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Stability indicating assay method for estimation of Empagliflozin using HPTLC

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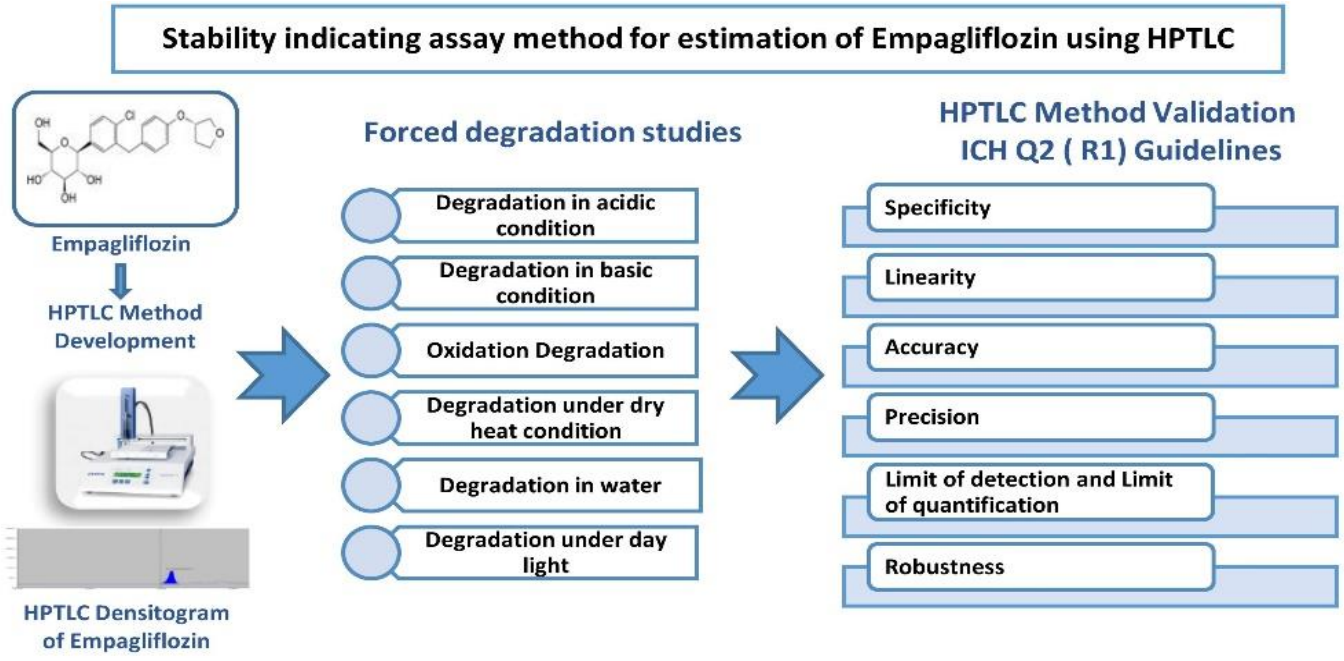
ABSTRACT

A planer chromatographic method capable of indicating the stability of the empagliflozin was developed and validated. Separations were achieved on the silica gel $60F_{254}$ as the planar stationary phase and a solvent mixture comprising of Chloroform, Toluene, Methyl alcohol, and Methanolic acid mixed in a proportion of 8:4:2:0.1 v/v/v/v. Examination of the band developed for empagliflozin indicated a densito metric value with an retardation factor of 0.33±0.02. The calibration data analysis through linear regression showed a statistically significant correlation ($R^2 > 0.990$) between peak area and concentration covering concentrations from 100 to 700 ng applied to the spot. The method was validated to check if it was accurate, precise, linear, and robust following the International Council for Harmonization guidelines. Detection and quantitation limit of the method also were determined. Empagliflozin was further subjected to forced degradation followed by analysis using the proposed method on the samples. The analytical method enabled precise, selective, and accurate analysis of Empagliflozin when its degradation products were present. The method developed can be used to quantitatively analyze Empagliflozin in bulk drug, formulations, and stability studies.



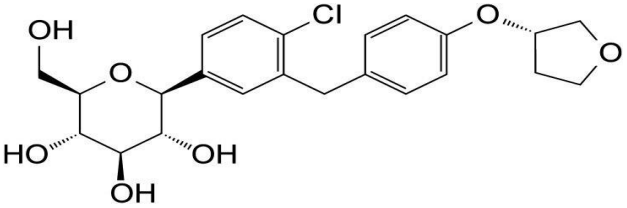
Keywords: Empagliflozin, Validation, Degradation, Robustness, linearity.

