quality by Design (QbD) framework, which incorporated systematic evaluation of risks, factorial design experiments, and statistical analysis via ANOVA and surface plots to pinpoint and refine essential method parameters, thereby ensuring robustness, accuracy, and adherence to regulatory standards.[19-22]

3. FACTORIAL DESIGN

To enhance the RP-HPLC technique for the concurrent analysis of Linagliptin and Empagliflozin, a 3^2 full factorial design was utilized, focusing on two independent variables: the concentration of methanol in the mobile phase and the flow rate. The methanol concentration was adjusted to three different levels including 60%, 70%, and 80% (v/v with acetonitrile), while the flow rate was evaluated at 0.8, 1.0, and 1.2 mL/min (table 1). This design led to nine experimental runs, enabling a thorough evaluation of how variations in methanol concentration and flow rate influenced critical chromatographic responses, including retention time, theoretical plate count, and tailing factor. The factorial design strategy facilitated the determination of optimal chromatographic parameters and offered insights into the interaction between the two variables, ensuring a method that is robust and reliable in accordance with QbD principles. The analysis of the experimental data was conducted using surface plots and ANOVA, which aided in visualizing the interaction effects of methanol concentration and flow rate while evaluating the statistical significance of each factor regarding the chromatographic responses.[23-26]

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 evaluating the experimental outcomes, retention time, theoretical plates, and tailing factor were identified as key response parameters to gauge the effectiveness and performance of the developed HPLC method. Retention time was utilized to observe the elution characteristics and separation of Linagliptin and Empagliflozin, ensuring that both analytes were sufficiently resolved within an appropriate run duration. Theoretical plates acted as an indicator of column efficiency, with a higher number of plates reflecting improved separation performance and reduced band broadening. Tailing factor was examined to evaluate peak symmetry, where values nearing 1.0 signify sharp and symmetrical peaks, crucial for precise quantification. During the factorial trials, these responses were assessed using ANOVA and surface plots to analyze the impact of methanol concentration and flow rate.[27]

Method Validation:

The validation of the analytical method for the simultaneous estimation of Linagliptin and Empagliflozin was performed following standard validation parameters. Calibration curves were created using standard solutions of Linagliptin and Empagliflozin at 20-80ug/ml to assess the linear relationship between peak area and analyte concentration, illustrating a strong correlation. Precision was evaluated through studies on repeatability and intermediate precision, demonstrating consistent results with low %RSD values. Accuracy was confirmed through recovery studies where 50%, 100% and 150% of standard were added to the sample matrix, and recoveries fell within the acceptable range of 98–102%, validating the method's reliability. Specificity was established by comparing the retention times of the sample peaks with those of the standard solutions, ensuring that the method could accurately differentiate Linagliptin and Empagliflozin from other components in the formulation.[28]

4. RESULTS AND DISCUSSION

Factorial design:

Table 2 summarizes the outcomes of a 3^2 full factorial design used to optimize chromatographic conditions for the simultaneous estimation of Linagliptin and Empagliflozin by RP-HPLC. The two independent variables examined were the % methanol in the mobile phase (60%, 70%, and 80%) and the flow rate (0.8, 1.0, and 1.2 mL/min). The dependent variables measured included retention time (RT), theoretical plate count (TPC), and tailing factor (TF) for both analytes. Results indicated that increasing the methanol concentration and flow rate led to decreased retention times for both drugs, resulting in faster elution. Theoretical plate count, reflecting column efficiency, varied across experiments, with higher values observed under optimized conditions, particularly at 70–80% methanol and a 1.0 mL/min flow rate. Tailing factors for both Linagliptin and Empagliflozin were mostly close to 1, signifying symmetrical peaks. Of all the experimental runs, the combination of 70% methanol and a 1.0 mL/min flow rate (Run 1) demonstrated the best overall chromatographic performance, producing sharp, well-resolved peaks, suitable retention times (4.71 min for Linagliptin and 6.691 min for Empagliflozin), high theoretical plate counts (7536.83 and 8358.88, respectively), and minimal tailing (1.063 and 1.060, respectively). Therefore, this condition was chosen as the optimized method for further validation.

Table 2: Optimization of parameters for analysis of Linagliptin and Empagliflozin by using 3^2 full factorial designs

Run	Factor 1 A:Mobile Phase (% Methanol)	Factor 2 B:Flow Rate	Response 1 RT (L)	Response 2 TPC (L)	Response 3 TF (L)	Response 4 RT (E)	Response 5 TPC (E)	Response 6 TF (E)
1	70	1	4.71	7536.83	1.063	6.691	8358.88	1.06
2	70	0.8	5.04	7150	1.08	6.88	7900	1.06
3	70	1.2	4.38	7300	1.07	6.29	8010	1.05
4	80	0.8	4.65	7650	1.05	6.72	8500	1.04

5	60	1	4.85	6905	1.1	6.76	7520	1.09
6	60	1.2	4.43	6730	1.09	6.31	7350	1.07
7	80	1.2	4.08	7600	1.02	6.01	8450	1.01
8	60	0.8	5.21	6800	1.12	7.14	7400	1.1
9	80	1	4.39	7800	1.03	6.38	8675	1.03

Optimized Condition Obtained:

Table 3 shows the optimized chromatographic conditions determined through a 3^2 full factorial design to achieve optimal results for simultaneous estimation of Linagliptin and Empagliflozin. The optimization focused on maximizing theoretical plate count, minimizing tailing factor, and ensuring appropriate retention times for both analytes, all evaluated using a desirability function. The ideal conditions were found to be 69.658% methanol with a flow rate of 0.843 mL/min, resulting in retention times of 4.911 min for Linagliptin and 6.862 min for Empagliflozin. Theoretical plate counts were 7255.947 and 7997.780, indicating high column efficiency. The tailing factors were 1.080 for Linagliptin and 1.067 for Empagliflozin, reflecting symmetrical peak shapes crucial for accurate quantification. A desirability score of 1.000 confirms these conditions provide the optimal balance among all response variables.

