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Development and Validation of an RP-HPLC Method for Determination of Empagliflozin in Empagliflozin Tablet

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Abstract:-

- ***Background:***

Reversed-phase HPLC (RP-HPLC) is a prevalent chromatographic technique in the pharmaceutical field. At present, there is no pharmacopoeial method to estimate Empagliflozin in Empagliflozin tablets, although a number of Empagliflozin brands in tablet forms are sold in local pharmacies. In this study, an RP-HPLC method has been developed and validated for estimation of Empagliflozin in Empagliflozin 10mg tablets. A C_{18} (250 mm × 4.6 mm, 5 µm particle size) column was employed to attain a satisfactory separation. The mobile phase contained 0.01 M NaH_2PO_4 (as buffer) and acetonitrile in a distinct proportion of 60 and 40 with a flow rate of 1.00 mL/min. Empagliflozin was detected at 210 nm.

- ***Results:***

The system was found to be precise, occupying an average retention time of 4.52 minutes with a % relative standard deviation (%RSD) value of 0.040% and %RSD for peak areas was 580%, theoretical plates 2517 and asymmetry factor 1.65. The precision of the method was ensured, and it was found that the %RSD for Day-1 was 0.644%, for Day 2, 1.045%, and the inter-day precision was 0.899%. The percentage recovery was obtained within 96.08 – 102.88%, which confirmed the proposed method's accuracy. The correlation coefficient was found 0.999 that indicated the linearity of the proposed method. Specificity and robustness of the method were also confirmed.

- ***Conclusion:***

The RP-HPLC method for estimation of Empagliflozin in Empagliflozin tablet has been developed and validated as per the ICH (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) guidelines with regard to the validation parameters.

***Keywords*:-** *Empagliflozin, RP-HPLC, Method Development, Validation, Tablet.*

I. INTRODUCTION

Empagliflozin is an oral antidiabetic drug used for type II diabetic patients to achieve a better glycemic control[1,2]. It is a potent sodium-glucose cotransporter 2 (SGLT2)-blocker[1,2]. In 2014, the drug got approval for medicinal use in the USA and EU[2,3]. Empagliflozin has the empirical formula $C_{23}H_{27}ClO_7$, and the IUPAC name is (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl] oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4, 5-triol. The MW of Empagliflozin is 450.9 g/mol. The drug is marketed as tablets in two strengths: 10mg and 25mg. Empagliflozin reduces renal reabsorption of glucose that is filtered, thus lowering its renal threshold. Consequently, its urinary excretion is increased and plasma levels are reduced[4]. In addition, the drug is also reported to reduce blood pressure and induce weight loss[5].

High-performance liquid chromatography is an excellent technique for separation, identification, quantification, and purification of the analytes in any mixture[6]. Reversed-phase HPLC is currently the most frequently applied method in the pharmaceuticals for routine analysis of drugs. A noteworthy convenience of RP-HPLC is its sustainability in the separation of molecules possessing a bit of hydrophobic nature[7]. The RP-HPLC method exhibits preparative applications along with analytical applications in separation and purification processes[6]. Some previously developed RP-HPLC methods for determination of Empagliflozin in Empagliflozin dosage forms have been reported[8-16].

Presently, there is no pharmacopoeial method that estimates Empagliflozin in Empagliflozin tablets, although many brands of Empagliflozin 10mg tablets are available in Bangladesh and other countries. Following the ICH guidelines, the present study aimed to develop an RP-HPLC method and validate the method to confirm that the proposed method is precise, accurate, specific, linear, robust, and can produce reproducible results[17].

