DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF EMPAGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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Abstract

For the simultaneous measurement of empagliflozin in pharmaceutical formulations, a quick, sensitive, and precise reverse-phase high-performance liquid chromatography (RP HPLC) technique was created and approved. A C18 was used to accomplish the chromatographic separation. Column (250 mm × 4.6 mm, 5 µm) with a mobile phase of 95% acetonitrile and 0.5% water (95:05 v/v) at a flow rate of 0.5 mL/min. Empagliflozin was detected at 254 nm with retention periods of 4.75 and 6.77 minutes, respectively. The technique showed linearity for both medications throughout concentration ranges of 5 to 30 ppm with correlation values greater than 0.999. ICH recommendations for accuracy, precision, specificity, linearity, robustness, and stability were used to validate the developed method. For both analytes, recovery experiments revealed mean recoveries of 0.99.67%. With RSD values, the approach demonstrated good precision.

Introduction

Effective therapeutic strategies are necessary because diabetes mellitus is a major worldwide health concern. Empagliflozin is a useful therapy option that combines complementary modes of action to improve glycemic control [1, 2]. In this case, a number of analytical parameters are routinely evaluated to determine the dosage form's quality medication regulatory governing bodies play an important part in the current situation and have placed a strong emphasis on medication manufacturing businesses to offer comprehensive information regarding the validation methodology of analytical methodologies [3].

The different analytes in any given mixture can be effectively separated, identified, quantified, and purified using high performance liquid chromatography

(HPLC). As a result, it has been confirmed that the current HPLC-UV methodology is one of the reliable and accurate analytical methods used to assess different pharmacological active principles in one has described. The measurement of empagliflozin in human plasma using the reverse-phase (RP) HPLC method. Research teams demonstrated the approved stability indicating approach for empagliflozin using a technique based on RP-HPLC [5].

However, references regarding the development of analytical methods and and validation of empagliflozin employing normal phase and trifluoroacetic acid as the mobile phase HPLC has not yet taken care of [6]. In this regard, the current paper outlined the crucial steps based on the technique's analytical life cycle from the early stages of creation to its validation [7]. The goal of the proposed study was to Requirements (ICH, 2005).[9].

create a straightforward, quick, dependable, sensitive, accurate, and robust method for measuring empagliflozin in both pharmaceutical and raw dose forms [8], along with a validation procedure carried out by the International Conference the Harmonization of Technical Guidelines for Pharmaceuticals for Human Use Registration

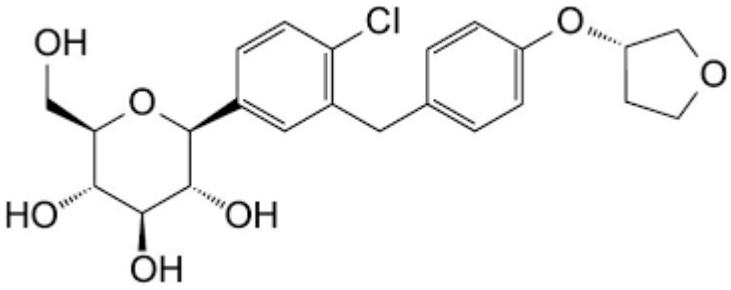


Fig.1 Structure of Empagliflozin

Empagliflozin's IUPAC name is (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-({4-[(3S)-oxolan 3-yloxy] phenyl} methyl) phenyl].-6-(hydroxymethyl) 3,4,5-triol oxane [10].

Material and Method

Chemical and reagent

The HPLC grade solvents used were procured from Merck (India) Ltd., Mumbai. HPLC grade methanol , water and Pottasium Dihydrogen Phosphate (Finar Ltd, Mumbai, India) and acetonitrile (Merck Ltd, India) were used in the analysis [11]. HPLC grade water was prepared using millipore purification system. Empagliflozin 99.8% purity reference criteria (Alkem Ltd,Mumbai, India) [12].

Instrumentation

An Agilent Zorbax 300 series HPLC system with a quaternary pump, autosampler, column thermostat, and UV-visible detector was used for the chromatographic study. ChemStation software was used for data collection and analysis. A Cosmos For separation, a C18 column (250 mm × 4.6 mm, 5 µm) was used [13].

Chromatographic condition

To find the ideal circumstances for the efficient separation and optimization of the sample, several combinations of solvent systems were tested based on the kind of medication and a thorough review of the literature [14].

The mobile phase consisting of

Solvent A – Acetonitrile (95%)

Solvent B – Water (05%)

Column	**Finepack Jasco 5µ (4.6 X 250 mm)**
Flow rate	1.0 mL/min.
Column temp	30°C ± 5°C
Sample temp	25° C ± 5°C
Injection volume	20 µl
Detector	UV at 254 nm
Run time	10 minutes
Retention time	About Approx. 4.5 minutes for Empagliflozin

Diluent : ACN:Water(95:05) (Mobile Phase/Diluent)

Preparation of Standard solution

A 10 mL volumetric flask was filled with precisely weighed 10 milligrams of empagliflozin. After adding the diluent, the ultrasonic water bath was sonicated for ten minutes. After cooling the solution, diluent was added to bring the volume up to par. After that, a 0.45 µ syringe filter was used. The final solution served as the test solution [15].

Procedure

Five replicates of the standard solution and one duplicate of the test solution were injected. The standard chromatogram described below was used to derive the system appropriateness parameters.

Theoretical plates for analyte peak	NLT 2000
Tailing factor for analyte peak	NMT 2.0
% RSD for replicate injections of standard	NMT 2.0

Table 1: Standard chromatogram

Study of Forced Degradation

When acidic deterioration occurs, the drug is treated with an acid solution (commonly 0.1–1 N HCl) and heated at a controlled temperature for a specific time, then neutralized and analyzed (usually by HPLC) to assess degradation [16].

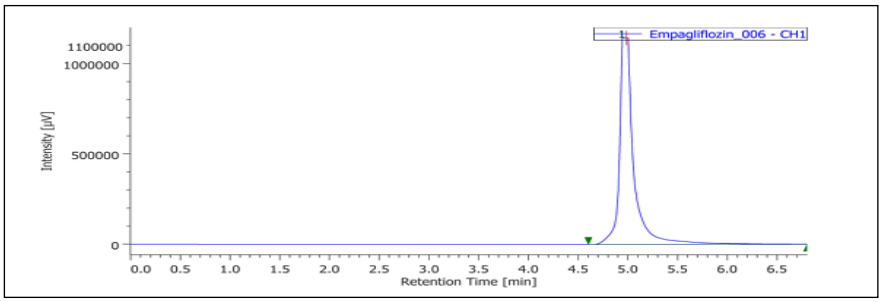


Fig. 1. In acidic degradation

In basic degradation, the drug is exposed to an alkaline solution (commonly 0.1–1 N NaOH), heated for a defined period, then neutralized with acid and analyzed (typically by HPLC) to evaluate degradation [17].

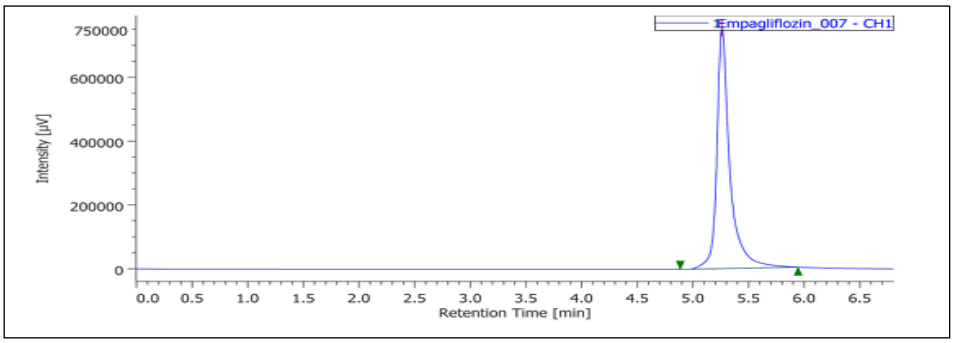


Fig. 2. In basic degradation

In oxidative degradation, the drug is exposed to an oxidizing agent (commonly 0.1–3% hydrogen peroxide) for a specified time at room or elevated temperature, then analyzed to identify oxidative degradation products.(18)

