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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF EMPAGLIFLOZIN IN API

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Keywords:

RP-HPLC, Empagliflozin, Validation, ICH guidelines, Sodium Acetate, ortho phosphoric acid.

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ABSTRACT: An accurate, precise and rapid RP-HPLC method was developed and subsequently validated for the determination of Empagliflozin in API. Better separation of the drug was achieved on Intersil column(150x40mm, 5 µm) with the mobile phase consisted of mixture of 0.01 M acetate buffer, methanol in ratio of (30:70v/v) at flow rate of 2ml/min, with detection at 260nm using PDA detector. The retention time was found to be 1.223min. The method was found to be linear in the range of 2-150ug/ml with a correlation coefficient (r2) of 0.999. The LOD and LOQ of the method were calculated to be 0.7 and 1.91µg/ml respectively. The Precision was estimated by employing repeatability; intra-day and inter-day studies and the results were calculated as %RSD values and were found to be within the limits. Recovery of Empagliflozin was found to be in the range of 99.7-100-% which confirms the accuracy of the method. The proposed HPLC method is validated using standard ICH guidelines.

INTRODUCTION: Empagliflozin (Jardiance, Boehringer Ingelheim) is an orally administered selective sodium glucose cotransporter-2 (SGLT-2) inhibitor, which lowers blood glucose in people with type 2diabetes by blocking the reabsorption of glucose in the kidneys and promoting excretion of excess glucose in the urine [1-2].

The sodium glucose cotransporter 2 (SGLT2), located in the proximal tubule of the nephron, is estimated to facilitate-90% of this reabsorption [3-4].

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The chemical name of empagliflozin is (empagliflozin; 1-chloro-4-[b-D-glucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran – 3 – yl -oxy) benzyl]-benzene **Fig.1** as a potent and selective competitive inhibitor of the SGLT2 protein [5]. Sodium-glucose co-transporter 2 (SGLT2) inhibitors offer an insulin-independent mechanism for improving blood glucose levels, since they promote urinary glucose excretion (UGE) by inhibiting glucose reabsorption in the kidney. In addition to glucose control, SGLT2 inhibitors are associated with weight loss and blood pressure reductions, and do not increase the risk of hypoglycaemia [6].

Lack of any published method for RP-HPLC estimation of Empagliflozin in API, therefore, provoked us to investigate the application of method development and validation of RP-HPLC method for the estimation of Empagliflozin in API.

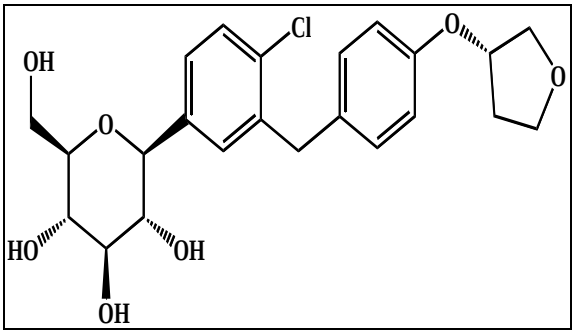

FIGURE 1: STRUCTURE OF EMPAGLIFLOZIN

MATERIALS AND METHODS:

Instrumentation:

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 260 nm with an Intersil C_{18}, (150mmx40mm, 5 µm) dimensions at ambient temperature.

Chemicals and reagents:

Empagliflozin was supplied as gift sample from Mylon laboratories, Hyderabad. Acetate buffer was obtained from Qualigens Fine Chemicals (Mumbai, India) and Methanol (HPLC grade) was purchased from local market.

Preparation of solutions:

Preparation of buffer: Accurately weighed about 2.86 grams of sodium acetate was taken into 1000ml beaker and dissolved and diluted to 1000ml with HPLC water and degassed in ultrasonic water bath and filtered through 0.45µm filter using vacuum filtration and the pH of 4 was adjusted by using diluted ortho phosphoric acid.

Preparation of mobile phase:

The optimized mobile phase consists of a mixture of acetate buffer and methanol in the ratio of 30:70 v/v.

The diluents:

The drug was first dissolved in methanol and further dilutions were made using methanol.