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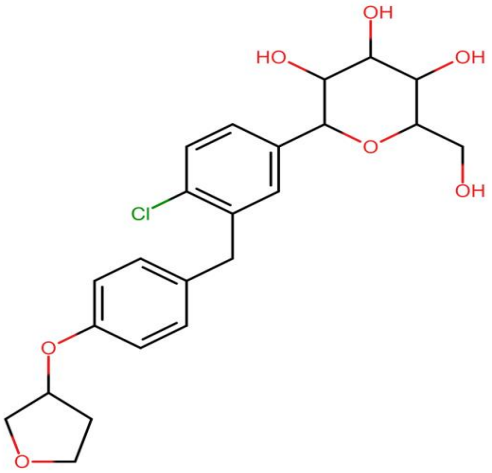

Fig 1 Structure of Empagliflozin (Drawn using RCSB PDB: Chemical Sketch Tool[18]).

II. MATERIALS AND METHODS

➢ *Chemicals and Reagents*

Empagliflozin working standard was received as a gift sample from Aristopharma Limited, Shampur, Dhaka. Empagliflozin 10 mg tablet dosage form was considered as sample in this study. The tablets were collected from a medicinal shop of the local market. Monobasic sodium phosphate (Merck, Germany) and HPLC-grade Acetonitrile (Merck, Germany). Milli-Q EQ 7000 Ultrapure Water Purification System (MilliporeSigma, USA) was used to purify water. All the solutions and solvents used in this study were filtered through a 0.2 µm size membrane filter (MilliporeSigma, USA) followed by degassing ultrasonically before using in the chromatography system. Each chemical and reagent used in this study was of analytical grade.

➢ *Equipment*

An analytical balance (Model: ML3002F, METTLER TOLEDO, Switzerland), Waters HPLC System (Model: e2695, Waters, USA), ThermoScientific HPLC System (Model: UltiMate 3000, ThermoScientific, USA), XTerra MS C_{18} column (USP L1, 250 mm × 4.6 mm, 5 µm particle size; Waters, USA), ThermoScientific Hypersil GOLD C_{18} column (250 mm × 4.6 mm, 5 µm particle size; ThermoScientific, USA) and an Ultrasonic bath (Elma, Germany) had been involved. The HPLC systems were connected to personal computers controlled by LabSolutions software that was used for generation of chromatograms and analysis of the data.

➢ *Preparation of Buffer and Solutions*

➢ *Preparation of Standard Solution*

About 25 mg of Empagliflozin working standard was accurately weighed and transferred into a 100 mL volumetric flask. About 70 mL of the diluting solvent that was mobile phase (0.01 M NaH_2PO_4 buffer and acetonitrile at 60:40 ratio) was added to it followed by sonication for around 20 minutes with intermittent shaking. Afterwards, the sample was kept at room temperature for cooling. Using the same solvent, 100 mL volume was made. 10 mL of this 100 mL solution was taken into a 50 mL volumetric flask, and the 50 mL volume was made with the same diluting solvent. Before injection, it was filtered through a 0.20 µm size membrane filter.

➢ *Preparation of Sample Solution*

The average weight of 20 randomly selected tablets was determined. 10 intact tablets were taken into a 100 mL volumetric flask. After addition of 10 mL of water to the tablets, the volumetric flask was shaken until the tablets were completely disintegrated. Afterwards, 70 mL of the diluent was added followed by sonication again with intermittent shaking for 20 minutes. It was kept at room temperature for to cool. Using the same solvent, the 100 mL volume was made. The sample was centrifuged, and 5 mL of the supernatant solution was transferred into a 100 mL volumetric flask. The volume was made up to the mark with the same solvent. The solution thus prepared was then passed through a 0.20 µm size membrane filter before injection.

III. METHOD VALIDATION

Validation of the proposed HPLC method was performed following ICH (2005) and US FDA (2001) guidelines[19,20].

➢ *System Precision/System Suitability*

An integral part of many analytical procedures is testing of system suitability. Parameters of the system suitability test depend on the type of procedure to be validated. System suitability testing was performed by measuring retention time, peak area, theoretical plate, resolution and asymmetry factor of standard solution that contains 100% working concentration (n = 6). The %RSD was calculated for retention time, and peak area, and the average value for theoretical plate and asymmetry factor. The results should be within the acceptance limit.

