RESEARCH PAPER

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF EMPAGLIFLOZIN USING QBD-APPROACH

Vipul M. Patil[1*], Prathmesh R. Demanna[2], Rushikesh V. Kadam[2], Shradha Lokare[3], Sindhutai Shedbale[3], Vivek U. Powar[2], Avdhut Talwar[4]

[1]Professor, Department of Pharmaceutical Quality Assurance, Ashokrao Mane College of Pharmacy, Peth-Vadgaon, Kolhapur 416112

[2]Research Student, Department of Pharmaceutical Quality Assurance, Ashokrao Mane College of Pharmacy, Peth-Vadgaon, Kolhapur 416112

[3]Lecturer, Women's College of Pharmacy, Peth-Vadgaon, Kolhapur 416118

[4]Research Student, Department of Pharmacology, Ashokrao Mane College of Pharmacy, Peth-Vadgaon, Kolhapur 416112

***Corresponding author: Vipul M. Patil, Professor, Department of Pharmaceutical Quality Assurance, Ashokrao Mane College of Pharmacy, Peth-Vadgaon, Kolhapur 416112**

Email: vipulpatil@amcoph.org

ABSTRACT

The present research work aimed to develop and validate a simple, rapid, accurate, precise, and stability-indicating RP-HPLC method for the estimation of Empagliflozin by applying the Analytical Quality by Design (AQbD) approach. The method development was carried out using systematic experimental design to optimize critical chromatographic parameters and to achieve robust analytical performance. Critical method variables such as mobile phase composition and flow rate were optimized using Design of Experiments (DoE) methodology. Their effects on chromatographic responses including retention time, peak area, theoretical plates, and tailing factor were evaluated statistically. Chromatographic separation was achieved using a C18 column with optimized mobile phase under isocratic conditions and detection was performed using UV detector at suitable wavelength. The developed method was validated according to ICH guidelines for parameters such as linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ). The method exhibited excellent linearity over the selected concentration range with satisfactory regression coefficient values. Accuracy and precision results were found within acceptable limits, indicating reliability of the method. Stability studies were performed under acidic, alkaline, oxidative, thermal, and photolytic stress conditions to evaluate the stability-indicating nature of the method. Empagliflozin showed degradation under different stress conditions, while the developed method successfully separated the drug peak from degradation products without interference. The results demonstrated that the proposed AQbD-based RP-HPLC method is simple, robust, reproducible, and suitable for routine quality control analysis and stability studies of Empagliflozin in bulk drug and pharmaceutical dosage forms.

Keywords: Empagliflozin, RP-HPLC, Stability-Indicating Method, Quality by Design (QbD), Analytical Method Optimization, Pharmaceutical Analysis.

How to cite this article: Patil VM, Demanna PR, Kadam RV, Lokare S, Shedbale S, Powar VU, Talwar A. Stability Indicating RP-HPLC Method Development and Validation of Empagliflozin Using QbD-Approach. Int J Drug Deliv Technol. 2026;16(57s): 1224-1239. DOI: 10.25258/ijddt.16.57s.120

Source of support: Nil.

Conflict of interest: None.

Introduction:

Quality by Design (QbD) is a systematic and scientific approach used in pharmaceutical development to ensure quality, reliability, and robustness of methods. Analytical Quality by Design (AQbD) involves predefined objectives, risk assessment, and optimization of critical method parameters using Design of Experiments (DoE). In RP-HPLC, factors such as mobile phase composition, flow rate, and pH can affect chromatographic performance. AQbD helps in understanding the effect of these variables on analytical responses like retention time, peak area, and resolution. Compared to conventional trial-and-error methods, AQbD provides better method

understanding, minimizes variability, and improves robustness and reproducibility. Therefore, AQbD was applied in the present study for the development and validation of a stability-indicating RP-HPLC method for Empagliflozin according to ICH guidelines.[1,2]

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is one of the most commonly employed analytical techniques in pharmaceutical analysis because of its high sensitivity, specificity, accuracy, reproducibility, and rapid separation capability. A stability-indicating RP-HPLC method is particularly important because it can effectively separate the active pharmaceutical ingredient from degradation products, impurities, and excipients formed under various stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic degradation. Stability studies play a vital role in determining the intrinsic stability of drug substances and ensuring the quality, safety, and efficacy of pharmaceutical products throughout their shelf life.[3]

Empagliflozin was selected due to its wide therapeutic use in the treatment of Type 2 Diabetes Mellitus and its growing pharmaceutical importance. As the drug may undergo degradation under different stress conditions, a reliable stability-indicating analytical method is necessary for routine quality control.[4] Moreover, limited AQbD-based RP-HPLC methods are available for Empagliflozin analysis. Therefore, the present study focuses on development and validation of a robust, accurate, and stability-indicating RP-HPLC method for Empagliflozin using QbD approach according to ICH guidelines.[5]

Zinman et al. (2015) reported the therapeutic importance of Empagliflozin in the treatment of Type 2 Diabetes Mellitus and highlighted its cardiovascular benefits. Several RP-HPLC methods have been reported for estimation of Empagliflozin in pharmaceutical dosage forms; however, most methods were developed using conventional trial-and-error approaches. Beg et al. (2015) and *Rozet et al. (2013)* emphasized the advantages of Analytical Quality by Design (AQbD) in chromatographic method development, including improved robustness, reliability, and method understanding through Design of Experiments (DoE). Literature survey revealed that limited AQbD-based stability-indicating RP-HPLC methods are available for Empagliflozin. Therefore, the present study focuses on development and validation of a robust stability-indicating RP-HPLC method for Empagliflozin using QbD approach according to ICH guidelines.[6,7]

Materials and method:

Instrumentation:

The analysis of the drug was carried out Agilent Technologies 1260 Infinity II with Quaternary Gradient HPLC Pump and Photo Diode Array Detector using a Reverse phase HPLC column. The output of signal was monitored and integrated using Chemstation Chromatogram Software.

Chemicals and Reagents :

The chemicals and reagents used during the development and validation of the stability-indicating RP-HPLC method for Empagliflozin were of analytical and HPLC grade. The pure drug sample of Empagliflozin was procured from Scienlogy Research Lab, Mumbai. The marketed formulation, EMPRIVO-10 mg tablets, manufactured by Ernst Pharmacia Pvt. Ltd., Panchkula, Haryana, India, was used for assay and analytical studies. HPLC grade Methanol were obtained from Loba Chemicals and were used as solvents and mobile phase components during chromatographic analysis. Ortho Phosphoric Acid (OPA, 0.1%) procured from Loba Chemicals was used for pH adjustment and preparation of the mobile phase. All reagents and solvents used in the study were of suitable analytical quality to ensure accuracy, precision, and reproducibility of the developed RP-HPLC method.