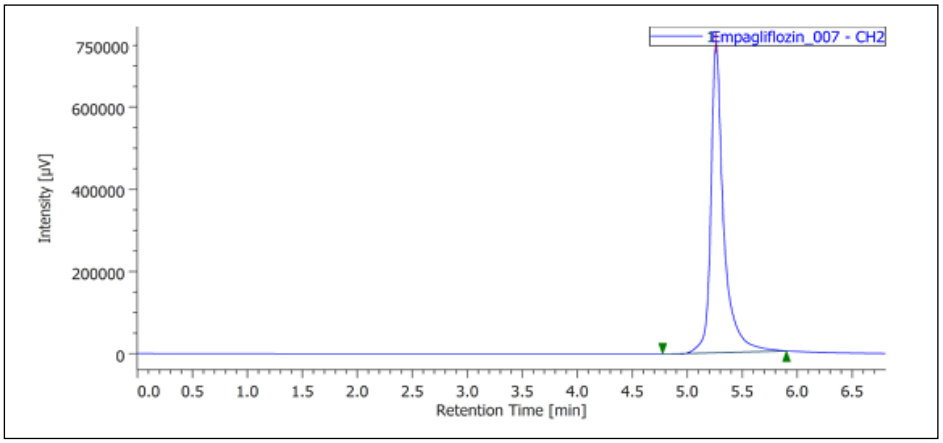


Fig.3. In oxidative degradation

In photolytic degradation, the drug or formulation is exposed to UV and visible light (as per ICH Q1B guidelines) to assess its light sensitivity, and the resulting samples are analyzed for degradation products.(19)

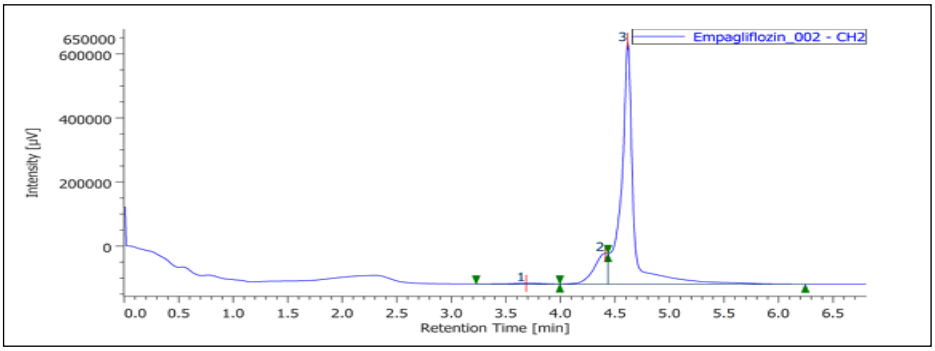


Fig.4.In photolytic degradation

In thermal degradation, the drug or formulation is exposed to elevated temperatures (usually 40–80 °C) in dry or humid conditions for a defined period, and the samples are analyzed to assess heat-induced degradation.(20)

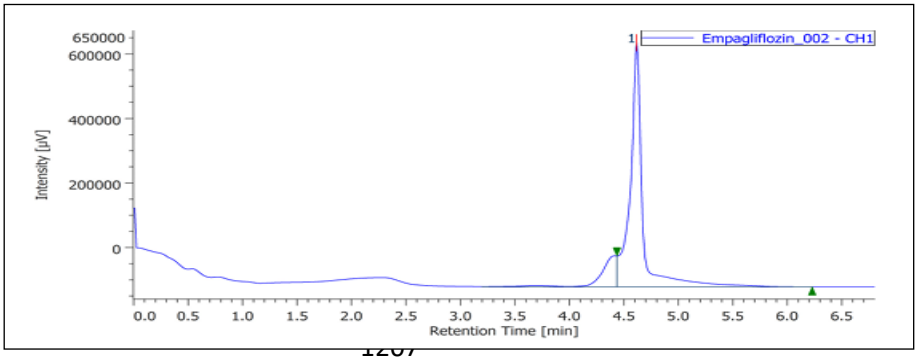


Fig.5. In thermal degradation

Forced degradation type	Actual peak Auc	Degraded peak Auc	% Degradation
Acidic degradation	7748486	6123405	79.02 %
Basic degradation	7748486	6157230	79.46 %
Oxidative degradation	7748486	6110387	78.85 %
Photolytic degradation	7748486	5866514	75.71 %
Thermal degradation	7748486	5920082	76.40 %

Table 2. Forced degradation study

Validation of methods

For the following parameters, the developed technique was validated in accordance with International Conference on Harmonization (ICH) recommendations Q2(R1).

1. System Suitability

The HPLC method's repeatability was confirmed by system appropriateness. Peak area, theoretical plate, and retention time repeatability were calculated and contrasted.(21)

2. Linearity

Concentration and mean area were found to have a linear relationship, which was supported by the regression coefficient of 0.9989. Consequently, it was concluded from the current study's data that the linearity experiment for this medication was effective within between 2 and 20 µg/ml.(22)

3. Accuracy

Analyzing sample solutions spiked with known concentrations of the bulk drug or standard at three different concentration levels—50, 100, and 150% of each at a certain limit—was how accuracy was determined.(23)

4. Precision

By diluting the volume corresponding to 0.3, 1.2, and 1.8 ml of standard stock solution (1000 µg/ml) to 100 ml with mobile phase to yield 3, 12, and 18 µg/ml working solutions, respectively.(24)

5. Limit of detection (LOD) and Limit of Quantization (LOQ)

Calibration standards were used to determine the LOD and LOQ of Mupirocin using the suggested techniques. The LOD and LOQ values were computed as indicated, where SD stands for standard deviation and D represents the calibration curve's slope. (25)

$$LOD = 3.3SD/D$$

$$LOQ = 10\ SD/D$$

6. Robustness and Ruggedness

Two distinct analysts examined the sample in order to determine the impact of slight variations in wavelength and robustness. (26)

7. Specificity and selectivity

In order to determine whether interfering substances were present in the empagliflozin working solution, specificity and selectivity were investigated. According to the findings, empagliflozin has a retention period of 2.675 minutes. (27) The compound's retention period is the same as that of the normal medication and is unaffected by formulation. solvent and excipient. This suggests that the technique for determining empagliflozin was determined to be specific and selective.[28]

Results and Discussion

Method development and optimization

The goal of the initial method development was to achieve the best possible separation of empagliflozin with minimal analysis time and sufficient resolution. During the process of optimizing the approach, different mobile phase compositions were assessed. Using methanol as an organic modifier resulted in improved peak forms in contrast to acetonitrile. Using acetonitrile and water (95:05 v/v) as the mobile phase at a flow rate of 0.9 mL/min, the finalized chromatographic conditions produced the best separation with retention periods of 5.0 and 6.5 minutes for empagliflozin, respectively. The method is appropriate for routine analysis because the whole run time was less than five minutes.(29)

Method validation

1. **Specificity :** The following solutions are injected into the HPLC apparatus to show the assay's specificity**.**

i. Diluent as a blank

ii. Test Solution

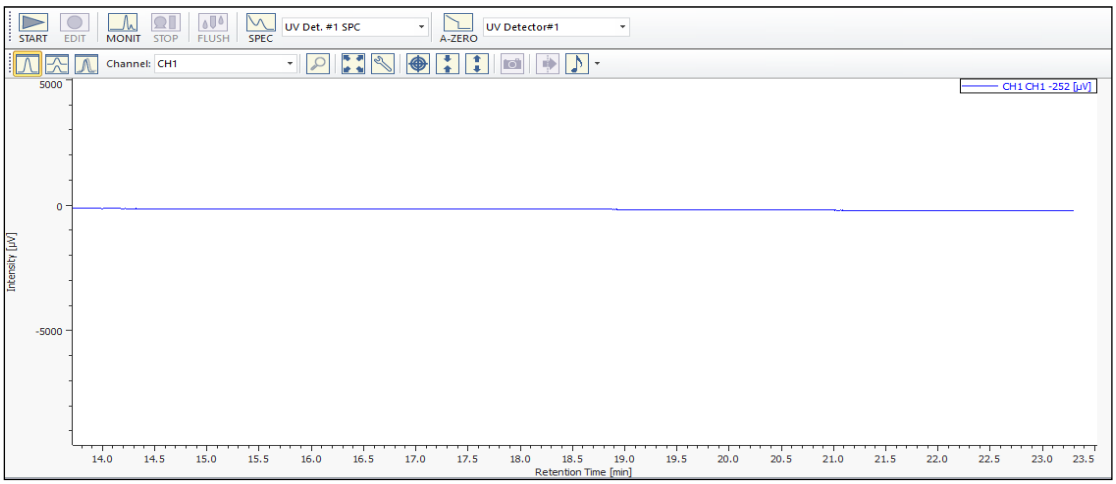


Fig. 6. HPLC Chromatogram of blank solution

2. System precision

Six replicate injections of the standard were used to assess system precision using the suggested method.