➢ *Method Precision*

Method precision was determined by assaying six separate samples that contained 100% of the working concentration, and the six samples were taken from the same batch. Percentages of results were calculated against the label claim.

➢ *Intermediate Precision*

Intermediate precision was determined by analyzing six separate samples that contained 100% of the working concentration, and the six samples were taken from the same batch, but analyzed on different day by different analysts using different equipment. Percentages of results were calculated against the label claim.

➢ *Accuracy*

Accuracy was determined by performing assay of nine sample solutions of Empagliflozin standard at 80%, 100%, and 120% concentrations (each concentration had three replicates) in a placebo mixture and the percent recovery of Empagliflozin was calculated from the solution containing placebo and the active ingredient. At each level, the range of

percent recovery must be within 95.0% - 105.0%[21]. Plotting the amount added against the amount recovered yielded a linear curve, and the correlation co-efficient was determined.

➢ *Specificity*

- *Identification*

Retention time observed for the major peaks generated in the chromatograms of the samples were compared to that of the chromatogram generated by the standard solution.

- *Placebo Interference*

Using the same excipients of the Empagliflozin 10 tablet, the solution of placebo was prepared in an identical way as the standard and sample. The solution of placebo should not generate any peak at the retention time of Empagliflozin.

➢ *Linearity*

Using Empagliflozin working standard, five standard solutions of 80%, 90%, 100%, 110% and 120% strengths were prepared and the peak areas generated by the five working standard solutions were recorded[21]. Plotting actual concentrations (µg/mL) against the peak areas yielded a linear curve from where the linear regression coefficient was determined.

➢ *Robustness*

Robustness of the proposed method was confirmed by performing analysis the same batch of Empagliflozin 10 mg tablets with minor deliberate changes in the optimized method parameters. The effect of variations in flow rate, ratio of the buffer and Acetonitrile in the mobile phase, and column oven temperature on the assay results was recorded. According to ICH guidelines, the % RSD should be less than 2[19].

IV. RESULTS AND DISCUSSION

Analytical technology in the pharmaceutical field is advancing day by day. To analyze a pharmaceutical formulation, the most vital and critical step is developing an effective and reliable assay method for quantification of the active ingredient(s) present in the formulation. A number of analytical methods have been used to quantify the active ingredient(s) of pharmaceutical formulations. However, the HPLC method is still the most prevalent and popular in pharmaceutical companies, and has been successfully used to analyze pharmaceuticals[19,20].

Several HPLC methods were applied in order to achieve the maximum separation of Empagliflozin. After a number of trials with acetonitrile, methanol, water, and buffer solutions at different ratios, the mobile phase was selected. Stationary phase, mobile phase, retention time, flow rate and other conditions in the optimized assay method are presented in Table 1.

Table 1 Chromatographic Parameters in the Optimized Assay Method of Empagliflozin

Parameters	Conditions
Stationary phase	C_{18} (250 x 4.6 mm, 5µm pore size)
Mobile phase	Phosphate buffer (0.01 M Sodium Dihydrogen Phosphate) : Acetonitrile (60:40v/v)
Flow rate	1.0 mL/min
Run time	10 min
Detector	UV
Wavelength	210 nm
Injection volume	10 µL
Column oven temperature	30°C ± 1°C