In Figure 1, the contour plot of desirability illustrates how various combinations of methanol concentration and flow rate influence the overall performance of the method, with the area of highest desirability shown as the darkest region representing the optimal zone. This structured optimization approach, grounded in Quality by Design (QbD), guarantees the robustness, reliability, and adherence to regulatory standards of the developed RP-HPLC method.

Table 3: Obtained solution for optimized trail of Linagliptin and Empagliflozin by using 3^2 full factorial design

Number	Mobile Phase (% Methanol)	Flow Rate	RT (L)	TPC (L)	TF (L)	RT (E)	TPC (E)	TF (E)	Desirability	
1	***69.658***	***0.843***	**4.911**	**7255.947**	**1.080**	**6.862**	**7997.780**	**1.067**	**1.000**	**Selected**

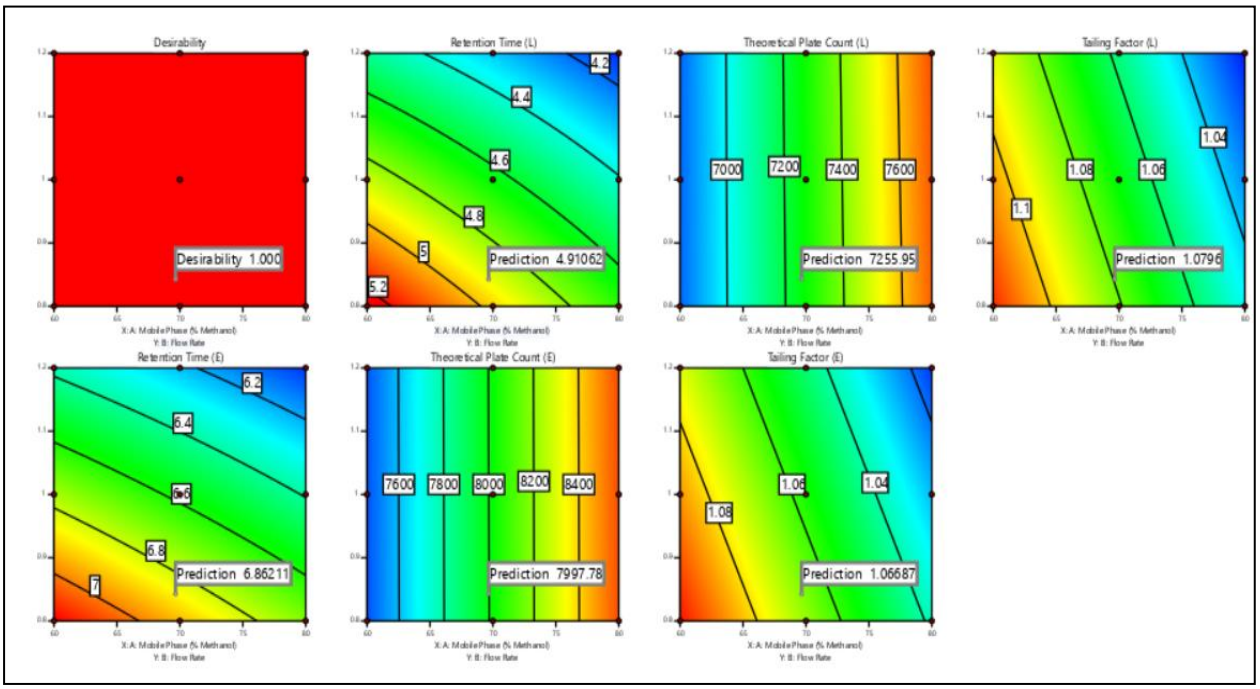

Figure 1: Contour plot of desirability for obtaining optimized trail

The effect of Retention Time of Empagliflozin:

The 3D surface plot shown in figure 3 illustrates how the composition of the mobile phase (% methanol) and the flow rate (mL/min) affect the retention time of Empagliflozin. It clearly shows that increasing the flow rate results in a decrease in retention time, suggesting that the analyte travels more quickly through the column when the flow rate is higher. Furthermore, a rise in the methanol percentage in the mobile phase also leads to a shorter retention time, which can be attributed to the stronger elution capacity of methanol, thus diminishing Empagliflozin's interaction with the stationary phase. The color gradient in the plot transitions from blue (indicating lower retention times around 4.08 minutes) to red (signifying higher retention times up to 5.21 minutes), illustrating how different experimental conditions affect the results. The red and pink points on the graph represent the design points from experiments that are either above or below the predicted surface, respectively, underscoring the reliability of the model fit. In summary, the plot indicates that both the concentration of methanol and the flow rate have a substantial effect on the retention time of Empagliflozin, with shorter retention times observed at increased methanol concentrations and flow rates, which is essential for optimizing methods in chromatographic analysis.