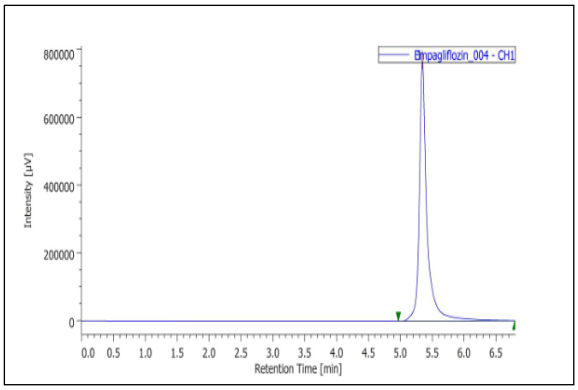


Figure 7. System precision injection 1

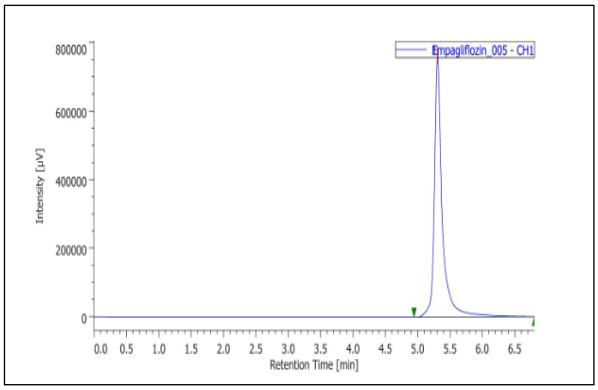


Figure 8. System precision injection 2

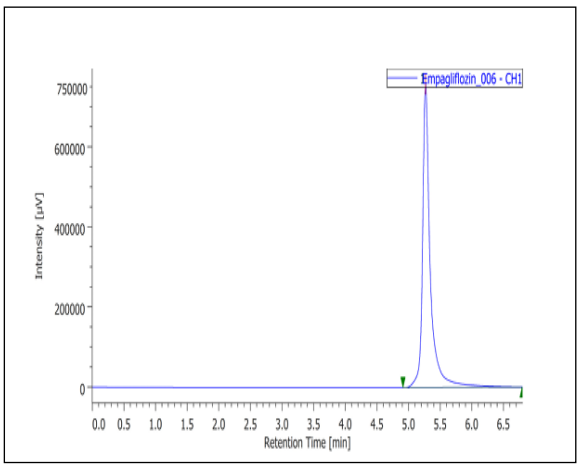


Figure 9. System precision injection 3

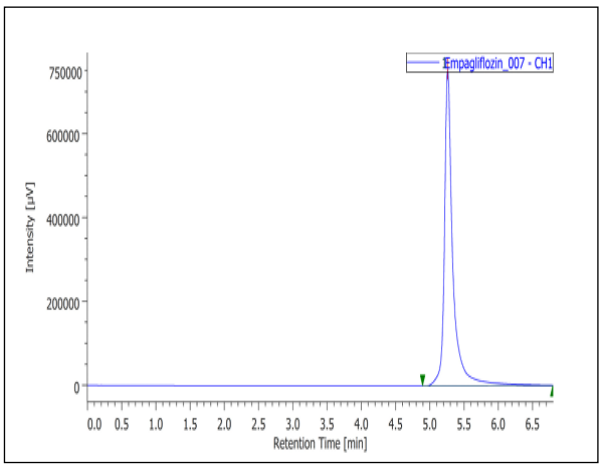


Figure 10. System precision injection 4

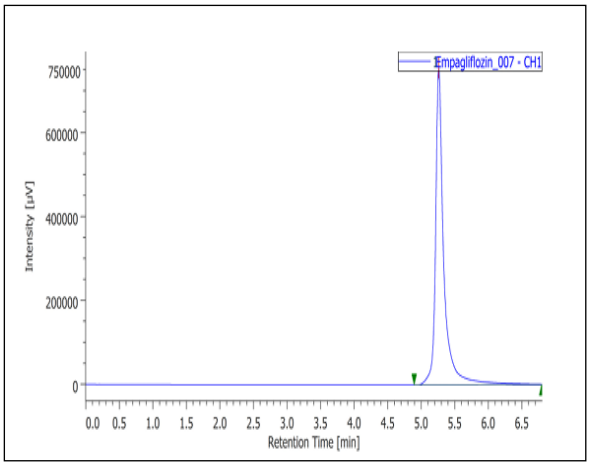


Figure 11. System precision injection 5

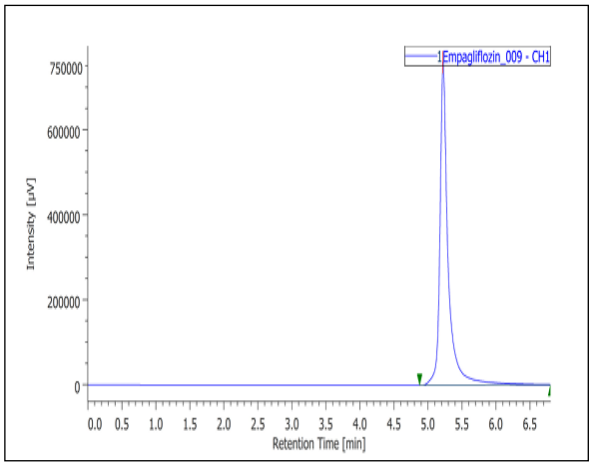


Figure 12. System precision injection 6

Table 3: System precision of Empagliflozin

Inject. No	Empagliflozin Peak Area
1	6440801
2	6401868
3	6473767
4	6477630
5	6456076
6	6448529
Mean	6449778.5
% RSD	0.426%

Acceptance Criteria:

The following ranges should apply to the relative standard deviation: % RSD for Empagliflozin Area < 2.0%,

Results and evaluation:

The % RSD observed within acceptable limit indicates the precision of the system.