Table No 1 : Experimental trial for choice of column

Column	Observation	Inference
C8	Poor Retention of Analyte	Broad and poor peak shape
C18	Improved Retention Of Analyte	Better peak shape

Table No 2 : Experimental trial for choice of mobile Phase

Mobile Phase Composition	Observation	Inference
Water : Methanol	No precision in Retention time. Broad Peak with tailing	Use of buffer required and use of methanol to improve peak shape
Methanol : 0.1 % Orthophosphoric Acid	No precision in Retention time. Good Peak shape	Use of buffer and methanol required.

Preparation of Standard solution:

A standard solution of empagliflozin (10 µg/mL) was prepared using methanol by a serial dilution method. Initially, 10 mg of empagliflozin was accurately weighed and transferred into a 10 mL volumetric flask, then dissolved and diluted up to the mark with methanol to obtain a stock solution of 1000 µg/mL. From this 1 mL was pipetted into another 10 mL volumetric flask and the volume was made up with methanol to produce an

intermediate solution of 100 µg/mL. Finally, 1 mL of this intermediate solution was further diluted to 10 mL with methanol to obtain the final working standard solution of 10 µg/mL.[8,9]

Chromatographic Conditions:

Chromatographic analysis was carried out using an Agilent Technologies 1260 Infinity HPLC system equipped with CHEMSTATION software. Separation was achieved on a C18 column (4.6 × 150 mm, 2.5 µm particle size). The mobile phase consisted of Methanol and 0.1% OPA in the ratio of 70.3:29.7 % v/v, delivered at a flow rate of 1.0 mL/min. Detection was performed at 224 nm with an injection volume of 20 µL. The analysis was carried out at a temperature of 25–28°C, providing satisfactory chromatographic performance for Empagliflozin analysis.[10,11]

Initial method development:

Choice of column:

In order to choose the appropriate column, initial experimental runs were carried out as shown in Table 1 and 2. According to the observations of above initial trials and its chromatograms, C18 column was selected for further trials.

Software aided method development:

A new Reverse Phase-HPLC method was developed for the determination of Empagliflozin by using QbD approach. A Quality by Design with Design of Experiments approach to the development of an analytical method mainly involves two phases as follows:

a) Screening Phase

b) Statistical Analysis and Final Optimization

Screening Phase :

A new Reverse Phase-HPLC method was developed for Empagliflozin using Design Expert 13 software. In this software, Central composite design statistical screening design was used to optimize the Critical Process Parameters (CPP) or Critical Method Parameters (CMPs) and to evaluate interaction effects of these parameters on the Critical Quality Attributes (CQAs).

Selection of Critical Method Parameters (CMPs) :

Critical Method Parameters are selected number of factors that impact on the analytical technique under development. So, the Critical Method Parameters selected for the study are Buffer (Orthophosphoric Acid) and Organic Modifier (Methanol). The results were analyzed using ANOVA and response surface methodology to study the effects of variables.[12]

Selection of Critical Quality Attributes (CQAs) :

Critical Quality Attributes are the responses that are measured to judge the quality of the developed analytical methods. So, the Critical Quality Attributes selected for the study are Retention time , Peak Area And Tailing Factor. These responses were monitored during the experimental trials.[13]

Experimental Trials :

As per the central composite statistical screening design, low, medium and high levels of the critical method parameters were selected based on the preliminary experimentation. Evaluation of all of the above critical method parameters with central composite design lead to 13 experimental trials due to permutation and combination of the three parameters. These 13 experimental trials were carried out using the aforementioned chromatographic conditions using the previously selected agilant C18 column.

Statistical Analysis and Final Optimisation :

The responses obtained after carrying out the above trial runs were feedback to Design Expert software and plots like 3D-response surface plots and Graph plots were plotted. These plots revealed the influence of critical method parameters on the selected quality attributes. The analysis of these plots was used to estimate as to which method parameter gave the most acceptable responses. Thus, based on these observations, the final critical method parameters of the method were determined and the optimized chromatographic conditions were finalized. Moreover, the evaluation of statistical analysis tool like ANOVA for each individual response was used to determine the significance of each method parameter selected for the study using the p value (probability).[14,15]

Analytical Method Validation:

System suitability : The testing is an essential part of the analytical method validation process. It was performed by injecting six replicate injections of standard Empagliflozin solution under optimized chromatographic conditions. Parameters such as retention time, theoretical plates, and tailing factor were evaluated to ensure the suitability and performance of the RP-HPLC system.

Limit of Detection (LOD) and Limit of Quantification (LOQ) : The LOD and LOQ of the developed RP-HPLC method for Empagliflozin were determined by injecting progressively lower concentrations of the standard solution. The concentrations producing signal-to-noise ratios of approximately 3:1 and 10:1 were considered as LOD and LOQ, respectively.[16]

Linearity: The linearity of the developed RP-HPLC method was evaluated over a suitable concentration range around the working concentration of Empagliflozin. Different concentrations of standard solutions were prepared and injected into the HPLC system. Calibration curves were constructed by plotting peak area versus concentration, and the correlation coefficient was determined to evaluate the linear relationship of the method.

Precision and Accuracy: Precision of the developed analytical method was evaluated in terms of intraday and interday precision and expressed as % Relative Standard Deviation (%RSD). Different concentration levels of Empagliflozin standard solutions were analyzed repeatedly on the same day and on different days to assess method precision. Accuracy of the method was determined by recovery studies at different concentration levels, and the percentage recovery of Empagliflozin was calculated to confirm the accuracy of the developed RP-HPLC method.[17]

Repeatability : Repeatability was evaluated by injecting six replicate injections of empagliflozin standard solution at a fixed concentration under the same chromatographic conditions. The system precision was expressed as %RSD of peak area. The %RSD value for empagliflozin was found to be within ICH acceptable limits (<2.0%)**,** indicating good repeatability of the method. In reported studies, the repeatability of empagliflozin ranged approximately from 0.2% to 0.9% RSD, confirming that the method produces highly consistent results.