The ANOVA analysis for the 2FI model regarding the Retention Time of Empagliflozin indicates that the overall model is statistically significant (F-value = 56.75, p = 0.0003). Both the mobile phase (% methanol) and flow rate are important factors influencing retention time, with p-values of 0.0019 and <0.0001 respectively. The minimal residual error reinforces the model's dependability.

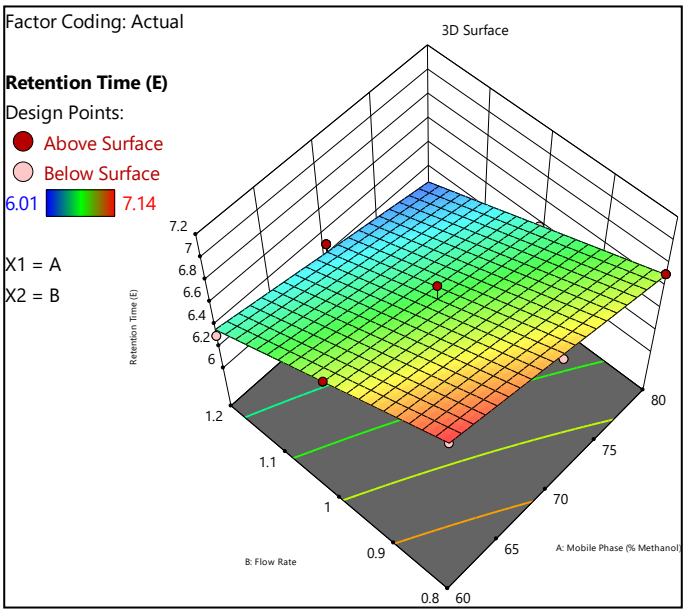

Figure 2: The effect of Retention Time of Empagliflozin

The effect of Theoretical plate of Empagliflozin:

The 3D surface plot demonstrates how the composition of the mobile phase (% methanol) and the flow rate affect the theoretical plate count of Empagliflozin, which serves as an indicator of column efficiency in chromatographic separation. From figure 4, it is clear that both factors have a significant impact on the theoretical plate count. An increase in methanol percentage combined with a decrease in flow rate results in a noticeable rise in the theoretical plate count, suggesting improved separation efficiency. The plot displays a gradient transitioning from blue (indicating lower plate counts around 7350) to red (indicating higher plate counts peaking at about 8675), showcasing the range of efficiency attained under various conditions. Design points positioned above and below the surface (marked by red and pink spheres, respectively) underscore the model's predictive accuracy. In summary, the figure reinforces that optimal chromatographic performance for Empagliflozin occurs with elevated methanol concentrations and reduced flow rates, as these conditions enhance the interaction between the analyte and the stationary phase, resulting in sharper and more distinct peaks.

The ANOVA for the 2FI model indicates that the model is statistically significant (F = 17.28, p = 0.0045), demonstrating its ability to explain variations in theoretical plate count reliably. Among the factors analyzed, only the mobile phase composition (% methanol) is markedly significant (p = 0.0008), demonstrating a strong effect on the response variable. On the other hand, the flow rate (p = 0.9837) and its interaction with methanol (p = 1.0000) do not exhibit significance, implying they have little to no impact. Consequently, it may be worth considering the refinement of the model by eliminating non-significant terms while maintaining hierarchy.

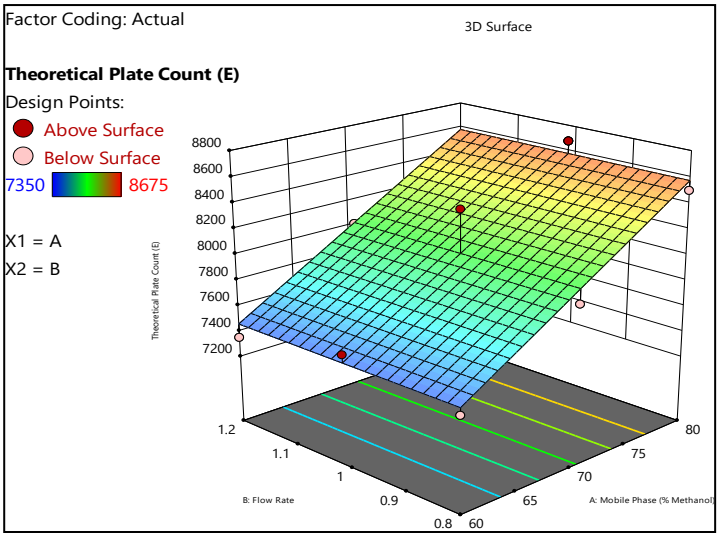

Figure 3: The effect of Theoretical plate of Empagliflozin

The effect of Tailing Factor of Empagliflozin:

The data illustrated in figure 4 clearly shows that both parameters have a slight effect on the tailing factor. Increasing the flow rate and the percentage of methanol tends to cause a minor rise in the tailing factor, as demonstrated by the gradual transition in color from blue (indicating lower tailing) to red (indicating higher tailing). Nevertheless, the variation remains limited (ranging from 1.01 to 1.1), suggesting that the peak shape stays relatively symmetrical under the tested conditions. The design points represented in red (above the surface) and pink (below the surface) further confirm the model's precision in predicting the response. Overall, the tailing factor stays within acceptable bounds across the various chromatographic conditions examined, indicating a robust method in terms of peak symmetry.