3. Method precision

Each of the six test solutions was prepared independently. Each was examined in

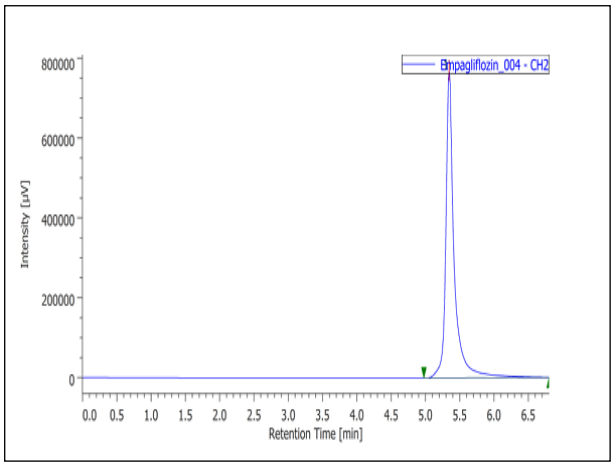


Figure 13. Method Precision injection 1

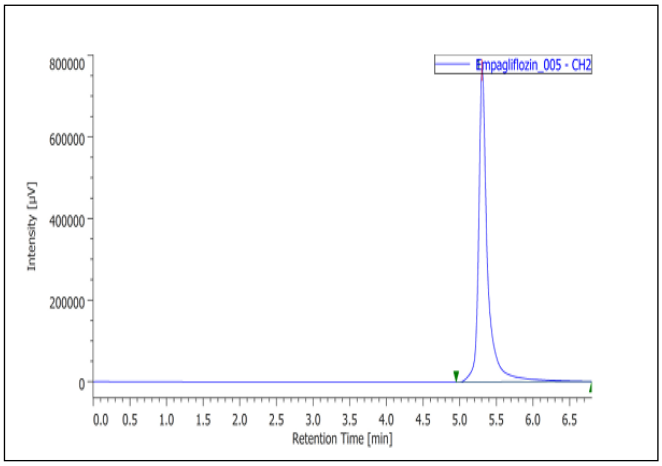


Figure 14. Method Precision injection 2

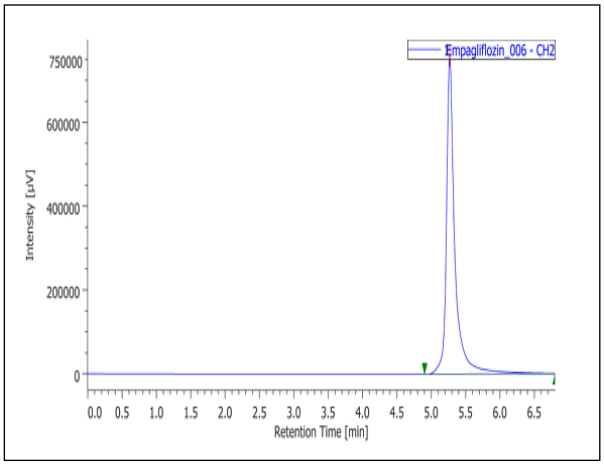


Figure 15. Method Precision injection 3

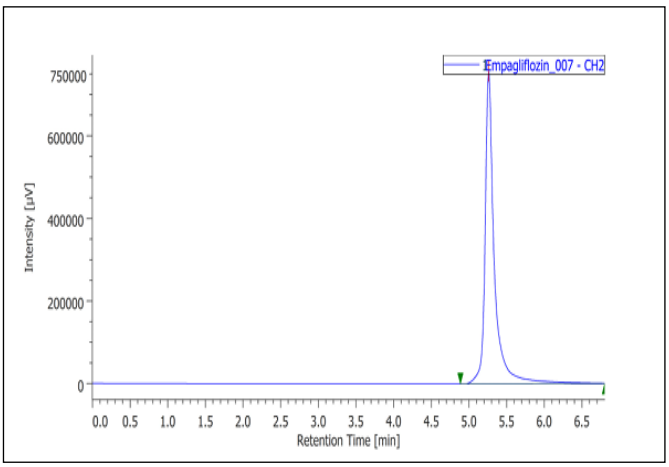


Figure 16. Method Precision injection 4

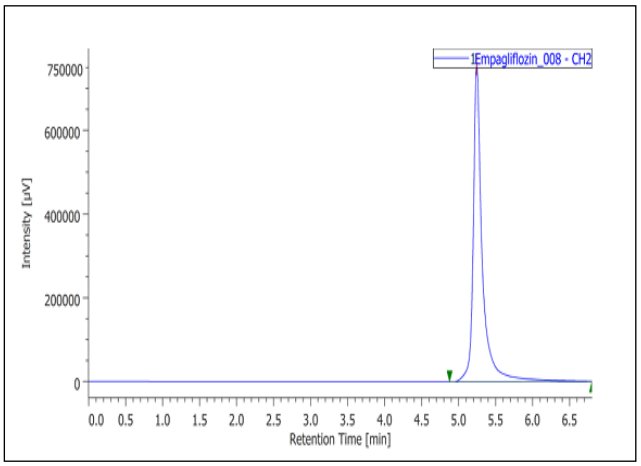


Figure 17. Method Precision injection 5

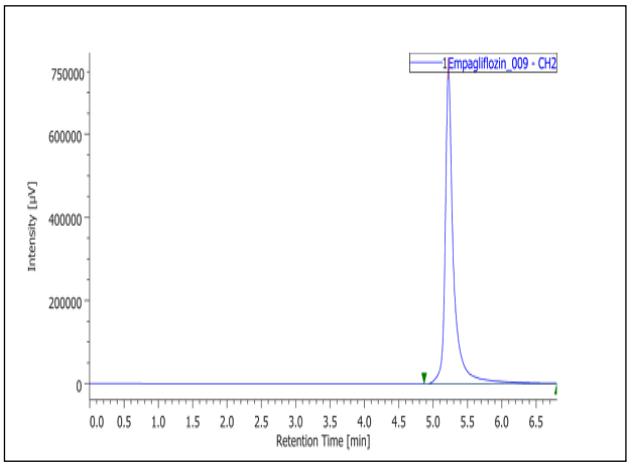


Figure 18. Method Precision injection 6

Table 4: Method precision data

Sample No.	% Sample Assay
1	100.41
2	99.80
3	100.96
4	100.94
5	100.65
6	100.53
Mean	100.54
% RSD	0.425%

Acceptance Criteria:

The relative standard deviation should be within the following limits % RSD for Assay of Sample < 2.0%,

Results and evaluation:

The % RSD was observed within the limit indicates that the method has an acceptable level of precision.

4. **Intermediate precision**

The six test solutions were prepared separately. Each was analyzed as per

proposed procedure (analyzed in morning, afternoon and evening i.e. intraday precision and today, tomorrow and day after tomorrow i.e. interday precision).

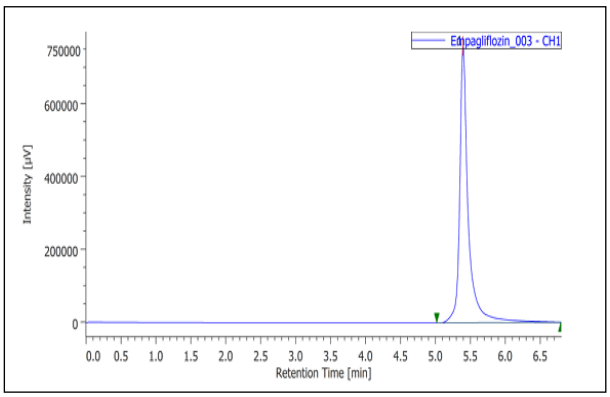


Figure 19: Intrday precisin morning

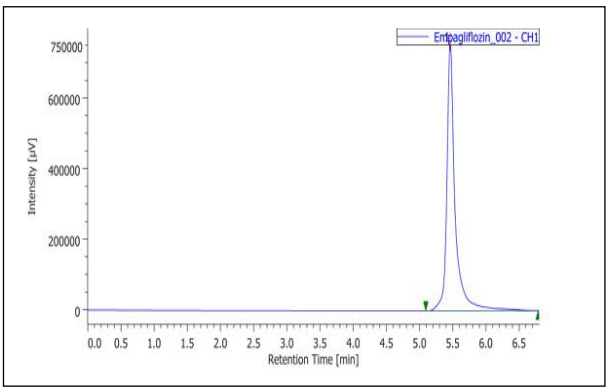


Figure 20: Interday precision afternoon

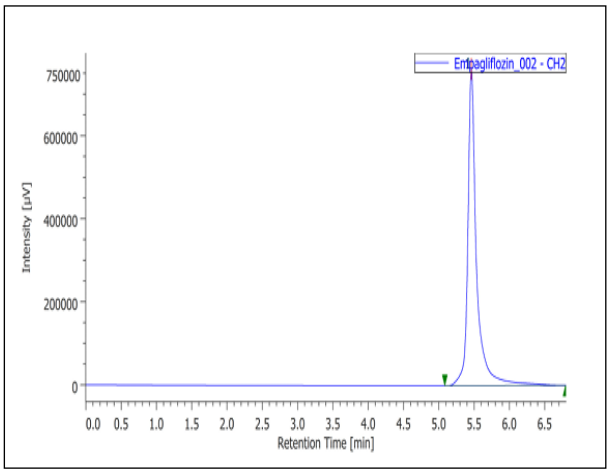


Figure 21. Intraday precision evening

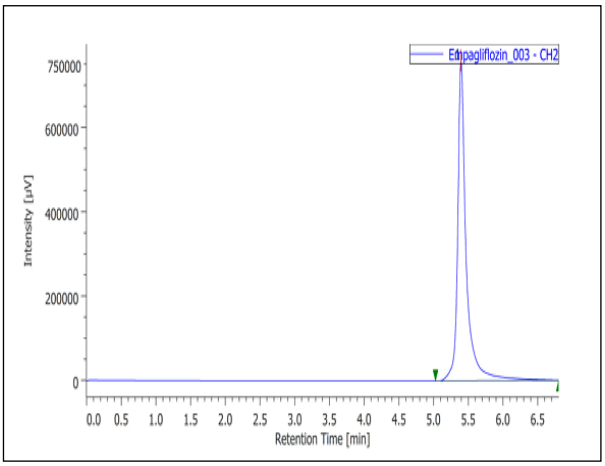
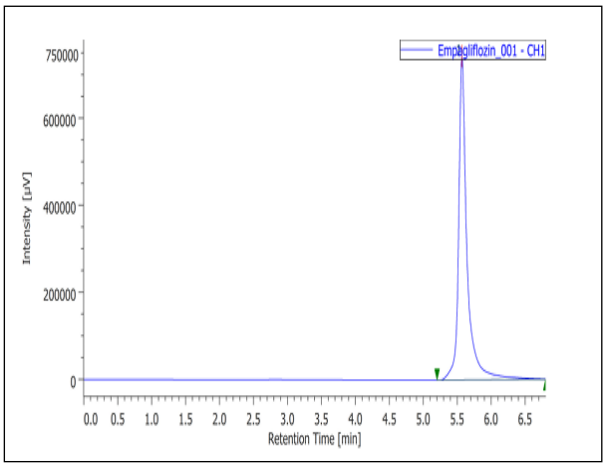