Robustness : The ability of an analytical method to remain unaffected by small, deliberate changes in chromatographic conditions such as flow rate, mobile phase composition, wavelength, and temperature. A robust RP-HPLC method for Empagliflozin gives consistent results with minimal variation in retention time, peak area, and assay values, confirming method reliability during routine analysis.

Ruggedness : The ability of a meth od to produce consistent results under different operating conditions such as different analysts, instruments, and days of analysis. For Empagliflozin, a rugged method shows low variation in results and %RSD within acceptable limits, ensuring reproducibility of the method.[18]

Analysis of marketed formulation: Twenty tablets of EMPRIVO-10 containing Empagliflozin were accurately weighed and finely powdered. An amount of tablet powder equivalent to 10 mg of Empagliflozin was transferred into a 10 mL volumetric flask and dissolved using the diluent. The solution was sonicated for 20 minutes to ensure complete extraction of the drug and then diluted up to the mark with diluent. The resulting solution was filtered through Whatman filter paper to remove insoluble excipients. A suitable aliquot of the filtrate was further diluted to obtain the desired working concentration of Empagliflozin. Finally, 20 µL of the prepared sample solution was injected into the RP-HPLC system under optimized chromatographic conditions for analysis.[19]

Stability Studies:[20]

Stability Indicating Assay of Empagliflozin :

To establish the stability-indicating nature of the developed RP-HPLC method, the standard stock solution of Empagliflozin was subjected to various stress conditions to induce degradation. The drug was exposed to different degradation conditions such as acidic, alkaline, oxidative, photolytic, and thermal stress studies. Standard and stressed sample solutions were prepared and analyzed under optimized chromatographic conditions to evaluate the ability of the method to separate Empagliflozin from its degradation products.

Preparation of Standard Solution :

An accurately weighed quantity of 5 mg of Empagliflozin working standard was transferred into a 50 mL volumetric flask and dissolved in diluent to obtain a standard stock solution having a concentration of 100 µg/mL. This solution was used as the untreated standard solution for analysis.

Acidic Degradation : For acidic degradation study, an accurately weighed quantity of Empagliflozin was transferred into a volumetric flask and treated with 0.1 N hydrochloric acid (HCl). The solution was kept at room temperature for 3 hours to promote acid hydrolysis of the drug. After completion of the degradation period, the stressed solution was neutralized with an appropriate quantity of sodium hydroxide solution to stop further degradation. The solution was then diluted with the mobile phase to obtain the desired concentration, filtered through a 0.45 µm membrane filter, and analyzed using the developed RP-HPLC method. The chromatogram was recorded to evaluate the extent of acidic degradation and the formation of degradation products.

Basic Degradation : For alkaline degradation study, an accurately weighed quantity of Empagliflozin was treated with 0.1 N sodium hydroxide (NaOH) solution and kept at room temperature for 3 hours. This study was carried out to evaluate the stability of the drug under basic conditions. After the specified time, the degraded solution was neutralized with an appropriate quantity of hydrochloric acid solution. The final solution was diluted with the mobile phase, filtered through a 0.45 µm membrane filter, and injected into the RP-HPLC system for chromatographic analysis. The obtained chromatogram was used to determine the degradation behavior of Empagliflozin under alkaline conditions.

Oxidative Degradation : For oxidative degradation, an accurately weighed quantity of Empagliflozin was treated with 3% hydrogen peroxide (H_2O_2) solution and kept at room temperature for 3 hours. The oxidative stress condition was applied to study the susceptibility of the drug towards oxidation. After completion of the degradation period, the solution was diluted appropriately with the mobile phase without neutralization. The prepared sample was filtered through a 0.45 µm membrane filter and analyzed using the developed RP-HPLC method. The chromatographic results were evaluated for the presence of oxidative degradation products and percentage degradation of the drug.

Photolytic Degradation : For photolytic degradation study, the prepared solution of Empagliflozin was exposed to UV/light conditions for 24 hours to investigate the effect of light on drug stability. After exposure, the solution was diluted with the mobile phase to obtain the required concentration for analysis. The sample solution was filtered through a 0.45 µm membrane filter before injection into the HPLC system. The chromatogram obtained after analysis was compared with that of the untreated standard solution to determine the extent of photolytic degradation and to confirm the stability-indicating nature of the developed RP-HPLC method. In all forced degradation studies, the percentage degradation and percentage recovery of Empagliflozin were calculated to evaluate the stability-indicating capability of the developed RP-HPLC method.

Results and discussion:

Choice of column:

Empagliflozin possesses moderately polar characteristics with aromatic and glucose moieties, making reverse-phase chromatography suitable for its analysis. Different reverse-phase columns were evaluated during method development, and the C18 column was selected due to its better retention, improved peak shape, and satisfactory chromatographic performance. Methanol and 0.1% Orthophosphoric Acid (OPA) were selected as the mobile phase components to obtain good resolution and reproducible chromatographic results. The optimized chromatographic conditions using the C18 column provided efficient separation and accurate analysis of Empagliflozin.

Result of UV method :

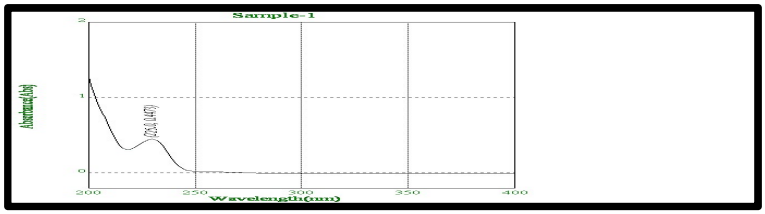


Fig No.1 : λ max of Empagliflozin at 225 nm

The standard solution was scanned between 200-400 nm wavelength and the λ max was observed. It was found at 225 nm wavelength as shown in Fig. No. 1.

Development and Optimization of New RP-HPLC Method for Empagliflozin Using QbD Approach : A Central Composite Design (CCD) using Design-Expert® version 13 was applied for optimization of the RP-HPLC method for empagliflozin by evaluating two critical factors: mobile phase composition (A) and flow rate (B). The design included 13 experimental runs comprising 4 factorial points, 4 axial points, and 5 center points. The factorial points evaluated the main effects, while the axial points assessed quadratic effects. The center points (70% mobile phase and 1.0 mL/min flow rate) were repeated five times to determine repeatability and experimental error. Based on desirability and chromatographic responses, the optimized condition was obtained

at 70% mobile phase composition and 1.0 mL/min flow rate, providing satisfactory retention time, peak area, and theoretical plates. Run 2 was identified as the optimized run due to its consistent chromatographic performance. Thus, CCD successfully established a reliable quadratic model and robust optimized conditions for empagliflozin analysis.as shown in table no 3.