The ANOVA results indicate that the overall model is statistically significant, featuring an F-value of 56.52 and a p-value of 0.0003, which reflects only a 0.03% likelihood that such a high F-value could arise from random variation. For the individual factors, both A (Mobile Phase % Methanol) and B (Flow Rate) show strong significance with p-values less than 0.05, implying they have a substantial effect on the tailing factor of Empagliflozin.

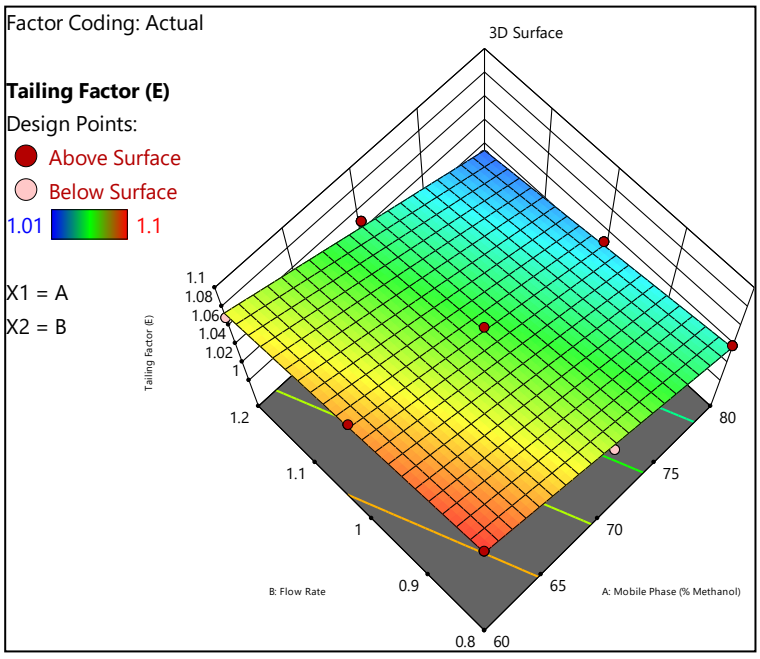

Figure 4: The effect of Tailing Factor of Empagliflozin:

The effect of Retention Time of Linagliptin:

The 3D surface plot (figure 5) demonstrates how the composition of the mobile phase (% methanol) and flow rate affect the retention time of Linagliptin. As illustrated in figure 5, an increase in either the methanol percentage or flow rate leads to a reduction in retention time. Lower levels of methanol and slower flow rates result in increased retention times, suggesting that Linagliptin stays on the column longer under these circumstances. In contrast, higher methanol concentrations and faster flow rates shorten the retention time, causing quicker elution. The smooth transition from red (indicating high retention time) to blue (indicating low retention time) effectively showcases this pattern. The design points, represented as red and pink dots, closely match the surface, confirming the model's accuracy. This analysis underscores the considerable impact of both variables on chromatographic behavior, which is essential for optimizing methods.

The ANOVA analysis for the 2FI model indicates that the model employed to forecast the retention time of Linagliptin is statistically significant, exhibiting an overall F-value of 67.66 and a p-value of 0.0002. This shows that the model is highly dependable, presenting only a 0.02% likelihood that this outcome is due to random chance. Both individual factors, Factor A (Mobile Phase: % Methanol) and Factor B (Flow Rate), are statistically significant, with p-values of 0.0005 and <0.0001, respectively, which reinforces that they exert a strong and independent influence on the retention time of Linagliptin.

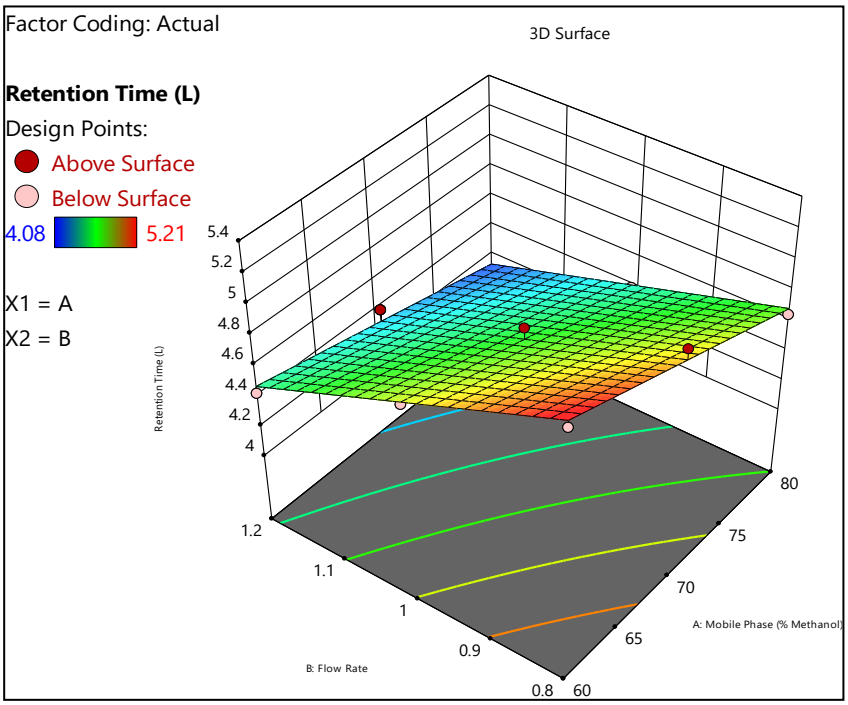

Figure 5: The effect of Retention Time of Linagliptin

The effect of Theoretical plate of Linagliptin:

The 3D surface plot (figure 6) displays how the composition of the mobile phase (% methanol) and the flow rate affect the theoretical plate count of Linagliptin, which indicates the efficiency of the column. The plot reveals a distinct upward trend in theoretical plate count as methanol concentration increases, suggesting that higher organic content leads to better column efficiency. In contrast, a lower flow rate also results in a greater number of theoretical plates, thereby improving the quality of separation. The color gradient, ranging from blue (indicating lower plate count) to orange (indicating higher plate count), visually reinforces this pattern. The peak efficiency is seen with high methanol concentration combined with a low flow rate, while the lowest efficiency occurs with low methanol and high flow rate. The design points (represented by red and pink dots) affirm the model's precision by aligning closely with the predicted surface. This figure illustrates that fine-tuning both the mobile phase composition and the flow rate is essential for optimizing column efficiency in the analysis of Linagliptin.

The ANOVA analysis for the two-factor interaction model shows that it is statistically significant in predicting the retention time of Linagliptin (F-value = 67.66, p = 0.0002). The mobile phase (% methanol) and flow rate both have a significant impact on retention time, with p-values of 0.0005 and <0.0001, respectively.

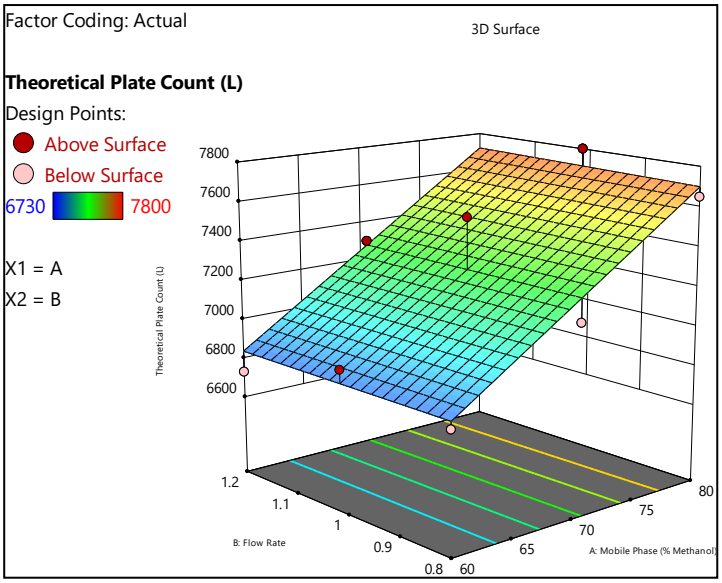

Figure 6: The effect of Theoretical plate of Linagliptin

The effect of Tailing Factor of Linagliptin:

The 3D surface plot in figure 7 depicts how the composition of the mobile phase (% methanol) and the flow rate influence the tailing factor of Linagliptin. It is clear from the plot that both an increase in methanol content in the mobile phase and a decrease in flow rate lead to a reduction in the tailing factor. The color gradient, ranging from red (indicating a higher tailing factor) to blue (indicating a lower tailing factor), effectively illustrates this relationship. The minimum tailing factor, approximately 1.02, occurs at elevated methanol concentrations and reduced flow rates, while a maximum tailing factor, around 1.12, is seen with decreased methanol levels and increased flow rates. The design points situated above and below the surface validate the model's ability to predict outcomes. This plot indicates that optimal peak symmetry for Linagliptin can be obtained by enhancing the organic modifier concentration and decreasing the flow rate.

The ANOVA analysis assessing the Tailing Factor (L) of Linagliptin reveals that the model is statistically significant, with an F-value of 49.16 and a p-value of 0.0004. This indicates a very low likelihood (0.04%) that the observed result is attributable to random chance. Among the factors examined, A (Mobile Phase % Methanol) and B (Flow Rate) have a significant impact on the tailing factor, with p-values below 0.05. Factor A demonstrates a notably strong effect (F = 132.72), whereas Factor B also displays a substantial influence (F = 14.75).

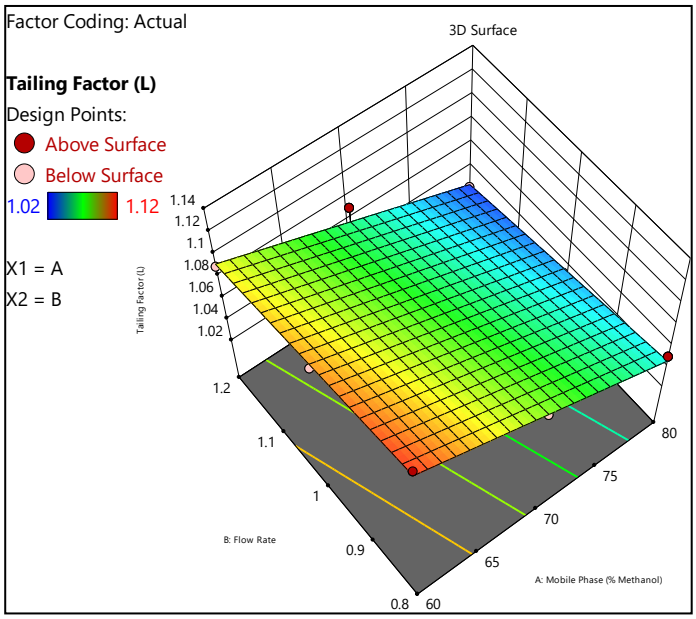

Figure 7: The effect of Tailing Factor of Linagliptin

5. ANALYTICAL METHOD VALIDATION

System suitability:

The collected information validates that the HPLC system operated effectively and produced dependable outcomes, as all system suitability parameters listed in Table 4 were found to fall within the predetermined acceptance criteria. This suggests the method's reliability and the instrument's excellent performance throughout the examination (figure 8).

Table 4: System suitability for Lobeglitazone Sulphate.

Sr. No.	Parameter	Linagliptin	Empagliflozin
1.	Theoretical plates (N)	7536.83	8358.88
2.	Retention Time (RT)	4.707	6.684
3.	Tailing factor (T)	1.063	1.060

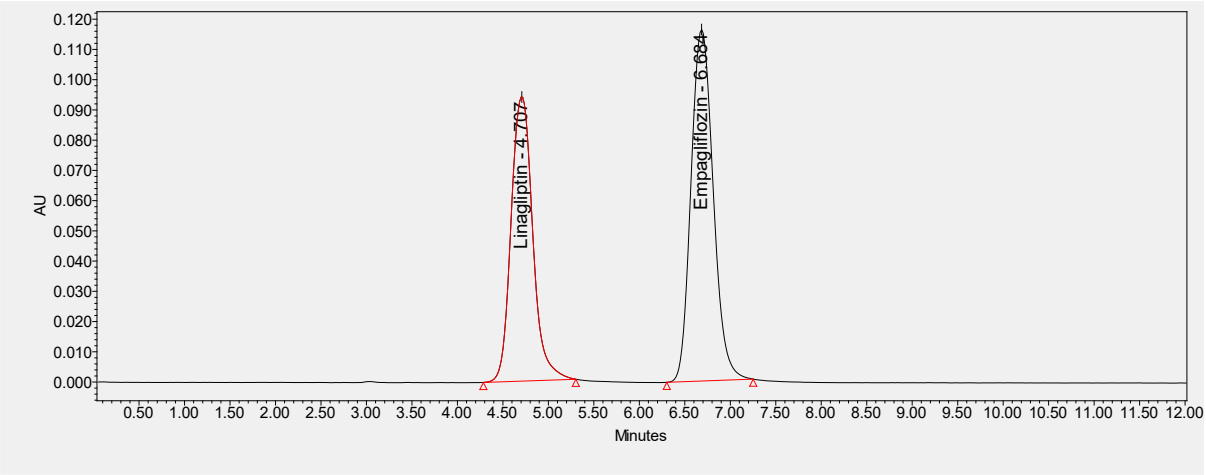

Figure 8: Suitability of the Device Standard Chromatogram

Specificity:

The specificity study indicated that the chromatograms for the Linagliptin and Empagliflozin samples displayed a distinct and positive response when compared to the reference standards. Conversely, the blank (diluent) presented no response or interference. This validates the method's accuracy and selectivity. Figures 9 and 10 illustrate the chromatograms of the standard and blank injections, respectively.

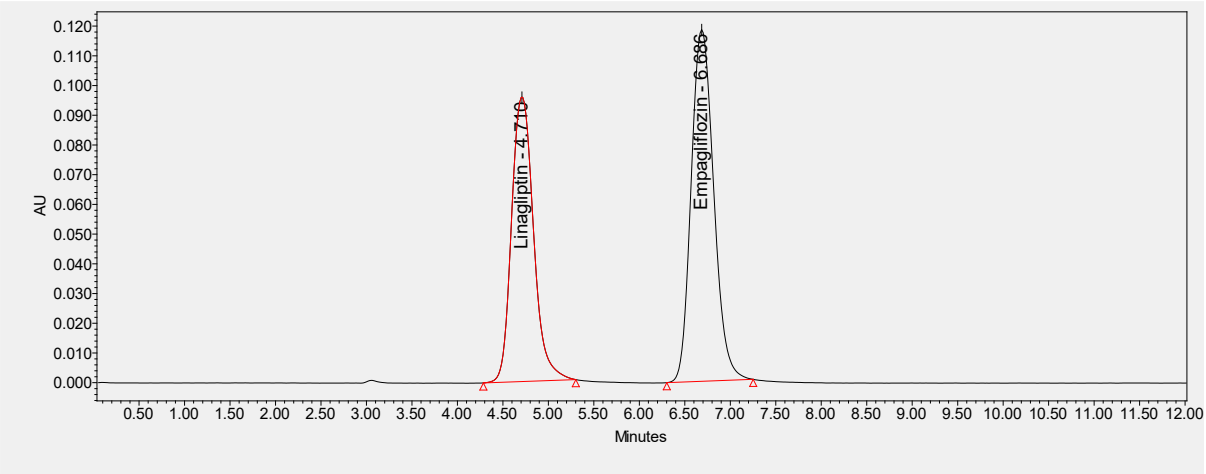

Figure 9: Chromatogram for Standard.