Figure 22. Interday precision today



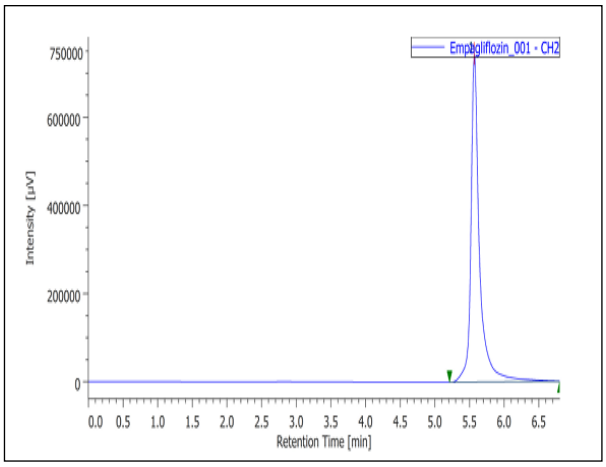


Figure 23. Interday precision tomorrow tomorrow

Figure 24. Interday precision after

Table 5. Intraday and Interday precision

Name of Analyte	Time interval	Intraday	Time interval	Interday
Empagliflozin	Morning	84.26	Today	84.37
	Afternoon	85.01	Tomorrow	82.94
	Evening	85.07	Day after tomorrow	82.98
	Average	84.78		83.43
	% RSD	0.532%		0.976%

Acceptance Criteria:

The following ranges should apply to the relative standard deviation from analyses 1 and 2. The total percentage RSD for the intraday and interday empagliflozin assay should be less than 2.0%.

Results and evaluation:

For intraday and interday analysis, the percentage RSD of the test findings from three to three determinations falls within the acceptable range.

1. **Linearity**

The sample's peak area response was found to be linear between 5 and 30 ppm of working concentration. Six distinct known concentrations of the sample's stock solutions were diluted. Concentration (as x-value) versus area (as y-value) was shown on a graph. The regression slope, y-intercept, and correlation coefficient (r2).

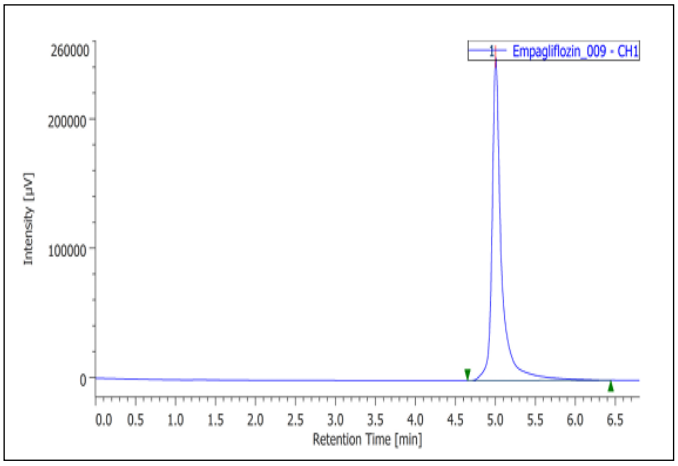


Figure 25. 5 PPM

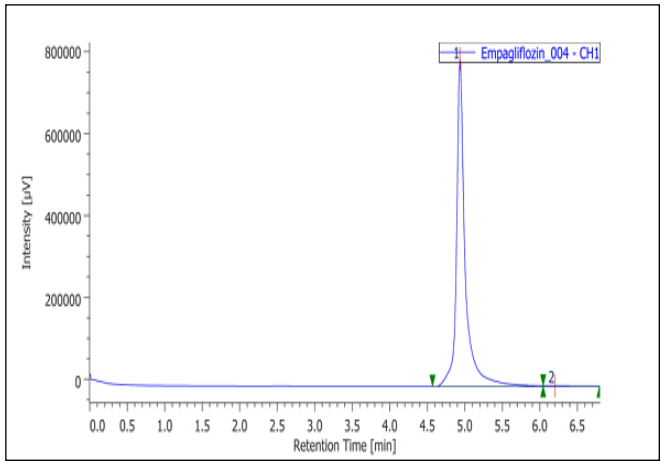


Figure 26.10 PPM

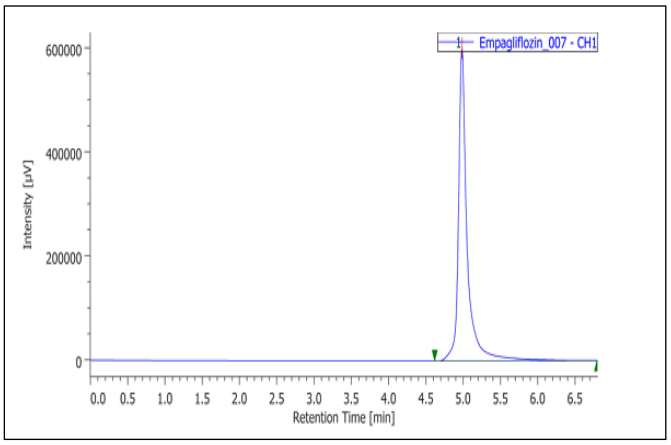


Figure 27. 15 PPM

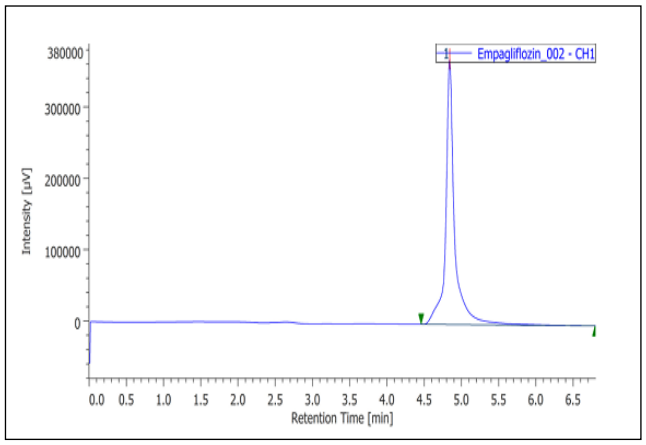


Figure 28. 20 PPM

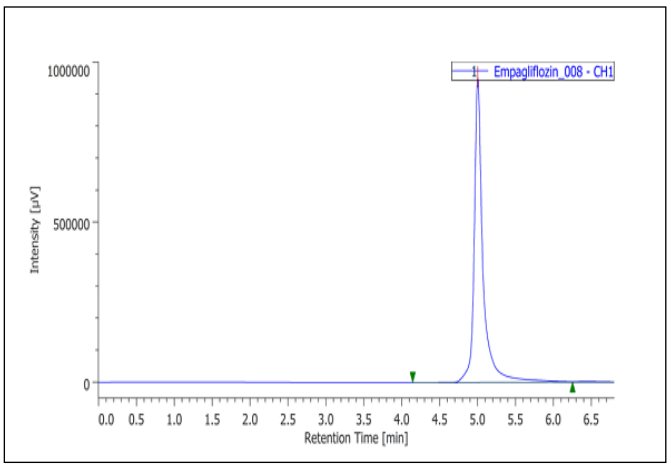


Figure 29 . 25 PPM

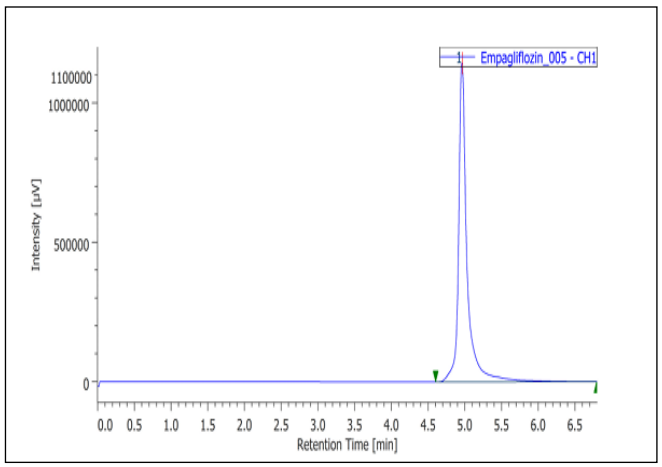


Figure 30. 30 PPM

Table 6. Linearity of Empagliflozin

Conc. of Sample (ppm)	Average Peak area
05	2067366
10	3106672
15	4887509
20	6423132
25	7748486
30	9270821
0.9967	