Table No. 3: QbD runs

		Independent Variables		**Dependent Variables**		
		Factor 1	**Factor 2**	**Response 1**	**Response 2**	**Response 3**
Std	**Run**	**A:MOBILE PHASE**	**B:FLOW RATE**	**R1(RT)**	**R2(AREA)**	**R3(TP)**
		%	Ml/min	min	AUC	TP
2	1	75	0.9	4.78	1107.22	5406
9	2	70	1	4.35	988.761	4343
5	3	62.9289	1	5.089	996.547	3340
7	4	70	0.858579	5.236	1174.7	4455
13	5	70	1	4.37	983.388	4419
1	6	65	0.9	5.52	1112.26	3778
8	7	70	1.14142	3.784	852.165	4270
6	8	77.0711	1	4.061	986.452	5333
10	9	70	1	4.373	983.292	4415
4	10	75	1.1	3.703	885.617	4990
11	11	70	1	4.363	987.293	4427
12	12	70	1	4.375	988.954	4435
3	13	65	1.1	4.303	884.379	3764

Fit Summary Response 1: R1(RT)

Table no 4 : ANOVA for Quadratic model

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	3.46	5	0.6925	418.74	< 0.0001	significant
A-MOBILE PHASE	0.9757	1	0.9757	590.00	< 0.0001	
B-FLOW RATE	2.36	1	2.36	1428.66	< 0.0001	
AB	0.0049	1	0.0049	2.96	0.1289	
A^2	0.0887	1	0.0887	53.62	0.0002	
B^2	0.0450	1	0.0450	27.19	0.0012	
Residual	0.0116	7	0.0017			
Lack of Fit	0.0112	3	0.0037	36.24	0.0023	significant
Pure Error	0.0004	4	0.0001			
Cor Total	3.47	12				

The model was analyzed using coded factor levels and Type III partial sum of squares. The Model F-value of 418.74 indicated that the model was highly significant, with only a 0.01% chance that the observed value occurred due to noise. P-values below 0.0500 confirmed that factors A, B, A^2, and B^2 were significant model terms, while higher P-values indicated insignificant terms. The Lack of Fit F-value of 36.24 was significant, suggesting that the model did not adequately fit the experimental data, with only a 0.23% probability of occurring due to noise. The **Predicted R^2** of 0.9770 is in reasonable agreement with the **Adjusted R^2** of 0.9943; i.e. the difference is less than 0.2.**Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 64.623 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors:

$$\textbf{Retention time} = 4.37 - 0.3492A - 0.5434B + 0.0350AB + 0.1129A^2 + 0.0804B^2$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

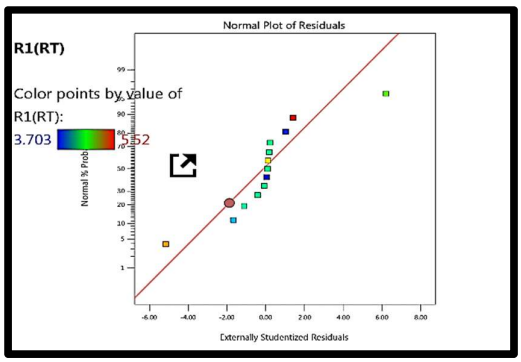
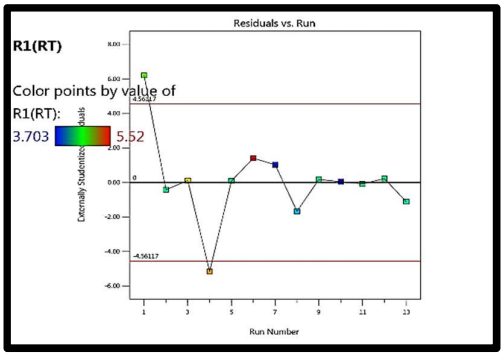


Fig. No. 2: Normal plot of Residuals



FigNo.3: Residuals vs. Run

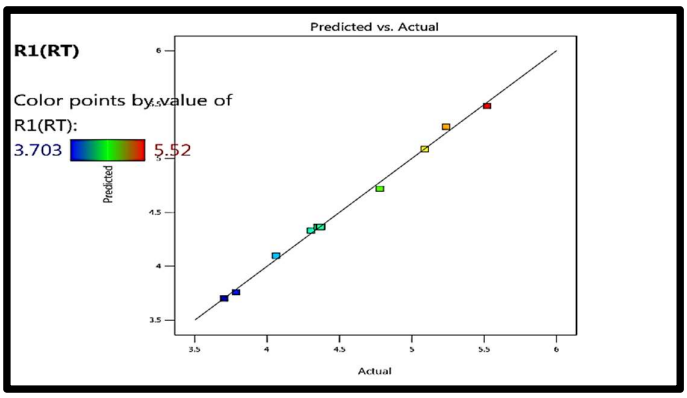


Fig.No.4: Predicted vs. Actual

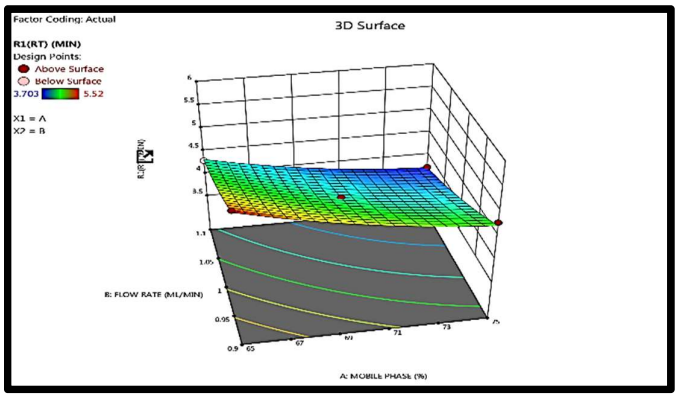


Fig. No.5: Counter & 3D plots of response 1 (RT)

Fit Summary - Response 2: R2(AREA)
ANOVA for Quadratic model

Table no 5: ANOVA for response 2

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.036E+05	5	20723.54	1411.88	< 0.0001	significant

A-MOBILE PHASE	40.88	1	40.88	2.78	0.1391	
B-FLOW RATE	1.025E+05	1	1.025E+05	6984.49	< 0.0001	
AB	9.86	1	9.86	0.6721	0.4393	
A^2	11.89	1	11.89	0.8099	0.3981	
B^2	1047.89	1	1047.89	71.39	< 0.0001	
Residual	102.75	7	14.68			
Lack of Fit	71.14	3	23.71	3.00	0.1580	not significant
Pure Error	31.60	4	7.90			
Cor Total	1.037E+05	12				

The model was evaluated using coded factors and Type III partial sum of squares. The Model F-value of 1411.88 indicated that the model was highly significant, with only a 0.01% chance of occurring due to noise. P-values below 0.0500 showed that B and B^2 were significant model terms, while higher values indicated insignificant terms. The Lack of Fit F-value of 3.00 was non-significant, indicating good agreement between the model and experimental data, with a 15.80% probability that the lack of fit occurred due to noise.