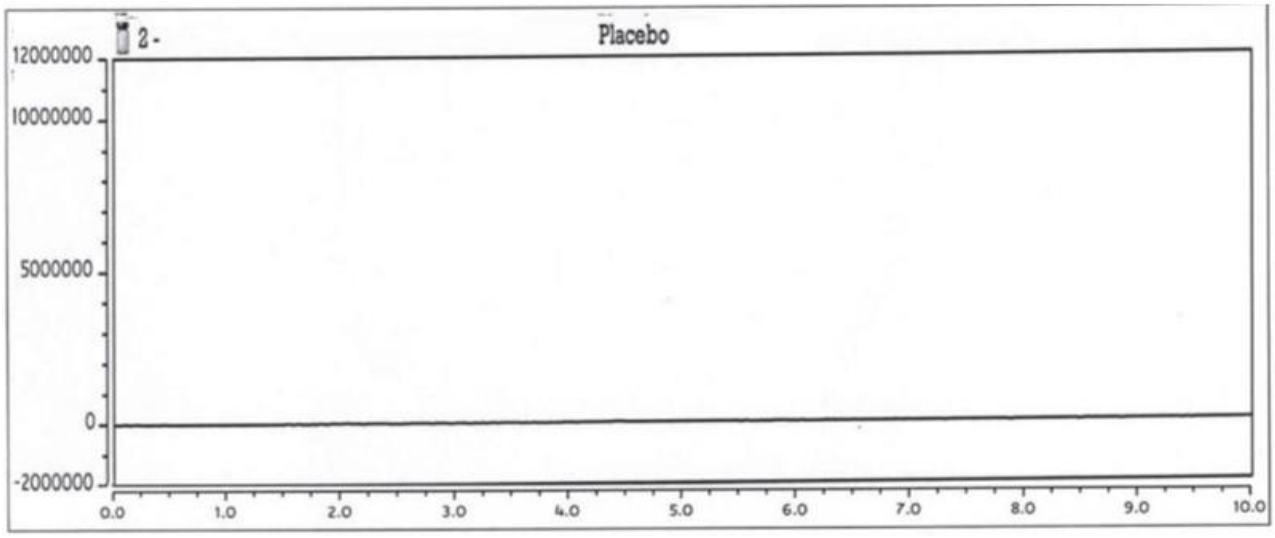

Fig 2(a): HPLC Chromatogram of Placebo

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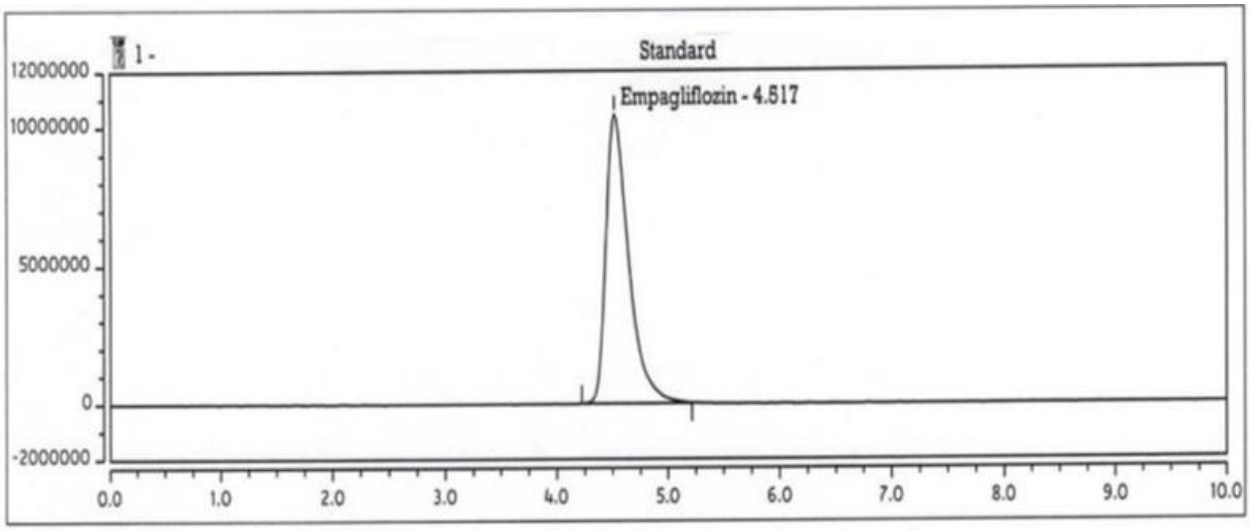