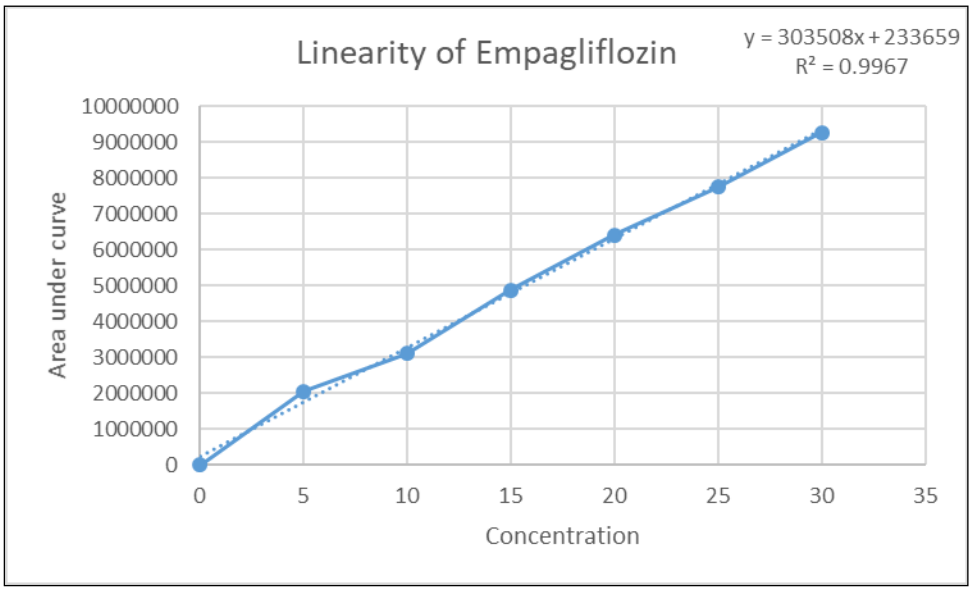


Figure 31. Calibration curve for Empagliflozin

Acceptance Criteria:

The Correlation coefficient (r^2) > 0.9967

Results and evaluation:

Since the correlation coefficient is within the acceptable range, the method is regarded linear.

5. Robustness

To show the method's resilience, the effects of slightly altered chromatographic

conditions were examined in accordance with ICH criteria. The tests are performed by injecting a blank and standard solution while changing the chromatographic settings listed below.

Sr. No	Parameters	Working parameter	- changes	+ changes
1	Flow	1 mL/minute	0.8 mL/minute	1.2 mL/minute
2	Wavelength	254 nm	252 nm	256 nm
4	Mobile phase	95:05 (ACN:Water)	93:07	97:03

Table 7. Robustness parameter

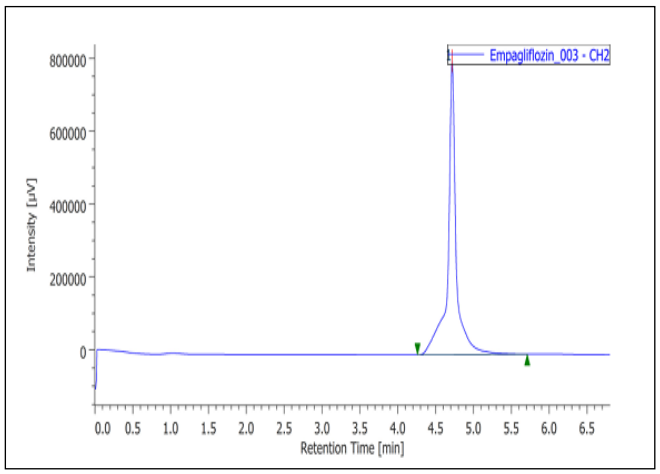


Figure 32. Wavelength +2 nm

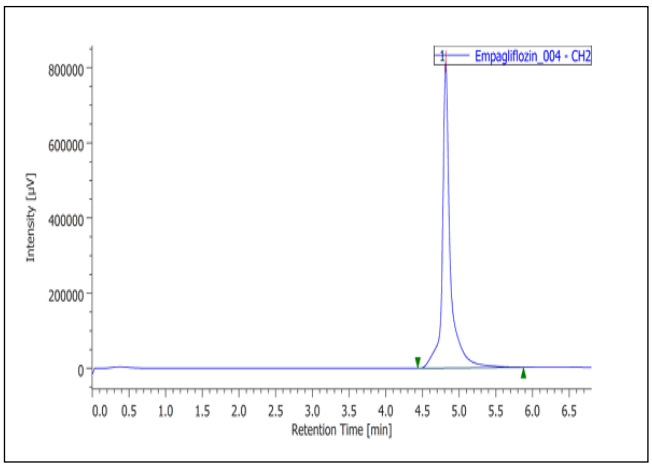
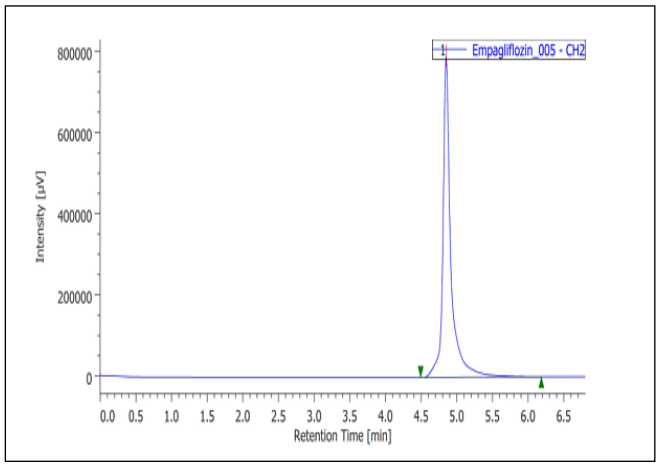
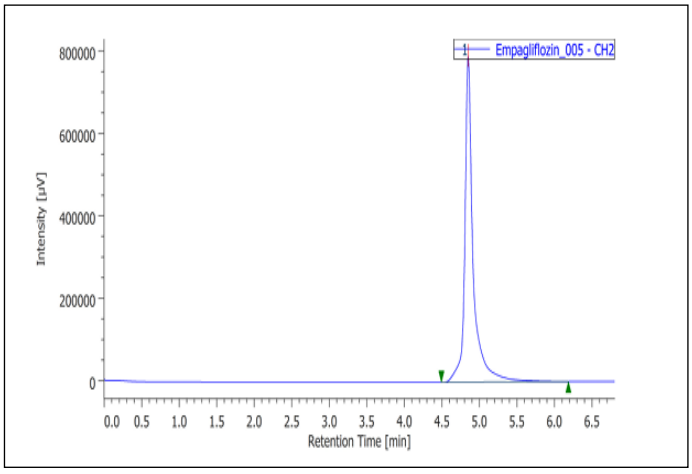