The **Predicted R^2** of 0.9946 is in reasonable agreement with the **Adjusted R^2** of 0.9983; i.e. the difference is less than 0.2. **Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 123.016 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors:

The coded equati **Area : 986.34-2.26A-113.20B+1.57AB+1.31A^2+12.27B^2** factor levels are represented as +1 and −1, respectively. It also helps in evaluating the relative effect of factors by comparing their coefficients.

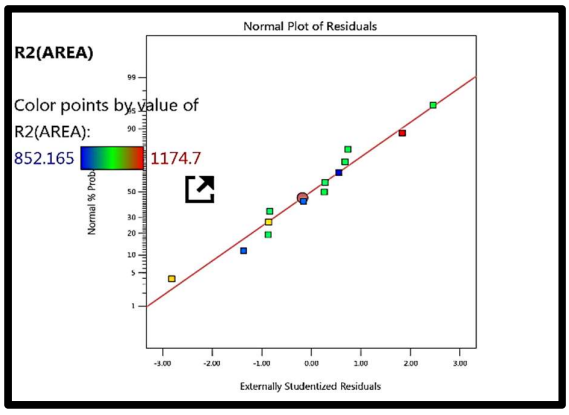


Fig. No.6: Normal plot of Residuals

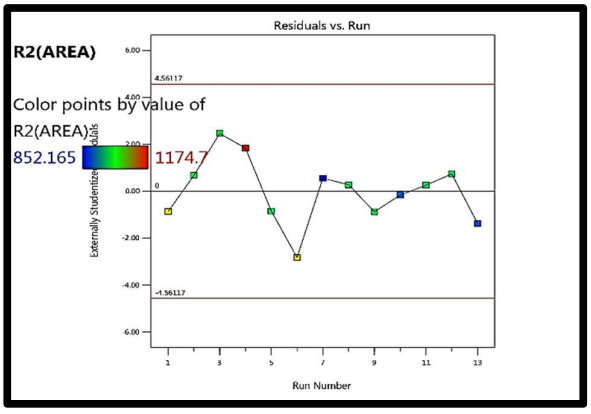


Fig No.7: Residuals vs. Run

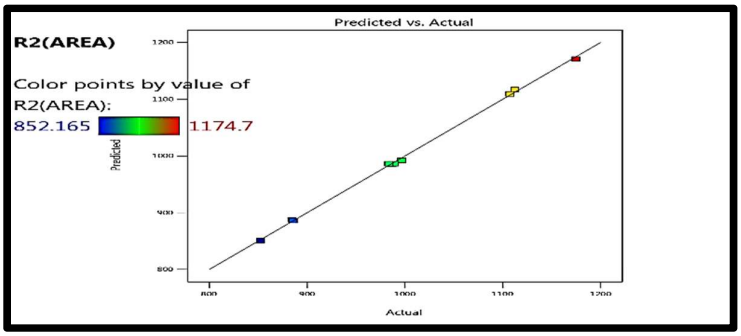


Fig. No.8: Predicted vs. Actual

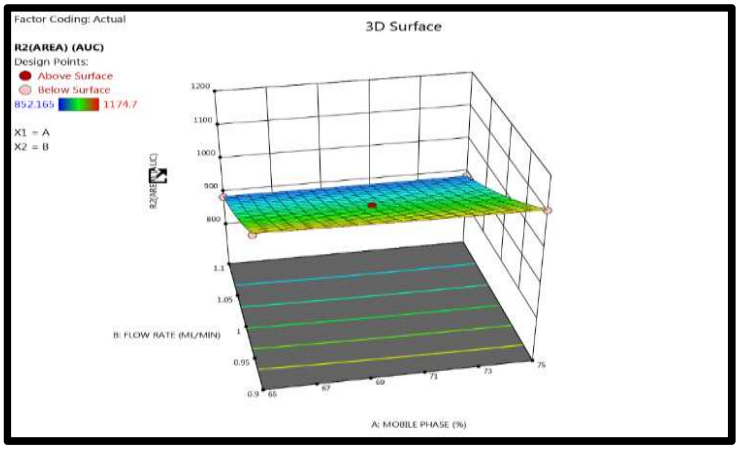


Fig. No.9: Counter & 3D plots of response 2 (Area)

Response 3: R3(TP)

ANOVA for Quadratic model

Table no 6: ANOVA for response 3

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.123E+06	5	8.247E+05	126.49	< 0.0001	significant
A-MOBILE PHASE	4.022E+06	1	4.022E+06	616.96	< 0.0001	
B-FLOW RATE	59793.92	1	59793.92	9.17	0.0192	
AB	40401.00	1	40401.00	6.20	0.0416	
A^2	25.11	1	25.11	0.0039	0.9522	
B^2	857.11	1	857.11	0.1315	0.7276	
Residual	45635.66	7	6519.38			
Lack of Fit	40150.86	3	13383.62	9.76	0.0260	significant
Pure Error	5484.80	4	1371.20			
Cor Total	4.169E+06	12				

The model was analyzed using coded factors and Type III partial sum of squares. The Model F-value of 126.49 indicated that the model was significant, with only a 0.01% chance of occurring due to noise. P-values below 0.0500 confirmed that A, B, and AB were significant model terms. The Lack of Fit F-value of 9.76 was significant, indicating poor model fit, with a 2.60% chance that the lack of fit occurred due to noise.

The **Predicted R^2** of 0.9295 is in reasonable agreement with the **Adjusted R^2** of 0.9812; i.e. the difference is less than 0.2.**Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 36.562 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

The coded [illegible] resented by +1 and [illegible] t on the response.