Preparation of standard stock solution:

Accurately weighed 10mg of Empagliflozin was taken in a 10ml standard volumetric flask and dissolved in few ml of methanol. Then the volume was made up to the mark with methanol. From the above solution, 1ml was diluted to 10 ml with mehanol to get a concentration of 100µg/ml of Empagliflozin

Method development selection of wavelength:

Stock solution of 100 mg/ml was prepared for Empagliflozin and further diluted to get the concentration of 10µg/ml of Empagliflozin was prepared with methanol. The wavelength was selected by scanning the above standard drug solution between 200 to 400nm. The scanned results showed that reasonable maximum absorbance was recorded at 260nm. Therefore 260nm was selected as the detection wavelength for the RP-HPLC investigation **Fig. 2**.

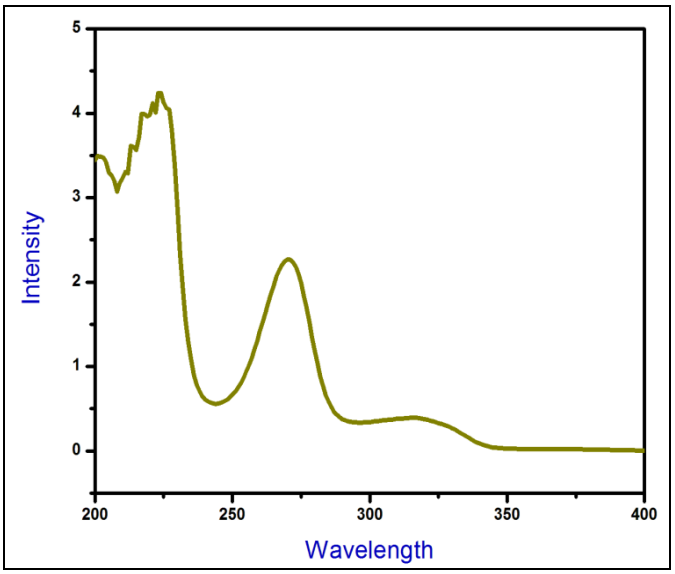

FIG. 2: UV SPECTRA OF EMPAGLIFLOZIN

Construction of calibration curve:

Aliquots of different concentrations of standard solution were prepared and their chromatograms were recorded at the optimized chromatographic conditions. The mean peak areas at different concentration levels were calculated from the chromatograms. Then the linearity plot was constructed using the mean peak areas at their respective concentrations.

Method validation:

The developed method was validated for linearity, accuracy, precision, and limit of detection, limit of quantitation, robustness and system suitability parameters as described in ICH guidelines.

Linearity: From the stock solution, 25, 50, 75, 100, 125, 150µg/ml solutions were made and their

chromatograms were recorded. From the recorded chromatograms, their respective mean peak areas were calculated and the linearity plot was constructed using the mean peak areas at their respective concentrations. The correlation coefficient was found to be 0.999. The linearity data of Empagliflozin was shown in **Table 1** and the calibration plot was shown in **Figure 3.**

TABLE 1: LINEARITY DATA OF EMPAGLIFLOZIN

S.no	Concentration (ug/ml)	Area
1	25	230190
2	50	461821
3	75	692730
4	100	923642
5	125	1164887
6	150	1385556

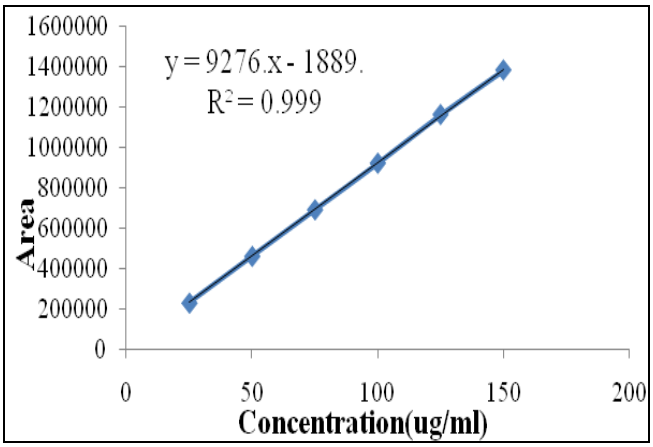

FIG. 3: CALIBRATION PLOT OF EMPAGLIFLOZIN

Accuracy:

Accuracy of the method was determined by calculating the % recovery of Empagliflozin by the method of standard edition (standard stock solution spiked into the placebo). The results of recovery studies were recorded in **Table 2.**

TABLE 2: RECOVERY STUDY RESULT

S.no	Level %	% Recovery
1	50	99.7
2	100	99.6
3	150	100

Precision:

The precision of the method was evaluated by carrying repeatability in the same day (intra-day) and inter-day precision studies. The percentage relative standard deviation (% RSD) of each study was calculated and was found to be less than 2% showing the method was precise. The results of intra-day and inter-day studies were shown in **Table 3.**

TABLE 3: PRECISION STUDY RESULTS

Concentrations 100ug/ml	Repeatability	Intra-day study	Inter day study	
			Day 1	Day 2
Avg.area	924838	918506	918510	915876
S.D	9845	7349	7082	6446
% RSD	1.064	0.8	0.77	0.7

LOD and LOQ:

Limit of detection (LOD) and limit of quantitation (LOQ) of the method were found to be 0.7µg/ml and 1.91µg/ml respectively.

Robustness:

Robustness of the method was determined by slightly changing the flow rate, temperature and mobile phase composition from the optimized chromatographic conditions. The results were shown in **Table 4**.

TABLE 4: ROBUSTNESS STUDY

Concentrations	Flow rate		Temperature		Mobile phase	
100ug/ml	1.2	0.8	35	25	2 ml	2 ml
Avg.area	910020	920363	915886	919754	919194	919504
S.D	8895	6331	6463	6950	7035	6596
% RSD	0.9	0.6	0.70	0.75	0.75	0.71

System suitability parameter:

System suitability was analyzed by giving six replicates and evaluated the chromatographic parameters like retention time, tailing factor, theoretical plates and peak area the results of system suitability was reported in the **Table 5.** The chromatogram of Empagliflozin standard was shown in **Fig. 4**. And the optimized chromatographic conditions were shown in **Table 6.**

TABLE 5: SYSTEM SUITABILITY PARAMETERS OF EMPAGLIFLOIN

Parameters	Values
λ max (nm)	260
Beer's law limit (µg/ml)	25-150ug/ml
Correlation coefficient (r2)	0.999
Theoretical plates	4864.4
Tailing factor	1.6
Retention time	1.223
LOD	0.7ug/ml
LOQ	1.91ug/ml

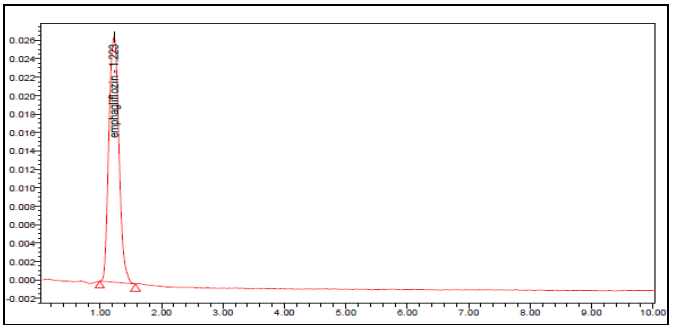

FIGURE 4: STANDARD CHROMATOGRAM OF EMPAGLIFLOZIN

TABLE 6: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

HPLC Condition	Results
Elution	Gradient
API conc.	100µg/ml
Column	Intersil C_{18}column (150mmx40mm, 5 µm)
Detector	Photo Diode Array detector
Wavelength	260 nm
Flow rate	2 ml/min
Run time	10 min
Retention time	1.223
Area	920363

RESULTS AND DISCUSSION: The present study was aimed to developing an accurate, precise and linear RP-HPLC method for estimation of Empagliflozin and in API as per ICG guidelines. The method was found to be linear in the range of 2-150ug/ml with a correlation coefficient (r^2) of 0.999. The LOD and LOQ of the method were calculated to be 0.7 and 1.91 µg/ml respectively. The Precision was estimated by employing repeatability; intra-day and inter-day studies and the results were calculated as %RSD values and were found to be within the limits. Recovery of Empagliflozin was found to be in the range of 99.7-100-% which confirms the accuracy of the method. The system suitability was studied with six replicates standard solution of Empagliflozin and results were found to be acceptance criteria.

CONCLUSION: In the present study, we have developed a new, rapid RP-HPLC Method and validated for different parameters linearity, accuracy, precision, and LOD, LOQ, Robustness and system suitability. By studying all these validation parameters we have concluded that the method was linear, accurate, precise, robust and rapid for the determination of Empagliflozin in API. Hence the method can be successfully applied for the estimation of Empagliflozin in API.

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