Figure 33. Wavelength +2 nm





nm

Figure 36. Wavelength +2 nm

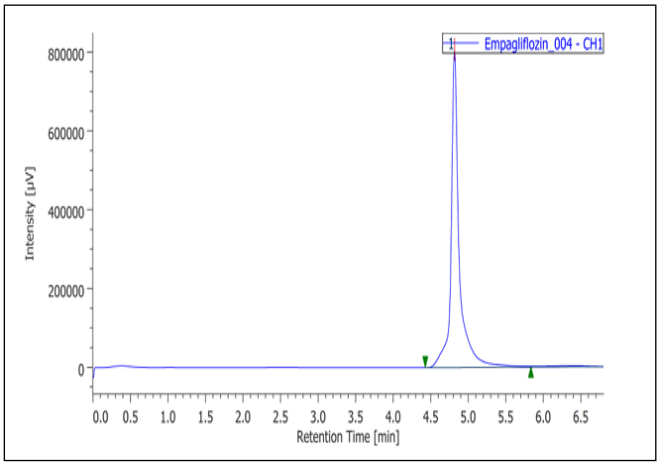


Figure 35. Wavelength -2 nm

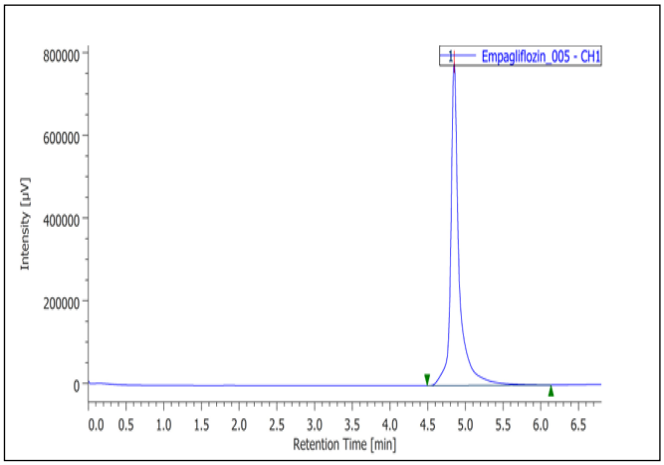
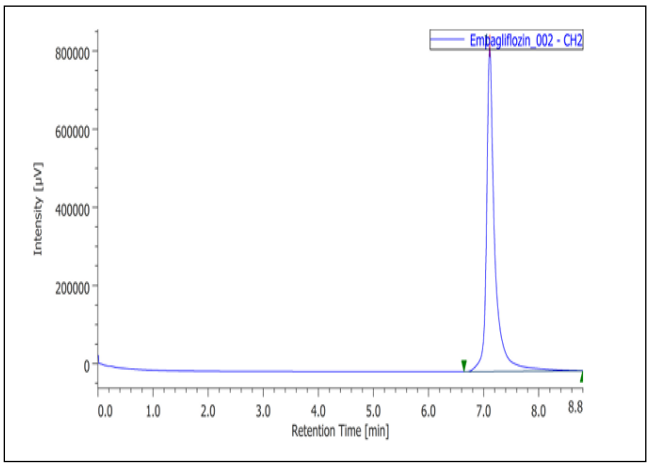
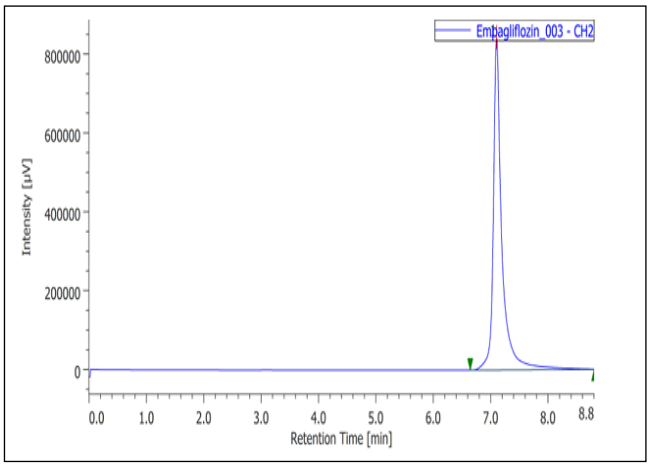


Figure 37. Wavelength -2 nm

Figure 38. Wavelength -2 nm





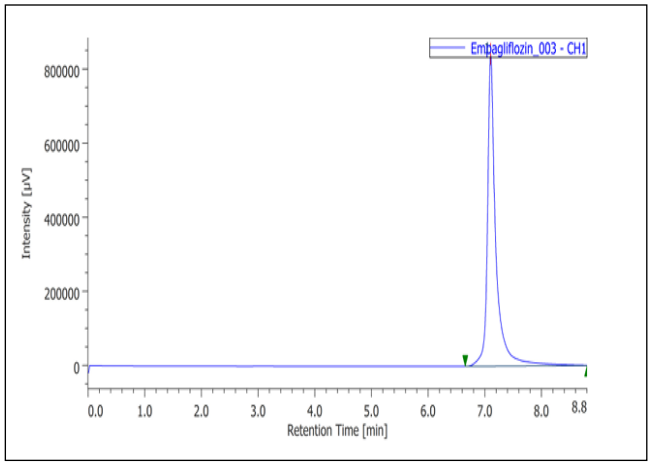


Figure 39. 0.8 ml/min flow rate

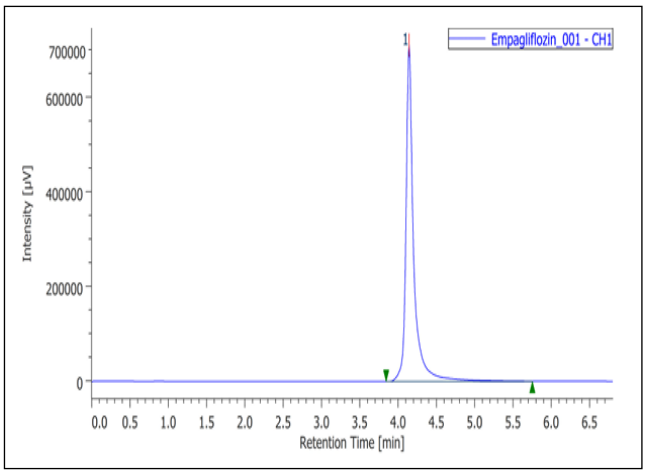
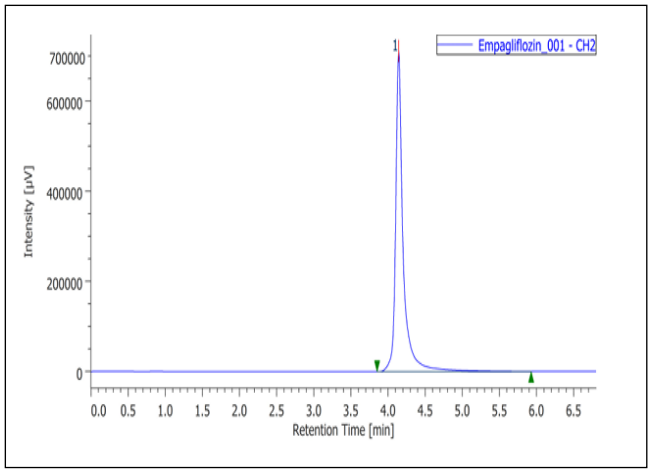


Figure 40, 0.8 ml/min flow rate

Figure 41, 0.8 ml/min flow rate

Figure 42, 1.2 ml/min flow rate



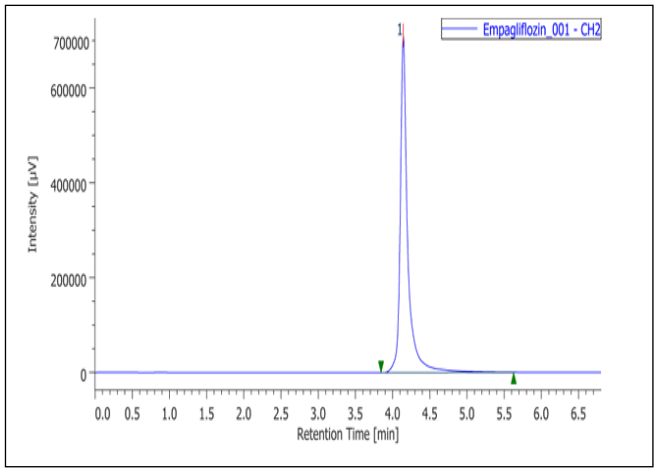
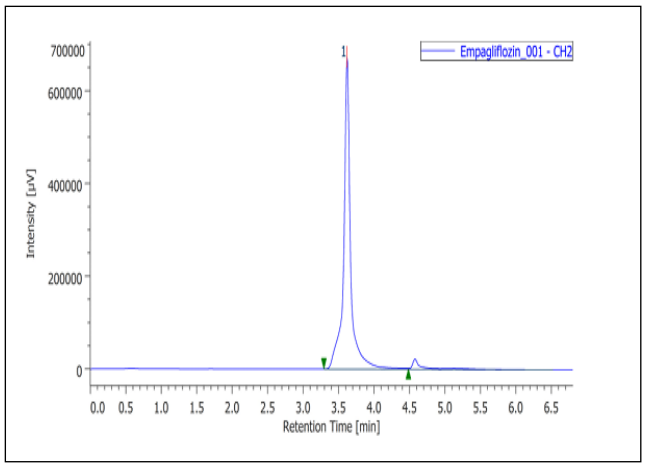