Therotical plate : $4407.80+709.07A-86.45B-100.50AB-1.90A^2+11.10B^2$

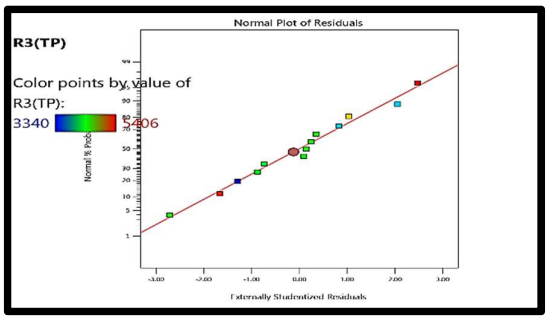


Fig. No.10: Normal plot of Residuals

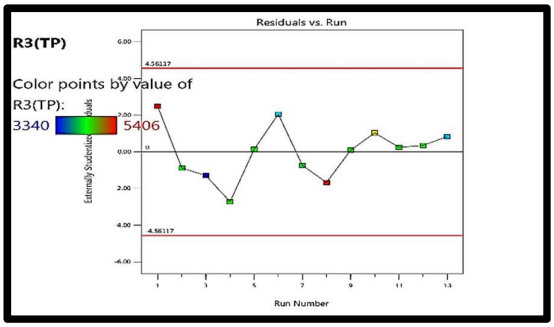


Fig No.11: Residuals vs. Run

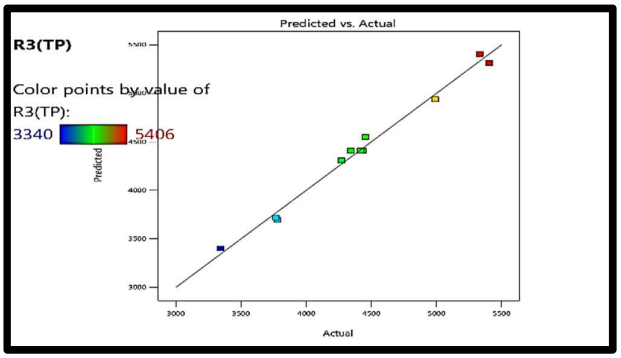


Fig. No.12: Predicted vs. Actual

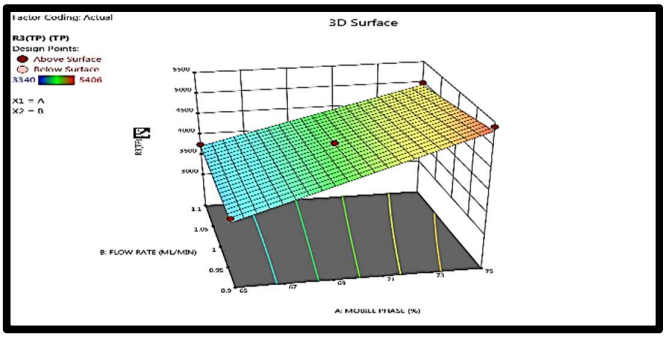


Fig. No.13: Counter & 3D plots of response 2 (TP)

Table 7 : Optimized Factors

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	Mobile phase	70.32% V/V	65.00	75.00	0.0000	Actual
B	Flow rate	0.9327 ml/min	0.9000	1.10	0.0000	Actual

Table 8 :Confirmation Location

Mobile phase (% v/v)	Flow rate (ml/min)
70.3171	1

Results of RP-HPLC method development:

Evaluation of System Suitability :
System suitability of the developed RP-HPLC method was evaluated by injecting six replicate injections of standard Empagliflozin solution. The %RSD obtained from peak areas of six injections was found to be less than 2.0%, indicating good precision of the system. The tailing factor was found to be less than 2.0 and the theoretical plate count was observed to be greater than 2000, confirming satisfactory column efficiency. Thus, all system suitability parameters were found to be within acceptable limits, indicating that the chromatographic system was suitable for the analysis of Empagliflozin as shown in above figures .

Limit of Detection (LOD) and Limit of Quantitation (LOQ) :
The method exhibits good sensitivity, as indicated by the low LOD and LOQ values. This confirms that the method is capable of detecting and quantifying the analyte at very low concentrations with acceptable accuracy and precision. As shown in table no 9 and 10.

LOD And LOQ :

Table No 9 : LOD

LOD	3.3 X SD/ Slope
	34.89298
	0.678456

Table No 10 : LOQ

LOQ=	10 X SD/ Slope
	105.7363
	2.055926

Linearity:
Linearity was evaluated in the range of 20% to 100% of the working concentration level i.e.,20µg/ml for empagliflozin. The Linearity was confirmed in the range of 20 µg/ml – 100 µg/ml. The Co-efficient of Co-relation (R2) was found to be 0.9996 and the equation of the line was y = 51.439x + 23.247 as evident from the below calibration curve. Thus, the data shows that the response is found to be linear table 11. This clearly indicates that an excellent correlation existed between the peak area and concentration of the analyte as shown in fig 14 .

Table No 11: Linearity

Conc	Area I	II	III	IV	V	VI	Mean	SD	%RSD
20	1039.185	1041.691	23.24	51.43	1017.20	19.78	1040.44	1.77	0.17
40	2056.845	2067.076	23.24	51.43	2038.72	39.64	2061.96	7.23	0.35
60	3133.483	3164.219	23.24	51.43	3125.61	60.77	3148.85	21.73	0.69
80	4157.514	4168.309	23.24	51.43	4139.67	80.49	4162.91	7.63	0.18
100	5144.154	5123.656	23.24	51.43	5110.67	99.37	5133.91	14.49	0.28
							Avrg SD	**10.57**	