Fig 2(b): HPLC Chromatogram of Standard

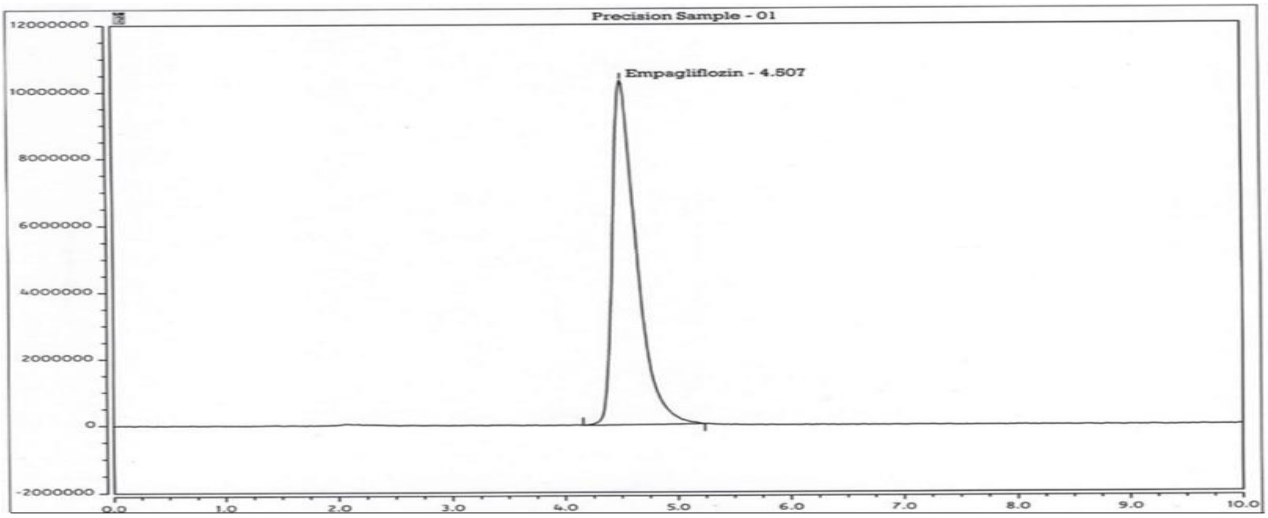

Fig 2 (c): HPLC Chromatogram of Sample

➢ *System Suitability/System Precision*

System Suitability data is presented in Table 2. The acceptable limit of % RSD for peak area and tailing factor is less than 2%, for theoretical plates not less than 2000, and for retention time it is not more than 1.0%[20,24]. In the system suitability test, the relative standard deviation observed was 0.040% (Not more than 1.0%) for retention time, 0.580% (Not more than 2.0%) for peak area, the average theoretical plate was 2517 (Not less than 2000), and the average asymmetry factor was 1.65 (Not more than 2.0). These findings indicated precision of the system to analyze Empagliflozin samples.

Table 2 Data for System Suitability/System Precision

Standard Concentration (µg/ml)	No. of Measurement	Retention Time (min)	Peak Area	Theoretical Plate	Asymmetry Factor
50 µg/ml	01	4.52	2474363	2390	1.67
	02	4.52	2481187	2432	1.65
	03	4.52	2454981	2487	1.65
	04	4.52	2441433	2560	1.65
	05	4.52	2467478	2576	1.66
	06	4.52	2459619	2656	1.63
Average		4.52	2463177	2517	1.65
%RSD		0.040%	0.580%	-	-

➢ *Method Precision*

The data for Precision on Day-1 is shown in Table 3. The %RSD was found 0.644% for six separate samples collected from a single batch, which was within the acceptable limit (Not more than 2.0%)[20,24] and confirmed precision of the proposed method to analyze Empagliflozin in Empagliflozin Tablets 10 mg.