Figure 43, 1.2 ml/min flow rate

Figure 44, 1.2 ml/min flow rate



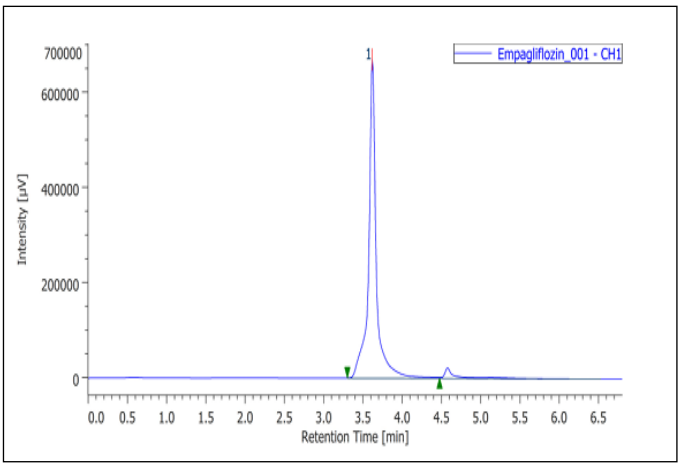


Figure 45. Organic mobile phase +2 +2

Figure 46. Organic mobile phase

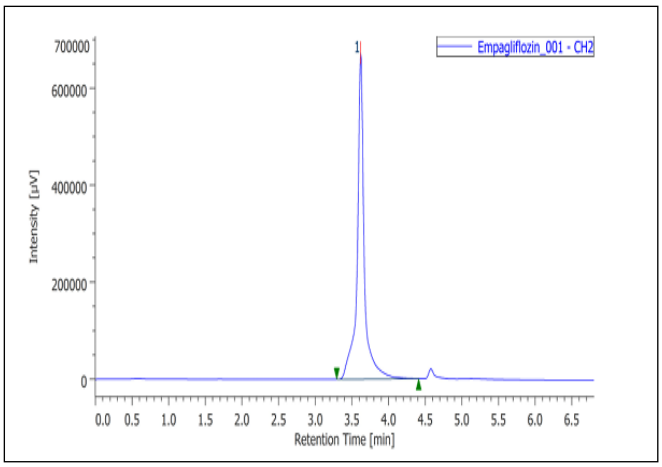


Figure 47. Organic mobile phase +2

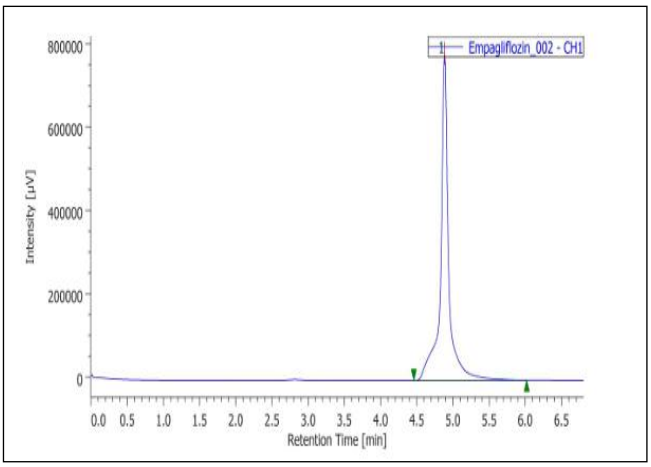


Figure 48. Organic mobile phase -2

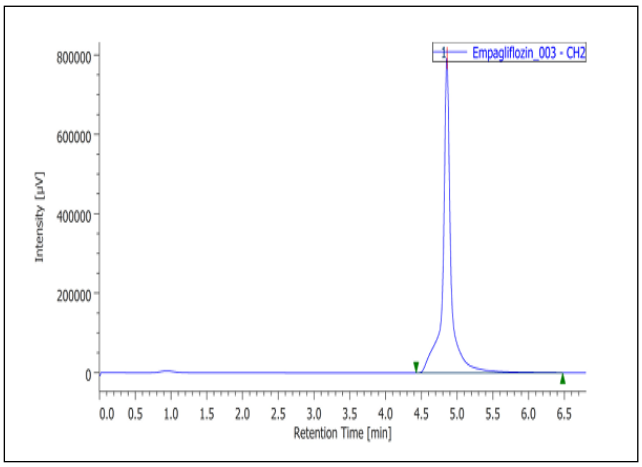


Figure 49. Organic mobile phase -2 -2

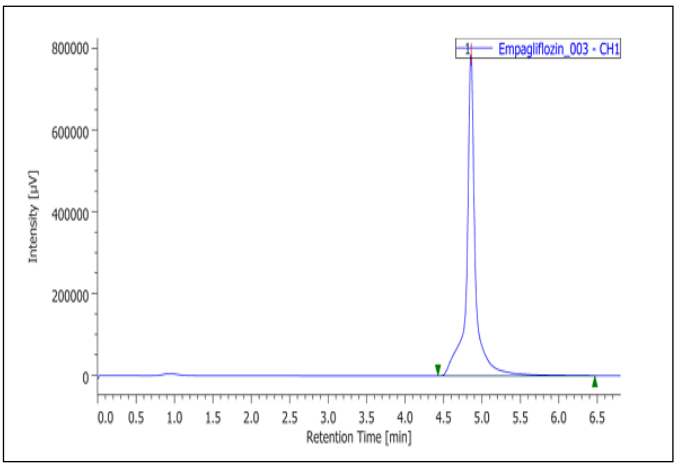


Figure 50. Organic mobile phase

Table 8: Robustness parameters

Robustness parameters		% RSD	Peak tailing	Theoretical plates	Remark
Sample					
Wavelength (+2 nm) and (-2 nm)	6119944	0.558%	0.89	21056	Pass
	6188532		1.19	18750	Pass
	6152109		1.35	16199	Pass
	6047513	0.432%	0.90	21027	Pass
	6094424		1.20	18514	Pass

	6091369		1.36	16664	Pass
Flow rate (0.8mL/min) And (1.2mL/min)	8710012	0.137%	1.51	17567	Pass
	8733912		1.54	18253	Pass
	8723434		1.54	18078	Pass
	5042700	0.210%	1.51	12363	Pass
	5060009		1.49	12708	Pass
	5040828		1.49	12709	Pass
Organic mobile phase (+2) And (-2)	4580331	0.982%	0.99	12703	Pass
	4575580		0.99	13263	Pass
	4500615		0.98	12724	Pass
	6269765	0.569%	0.90	19213	Pass
	6212151		0.90	19184	Pass
	6205144		0.90	19301	Pass

Acceptance Criteria:

The system suitability requirements should be met and the percentage RSD of peak area response resulting from three replicate injections of the sample solution should be less than 2.0%.

Results and evaluation:

For findings obtained under different chromatographic settings, the system suitability parameters and percentage RSD are within acceptable bounds. As a result, the approach is determined to be reliable.

Conclusion

A rapid, sensitive, and specific RP-HPLC technique was developed and validated for the simultaneous measurement of empagliflozin in pharmaceutical formulations.

Using a straightforward mobile phase composition, the technique produced the best separation in less than five minutes of analytical time. Its simple mobile phase composition, rapid analysis time, and ambient temperature operation make it cost-effective and eco-friendly.

Validation experiments demonstrated excellent linearity, precision, accuracy, and robustness. Investigations into forced deterioration confirmed the approach's stability-indicating nature.

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