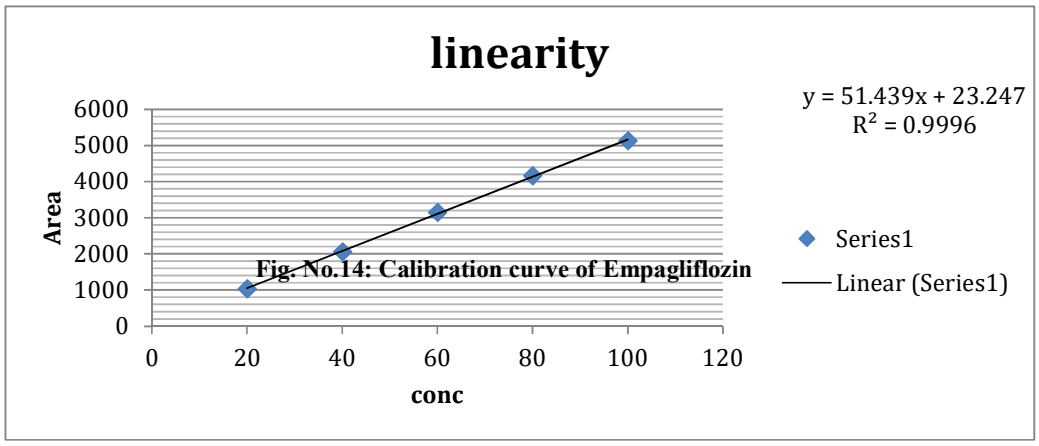


Fig. No.14: Calibration curve of Empagliflozin

The linearity of Empagliflozin represented graphically and R2 value was calculated. It was found to be 0.999 which is within the limit.

Precision:
Precision of the developed RP-HPLC method was expressed in terms of Relative Standard Deviation (%RSD) for both intraday and interday studies. Intraday precision was evaluated by analyzing replicate injections of standard Empagliflozin solutions within the same day, while interday precision was determined by analyzing the solutions on different days. The obtained %RSD values for both studies were found to be within the acceptable limit of less than 2.0%, indicating good precision and reproducibility of the method shown in table no 12 and 13 .

Table no 12 : Interday Precision

Conc	Area	II	III	IV	V	Mean	Amt Found	AM	% Amt Find	SD	%RSD
20	1033.957	1050.006	23.24	51.43	1018.74	1041.98	19.81	0.990415	99.04	11.35	1.09
60	3140.478	3142.528	23.24	51.43	3118.26	3141.50	60.63	1.01052	101.05	1.45	0.05
100	5132.24	5162.512	23.24	51.43	5124.14	5147.38	99.63	0.996332	99.63	21.41	0.42

Table no 13: Intraday Precision

Conc.	Area	II	III	IV	V	Mean	Amt Found	AM	% Amt Fnd	SD	%RSD
20	1064.504	1066.2	23.24	51.43	1042.11	1065.35	20.26	1.013136	101.31	1.20	0.11
60	3104.175	3117.777	23.24	51.43	3087.74	3110.98	60.04	1.000627	100.06	9.62	0.31
100	5186.261	5197.489	23.24	51.43	5168.64	5191.88	100.50	1.004984	100.50	7.94	0.15

Repeatability: For the concentration level of 40 µg/mL, the chromatographic responses obtained from replicate injections showed peak areas of 2114.467, 2107.189, 2123.24, 2151.43, and 2087.59, with a mean peak area of 2110.83. The amount of drug found was calculated to be 40.59 µg/mL with an average mean value of 1.014771. The percentage amount found was 101.48%, indicating good accuracy of the developed RP-HPLC method. The standard deviation was found to be 5.15 and the %RSD value was 0.24%, which was well within the acceptable

limit of less than 2%, confirming the precision and reproducibility of the analytical method for Empagliflozin estimation as shown in table no 14.

Table no 14 : Repeatablity

Conc.	Area	II	III	IV	V	Mean	Amt Found	AM	%Amt Fnd	SD	%RSD
40	2114.467	2107.189	23.24	51.43	2087.59	2110.83	40.59	1.014771	101.48	5.15	0.24

Accuracy:

For the recovery study at the 80% concentration level, a standard amount of 32 µg/mL of Empagliflozin was added to the pre-analyzed sample solution containing 40 µg/mL of the drug. The chromatographic analysis produced peak areas of 3727.00 and 3724.432 for the replicate injections. The corresponding amounts of drug found were 72.02 µg/mL and 71.97 µg/mL, respectively.The amount of drug recovered was calculated as 32.02 µg/mL and 31.97 µg/mL, with percentage recoveries of 100.05% and 99.89%, respectively. The mean percentage recovery was found to be 99.97%, indicating excellent accuracy of the developed RP-HPLC method. The standard deviation for percentage recovery was 0.11 and the %RSD value was 0.11%, which was well within the acceptable limit of less than 2%, confirming the precision and reliability of the recovery method for estimation of Empagliflozin as shown in table no 15

Table No 15 : 80% Accuracy

Sr no.	µgm/ml	Amt added	Area	C (y-intercept)	M (Slope)	CM	Amt found	Amt recvd	Recv	% Recv
1	40	32	3727.00	23.24	51.43	3703.76293	72.02	32.02	1.00	100.05
2	40	32	3724.432	23.24	51.43	3701.19237	71.97	31.97	1.00	99.89
			Mean				**71.99**	**31.99**		**99.97**
			SD				**0.035**	**0.035**		**0.11**
			%RSD				**0.049**	**0.110**		**0.11**

For the recovery study at the 100% concentration level, 40 µg/mL of standard Empagliflozin was added to the sample solution. The obtained peak areas were 4140.62 and 4154.656, with corresponding amounts found of 80.05 µg/mL and 80.33 µg/mL, respectively. The percentage recovery values were found to be 100.14% and 100.83%, with a mean recovery of 100.49%. The %RSD value was 0.48%, which was within the acceptable limit, confirming the accuracy and precision of the developed RP-HPLC method as shown in table 16.

Table No 16 : 100% Accuracy

Sr no.	µgm/ml	Amt added	Area	C (y-intercept)	M (Slope)	CM	Amt found	Amt recvd	Recv	% Recv
1	40	40	4140.62	23.24	51.43	4117.380112	80.05795	40.05795	1.00	100.14
2	40	40	4154.656	23.24	51.43	4131.415776	80.33085	40.33085	1.01	100.83
			Mean				**80.19**	**40.19**		**100.49**
			SD				**0.193**	**0.193**		**0.48**
			%RSD				**0.241**	**0.480**		**0.48**

For the recovery study at the 120% level, 48 µg/mL of standard Empagliflozin was added to the sample solution. The obtained peak areas were 4576.742 and 4578.849, with corresponding amounts found of 88.53 µg/mL and 88.57 µg/mL, respectively. The percentage recoveries were 101.12% and 101.21%, with a mean recovery of 101.16%. The %RSD value was 0.06%, indicating good accuracy and precision of the developed RP-HPLC method for Empagliflozin estimation as shown in table no 17