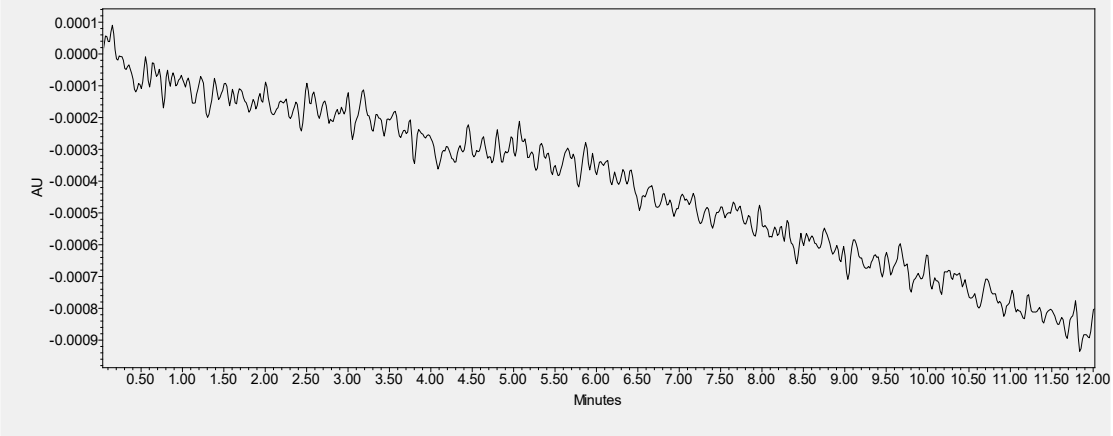

Figure 10: Chromatogram for blank injection

Linearity:

The developed HPLC method demonstrated linearity for both Linagliptin and Empagliflozin within the concentration range of 20–80 µg/mL. Each drug was analyzed at six different concentrations, and their peak areas were graphed against the corresponding concentrations. The calibration curves for both compounds exhibited a robust linear relationship with a regression coefficient (R^2) of 0.999, signifying that the method is very linear and appropriate for the accurate quantification of both Linagliptin and Empagliflozin (refer to Table 5 and Figures 11 and 12).

Table 5: Linearity results for Linagliptin and Empagliflozin

Concentration (ppm)	Area (Linagliptin)	Area (Empagliflozin)
20	197821	1130470
30	296731	1695705
40	395642	2260940
50	489132	2792153
60	593463	3391410
70	692373	3656645
80	791284	4521880
Slope	9884	56481
y-Intercept	-380.3	2387.5
Correlation Coefficient	0.999	0.999

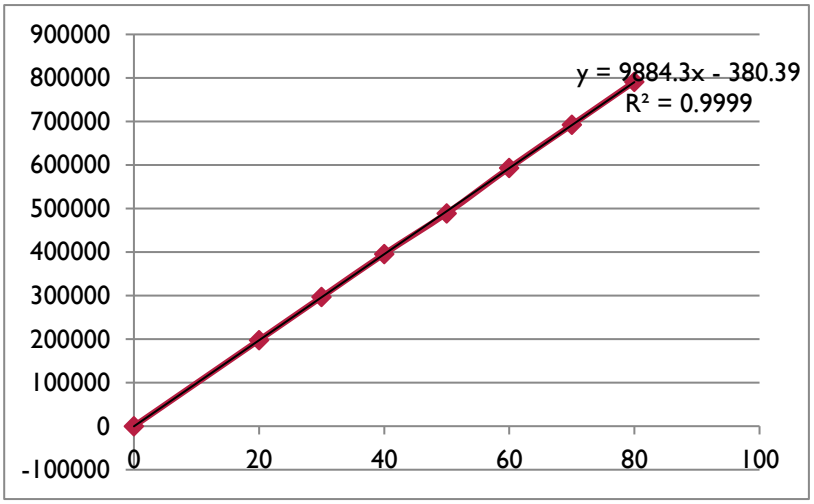

Figure 11: Linearity graph of Linagliptin

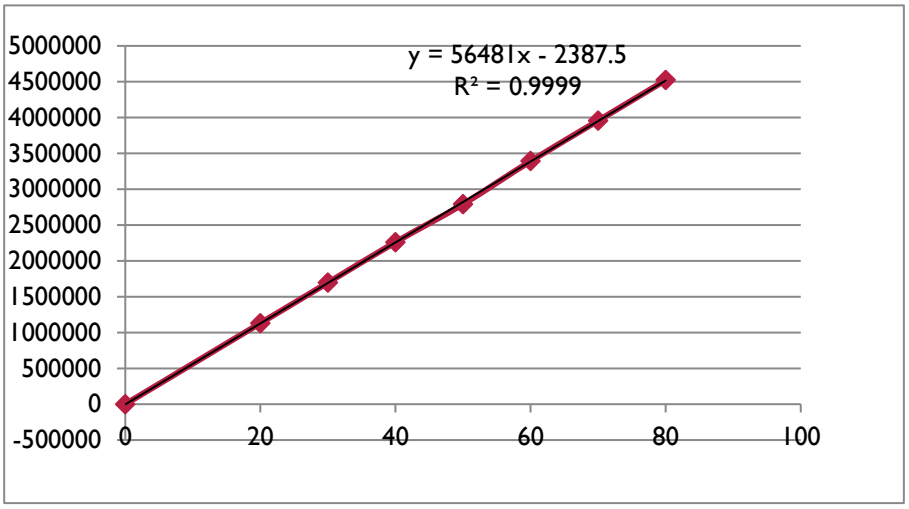

Figure 12: Linearity graph of Empagliflozin

Accuracy:

The method's accuracy was assessed via recovery studies conducted at three distinct concentration levels (usually 50%, 100%, and 150% of the target concentration). The average recovery values for both Linagliptin and Empagliflozin fell within the permissible range of 98% to 102%, demonstrating that the method is both accurate and dependable for measuring the quantities of both drugs in pharmaceutical formulations (Tables 6).