Table 3 Data for Precision on Day-1

Sample No.	Weight of Sample (mg)	Assay Result (mg/Tablet)	% of Label Claim	% of Relative Standard Deviation (%RSD)
01	1022.4	10.183	101.83	0.644%
02	1018.4	10.066	100.66	
03	1022.7	10.178	101.78	
04	1017.0	10.224	102.24	
05	1016.5	10.223	102.23	
06	1027.5	10.101	101.01	

➢ *Intermediate Precision*

The data for Precision on Day-2 is shown in Table 4. On Day-2, the %RSD was found 1.045% for six separate samples collected from the same batch of Day-1, which was within the acceptable limit (Not more than 2.0%)[20,24]. The findings also indicated precision of the proposed method.

Table 4 Data for Precision on Day-2

Sample No.	Weight of Sample (mg)	Assay Result (mg/Tablet)	% of Label Claim	% of Relative Standard Deviation (%RSD)
01	1023.4	10.339	103.39	1.045%
02	1020.5	10.194	101.94	
03	1017.8	10.098	100.98	
04	1017.2	10.16	101.60	
05	1024.9	10.22	102.20	
06	1019.5	10.377	103.77	

Table 5 Data for Intermediate Precision

Sample No.	% of Label Claim	
	Day-1	Day-2
01	101.83	103.39
02	100.66	101.94
03	101.78	100.98
04	102.24	101.60
05	102.23	102.20
06	101.01	103.77
% RSD	0.644%	1.045%
% RSD of 12 samples	0.899%	

To determine Intermediate Precision, the data for Precision on Day-1 and Day-2 was compared and shown in Table 5. The %RSD of the twelve separate samples from the same batch was observed to be 0.899%, which was less than the limit of 2.0%[20,24]. The findings of the intermediate precision test confirmed precision of the proposed method to analyze Empagliflozin in Empagliflozin tablets 10 mg.

➢ *Specificity*

HPLC data obtained in this study showed that the retention time of the major peak generated by the sample matched that generated by the standard. No interference was observed for placebo. The placebo did not show a peak at the retention time of Empagliflozin. Thus, specificity of the method was confirmed.

➢ *Accuracy*

All nine determinations for determining accuracy of the assay method were found within the limit of 95% – 105%[21]. Accuracy data is shown in Table 6.

Table 6 Data for Accuracy

Concentration level	Sample	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	Statistical parameters
80%	Sample-1	41.2	39.66	96.26	Mean = 97.64
	Sample-2	41.6	39.97	96.08	SD = 2.55
	Sample-3	39.6	39.83	100.58	%RSD = 2.61
100%	Sample-1	50.0	49.38	98.76	Mean = 98.31
	Sample-2	51.2	50.05	97.75	SD = 0.52
	Sample-3	50.8	50.00	98.43	%RSD = 0.53
120%	Sample-1	58.4	60.08	102.88	Mean = 99.77
	Sample-2	61.2	59.67	97.50	SD = 2.79
	Sample-3	60.4	59.76	98.94	%RSD = 2.80