Table No 17 : 120% Accuracy

Sr no.	Conc. µgm/ml	Amt added	Area	C (y-intercept)	M (Slope)	CM	Amt found	Amt recvd	Recv	% Recv
1	40	48	4576.742	23.24	51.43	4553.5017	88.53785	48.53785	1.01	101.12
2	40	48	4578.849	23.24	51.43	4555.60863	88.57882	48.57882	1.01	101.21
			Mean				**88.56**	**48.56**		**101.16**
			SD				**0.029**	**0.029**		**0.06**
			%RSD				**0.033**	**0.060**		**0.06**

Robustness:
Robustness of the developed RP-HPLC method was evaluated by making a slight change in the detection wavelength from 224 nm to 226 nm. At 224 nm, the peak areas obtained for 20 µg/mL concentration were 1094.857 and 1092.816, with a mean area of 1093.8, standard deviation of 1.44, and %RSD of 0.13%. Similarly, at 226 nm, the peak areas obtained were 1058.999 and 1057.204, with a mean area of 1058.10, standard deviation of 1.27, and %RSD of 0.12%. Since the %RSD values were found to be less than 2%, the developed RP-HPLC method was considered robust against small changes in wavelength as shown in table no 18.

Change in MP Composition:

Table No 18 : Robustness (change in Wavelength)

Wave length change: 224 nm			Wave length change: 226		
Sr No.	**Conc.µgm/ml**	**Area**	**Sr No.**	**Conc. µgm/ml**	**Area**
1	20	1094.857	1	20	1058.999
2	20	1092.816	2	20	1057.204
	Mean	1093.8		**Mean**	1058.10
	SD	1.44		**SD**	1.27
	%RSD	0.13		**%RSD**	0.12

Ruggedness: Ruggedness of the developed method was evaluated by performing the analysis under varied conditions such as change in analyst. For the concentration level of 40 µg/mL, the obtained peak areas were 2057.615 and 2052.157, with corresponding amounts found of 39.55 µg/mL and 39.45 µg/mL, respectively. The percentage label claims obtained were 98.89% and 98.63%, with a mean value of 98.76%. The %RSD value was found to be 0.19%, which is well within the acceptable limit of less than 2%, indicating that the method is rugged and capable of producing consistent and reliable results under normal operating variations as shown in table 19.

Table No 19 : Ruggedness (Change in Analyst)

Conc.	Area	C (y-intercept)	M (Slope)	CM	Amt Found	Label Claim	%Label Claim
40.00	2057.615	23.24	51.43	2034.38	39.5562	0.98890506	98.89
40.00	2052.157	23.24	51.43	2028.92	39.45007	0.98625181	98.63
Mean	2054.89				39.50		98.76
SD	3.860				0.075		0.188
%RSD	0.188				0.190		0.190

The %RSD value is less than 2%, indicating very low variability between different conditions (e.g., analyst). Therefore, the method is rugged and produces consistent and reliable results under varied conditions.

Application on Marketed Formulation : The developed stability-indicating RP-HPLC method was successfully applied for the estimation of Empagliflozin in the marketed formulation EMPRIVO-10 mg tablets. The analysis revealed that the formulation contained 99.15% of the labeled claim, indicating good agreement with the declared amount. The results confirm the suitability of the developed method for routine quality control analysis of Empagliflozin in pharmaceutical dosage forms as shown in table no 20.

Table No 20: Assay

Conc.	Area	C (y-intercept)	M (slope)	CM	Amt. Found	LC (Label claim)	% Label Claim
40.00	2069.797	23.24	51.43	2046.56	39.79307	0.99482664	99.48
40.00	2056.013	23.24	51.43	2032.77	39.52504	0.98812606	98.81
Mean	**2062.91**				39.66		99.15
SD	**9.747**				0.190		0.474
%RSD	**0.472**				0.478		0.478

The % labelled claim of drug represents that test method has an acceptable level (within 98 – 102% of % labelled claim.

Stability testing:

Table No 21 : Stablity Study

Sr. No.	Method	Area of Standard	Area of degraded Sample	Drug Concentration %	% Degradation
1.	Acidic Degradation	2061.96	1837.29	89.10	10.90
2.	Basic Degradation	2061.96	1739.65	84.37	15.63
3.	Oxidative Degradation	2061.96	1904.23	92.35	7.65
4.	Photolytic Degradation	2061.96	2006.21	97.30	2.70

The forced degradation study of Empagliflozin confirmed that the developed stability-indicating RP-HPLC method effectively separated the drug from its degradation products under acidic, basic, oxidative, and photolytic stress conditions. Among all conditions, maximum degradation was observed under alkaline hydrolysis (15.63%), indicating higher susceptibility of the drug to basic conditions, followed by acid degradation (10.90%). Oxidative degradation using H_2O_2 showed 7.65% degradation, while the least degradation was observed under photolytic conditions (2.70%), indicating good photostability of Empagliflozin. The drug peak was well resolved from degradation peaks without interference, demonstrating the specificity and selectivity of the method. The obtained degradation results were within acceptable limits, confirming the suitability of the developed RP-HPLC method for routine stability testing and quantitative analysis of Empagliflozin in pharmaceutical dosage forms as shown in table 21.

Conclusion:

A simple, precise, accurate, and reliable stability-indicating RP-HPLC method was successfully developed and validated for the estimation of Empagliflozin using the Quality by Design (QbD) approach. The application of QbD principles helped in systematic method development by identifying and optimizing critical method parameters to achieve desired chromatographic performance. The optimized method provided good resolution, sharp peak shape, and satisfactory system suitability parameters under selected chromatographic conditions. The method was validated as per ICH guidelines and demonstrated excellent linearity over the studied concentration range with high correlation coefficient. The results of precision and accuracy studies confirmed the reproducibility and reliability of the method, as %RSD values were within acceptable limits and recovery studies showed results close to 100%. Robustness and ruggedness studies further confirmed that the method remains unaffected by small deliberate variations in analytical conditions. Stablity studies revealed that Empagliflozin undergoes degradation under acidic, basic, and oxidative conditions, while the degradation products were well resolved from the main drug peak, confirming the stability-indicating nature of the method. The developed method was successfully applied for the analysis of marketed formulation, showing satisfactory assay results. Hence, the proposed RP-HPLC method is suitable for routine quality control, stability testing,

and regulatory analysis of Empagliflozin in pharmaceutical dosage forms.

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