Table 6: Accuracy study for Linagliptin and Empagliflozin

Linagliptine					
Concentration % of the spiked level	**Amount added (ppm)**	**Amount found (ppm)**	**% Recovery**	**Statistical Analysis of % Recovery**	
50 %	20	20.03	100.27	**Mean**	100.18
		20.03	100.17	**%RSD**	0.096
		20.01	100.08		
100 %	40	40.07	100.19	**Mean**	100.15
		40.05	100.12	**%RSD**	0.034
		40.06	100.15		
150 %	60	60.08	100.13	**Mean**	100.17
		60.10	100.17	**%RSD**	0.034
		60.12	100.20		
Empagliflozin					
50 %	20	20.05	100.28	**Mean**	100.31
		20.06	100.32	**%RSD**	0.030
		20.06	100.34		
100 %	40	40.15	100.38	**Mean**	100.41
		40.21	100.58	**%RSD**	0.104
		40.13	100.33		

150 %	60	60.09	100.15	**Mean**	100.17
		60.10	100.18	**%RSD**	0.017
		60.10	100.18		

Precision:

The relative standard deviation (RSD) for the repeatability of the standard preparation was determined to be under 2%, demonstrating excellent reliability. This low RSD value verifies that the HPLC system produces dependable and consistent results with repeated injections of standard solutions. Consequently, the established HPLC method is appropriate and robust for the routine quantitative analysis of Empagliflozin and Linagliptin in various sample types (Table 7).

Table 7: Repeatability data for Linagliptin and Empagliflozin

Sr. No	**Linagliptin**		**Empagliflozin**	
	Area	**%Assay**	**Area**	**%Assay**
1.	395421	100.19	2264848	100.35
2.	395748	100.11	2263398	100.28
3.	395864	100.22	2265848	100.39
4.	395660	100.17	2264588	100.34
5.	395508	100.13	2265650	100.38
	395285	100.07	2266875	100.44
Mean	395581	100.15	2265201	100.36
SD	215.760	0.054	1195.804	0.052
%RSD	0.054	0.054	0.052	0.053718

Intermediate precision:

The data displaying the % RSD values are shown in Table 8 and 9.

Table 8: Intermediated precision study for Linagliptin

Analyst	**1**			**2**		
Sr.No	**Conc. (µg/ mL)**	**AUC**	**Assay (%)**	**Conc. (µg/ mL)**	**AUC**	**Assay (%)**
1.	40ppm	395480	100.12	40ppm	2026885	100.37
2.		395846	100.21		2028854	100.47
3.		395445	100.11		2027845	100.42
4.		395560	100.14		2023846	100.22
5.		395609	100.15		2027845	100.42
Statistical Analysis	**Mean**	395551	100.14	**Mean**	2026775	100.37
	SD	167.472	0.042	**SD**	1852.73	0.091
	%RSD	0.042	0.042	**%RSD**	0.091	0.091

Table 9: Intermediated precision study for Empagliflozin

Analyst	**1**			**2**		
Sr.No	**Conc. (µg/ mL)**	**AUC**	**Assay (%)**	**Conc. (µg/ mL)**	**AUC**	**Assay (%)**
1.	40ppm	2266544	100.42	40ppm	2264550	100.34
2.		2267878	100.48		2266641	100.43
3.		2265850	100.39		2265568	100.38
4.		2266982	100.44		2267064	100.45
5.		2268740	100.52		2264874	100.35
Statistical Analysis	**Mean**	2267183	100.45	**Mean**	2266091	100.40
	SD	1013.756	0.044	**SD**	1301.467	0.057
	%RSD	0.044	0.044	**%RSD**	0.057	0.057

Sensitivity:

Both Linagliptin and Empagliflozin demonstrated good sensitivity according to the method, as the LOD and LOQ values indicate the method's appropriateness for trace-level detection and quantification (table 10).

Table 10: LOD and LOQ values

	Linagliptin	Empagliflozin
LOD (ug/ml)	0.070	0.096
LOQ (ug/ml)	0.212	0.293

6. CONCLUSION

A QbD-driven analytical technique was successfully developed and validated for the simultaneous measurement of empagliflozin and linagliptin using RP-HPLC. To ensure robustness and dependability, a DoE approach was used to modify important method parameters, such as the composition of the mobile phase and flow rate. The technique met all ICH validation requirements and demonstrated remarkable linearity, accuracy, precision, specificity, and sensitivity. The method's sensitivity was validated by the low LOD and LOQ, and its accuracy and dependability were confirmed by recovery and repeatability studies. The technique is reliable and suitable for regular quality control analysis of pharmaceutical dosage forms and bulk forms of linagliptin and empagliflozin.

7. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

8. AUTHOR CONTRIBUTIONS

All authors have contributed equally.

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