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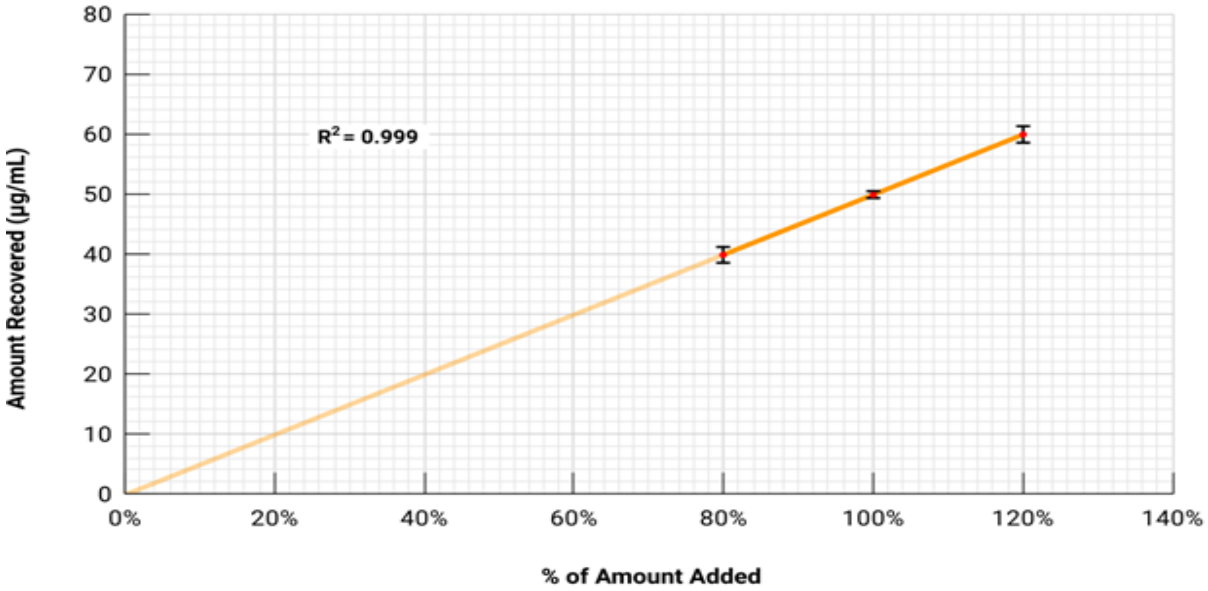

Fig 3 Graphical Representation for Accuracy of Empagliflozin

A plot of the amount added against the amount recovered showed linearity (Figure 3). Thus, the proposed method was considered accurate for analyzing Empagliflozin in Empagliflozin tablet 10 mg.

➢ *Linearity*

The linearity data is presented in Table 7.

Table 7 Data for Linearity

Concentration level	Concentration (µg/mL)	Peak Area	Correlation coefficient
80%	39.68	1951957	0.999
90%	44.64	2217938	
100%	49.60	2456177	
110%	54.56	2721961	
120%	59.52	2959651	

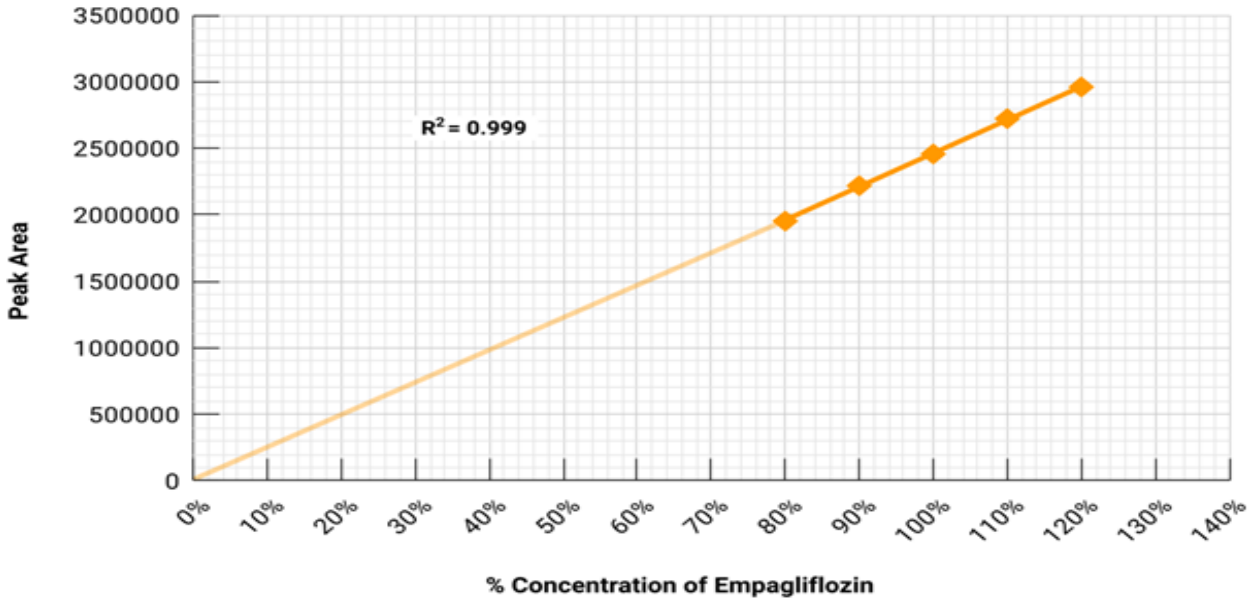

Fig 4 Graphical Representation for Linearity of Empagliflozin

By plotting the drug concentration against mean peak area, linearity of the optimized analytical method was observed, as shown in Figure 4. The findings obtained correlated with the calibration curve (Figure 3). The linear regression coefficient of determination was found to be 0.999, which was within the limit (Greater than or equal to 0.995)[21]. Therefore, the graph confirmed linearity of the proposed method within 80% - 120%.

➢ *Robustness*

The analytical method was found to be unaffected by minute changes in flow rate, composition of the mobile phase and column oven temperature. The findings are presented in Table 8 and, confirmed that the developed assay method for Empagliflozin was robust.

Table 8 Data for Robustness

Sl. No.	Changing Parameters	Assay Results (%)
01.	Flow rate (actual): Flow rate 1.00 ml per minute	101.83±0.23
	Flow rate changed to 0.80 ml per minute	101.05±0.38
	Flow rate changed to 1.20 ml per minute	100.54±0.59
02.	Ratio (actual): Buffer and Acetonitrile in the ratio of 60:40	101.83±0.41
	Ratio changed to Buffer and Acetonitrile in the ratio of 65:35	101.66±0.55
	Ratio changed to Buffer and Acetonitrile in the ratio of 55:45	101.48±0.63
03.	Column oven temperature (actual): 30°C	101.83±0.37
	Column oven temperature changed to 25°C	100.22±0.40
	Column oven temperature changed to 35°C	100.36±0.49

Various research articles have been reported on the same drug. Empagliflozin was measured earlier using a combination of orthophosphoric acid and acetonitrile, acetonitrile and water, and methanol with phosphoric buffer[25-28].

Currently, there is no pharmacopoeial method available for determining Empagliflozin in Empagliflozin tablets, while several brands of Empagliflozin are on the commercial market. The analytical method developed in this study for estimation of Empagliflozin in Empagliflozin 10 mg tablet has been validated according to the ICH guidelines. In the study, the results are accurate, precise, specific, robust, linear, and free from interference of the tablet excipients.

V. CONCLUSION

In the present study, an RP-HPLC based method has been developed to quantify Empagliflozin in Empagliflozin 10 mg tablets with no interference of the tablet excipients. The proposed method has been validated as per the ICH guidelines. The validation parameters, namely system suitability, accuracy, method precision, intermediate precision, specificity, linearity and robustness were necessarily examined to validate the proposed RP-HPLC method. Reproducibility is a matter of great concern, and the method was found to yield reproducible results. Compared to the other proposed methods already reported in the literature, this new method is simple, economic, and uses a mobile phase containing less organic solvent, is user-friendly and convenient. It can be concluded that this proposed RP-HPLC method could be an ideal assay method for routine analysis of Empagliflozin in Empagliflozin tablets.

➢ *Conflicts of Interest*

The authors declare no conflicts of interest.

➢ *Data Availability*

All data are available in the manuscript.

➢ *Acknowledgement*

The authors thank Aristopharma Limited for providing Empagliflozin working standard.

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