INTRODUCTION

Drug stability is a critical asset for pharmaceutical drug products that refers to the drug's capacity to stay unchanged or maintain its chemical, physical, pharmaceutical, microbial properties throughout the product lifecycle [1]. Drug product stability is affected due to chemical change which is commonly referred to as a degradation process that occurs during handling, processing, manufacturing, transportation, and storage in response to change in environmental factors, the action of unrelated compounds or due to inherent characteristics of the active pharmaceutical substance [2,3]. It is the process in which the drug forms a related molecule or may turn into a different compound commonly known as a degradation product. These degradants are impurities that may or may not be similar in action with the original drug product which leads to alteration of efficacy, safety, and overall quality of the product. Thus, it is essential to conduct stability testing at every phase of drug development for ensuring the quality and safety of the pharmaceutical product.

Studying forced degradation provides a method to assess the stability of pharmaceutical drug samples in the industry. This study also provides detailed insights about the possible impurities, their properties, pathway and assessing tools for analyzing these impurities, which helps in the proper selection of shelf life, storage condition, formulation condition, and retest period for avoiding the quality or stability related issue of a pharmaceutical drug product.

Figure 1: Chemical structure of Empagliflozin



The present research work focuses on Empagliflozin which is also recognized as gliflozin. It inhibits the Sodium-glucose co-transporter 2 (SGLT2). This medication is prescribed to treat non-insulin dependent diabetic mellitus [4]. Empagliflozin chemical name is (2*S*, 3R, 4R, 5S, 6R)-2-[4-chloro-3-[[4-[(3*S*)-oxolan-3yl] ox phenyl] methyl] phenyl]-6-(hydroxymethyl)-oxane-3, 4, 5-triol, with its single mole weight of 450.9 g with an empirical formula of $C_{23}H_{27}ClO_7$. The structural formula of Empagliflozin is depicted in Figure 1 [5]. Empagliflozin is the most specific SGLT2 inhibitor among all the currently available SGLT2 inhibitors. This inhibitory action reduces reabsorption of glucose from the kidney causing increased removal of glucose from urine. It provides beneficial effects beyond just managing glucose by consistently lowering body weight, pressure of blood, and uric acid levels in serum [6, 7].

Empagliflozin determination from drug substance and drug products, is reported to be performed using different analytical techniques such as UV spectroscopy [8-10], HPTLC [11-13], HPLC [14-18], and Mass spectrometry [19_20] either alone or in combination with some other active chemical entity. Few bio analytical methods are also reported for empagliflozin [21-23]. With reference to the stability indicating assay methods, HPLC [24-31] and HPTLC methods [13, 32] are reported for Empagliflozin. Today HPTLC has become the preferred method for analysis in the field due to its ease of use and cost-effectiveness associated with the reduction in time without affecting the accuracy and sensitivity of the analysis [33]. Thus, the current work aimed at developing and validating novel HPTLC analytical method which could be helpful in indicating the drug stability and gives precise, accurate, and reliable results. The method could be used for quantitative estimation of Empagliflozin which can be also significant in cross validation studies of Empagliflozin.

MATERIALS AND METHODS

Materials

Reference standard of Empagliflozin (99.8 %) was procured from Dr. Reddy, Telangana, Karnataka. The drug formulation, Empagliflozin tablets were purchased from chemist shop. The study was performed using analytical grade reagents and chemicals ordered from S. D. Fine Chemical Ltd. in Mumbai, Maharashtra, India.

Instruments and Software's

The chromatographic studies utilized the HPTLC system from CAMAG, Switzerland, which includes a semiautomatic spotting device called Linomat 5, the TLC Scanner IV (CAMAG Muttenz, Switzerland), a twintrough developing chamber (10 x 10 cm), a UV cabinet provided with dual wavelength UV lamps, Win-CATS software, and a syringe from Hamilton with a capacity of 100 µL.

Stock Solution and Working Solution Preparation

Methyl alcoholic stock solution of empagliflozin having concentration of 1000 µgmL^{-1} was prepared. Further working standard solutions having concentrations of 10, 20, 30, 40, 50, 60 and 70 µgmL^{-1} were prepared from empagliflozin using methyl alcohol for dilution.

Chromatographic Separation

Merck precoated silica gel 60F_{254} TLC plates (10.0 × 10.0 cm with 250 mm layer thickness) from E. Merck, Germany were used for the chromatographic separation of the drug. Sampling was carried out by creating bands that were 6 mm wide. The distance between two bands was kept 10 mm. This was done using a Linomat 5 semi-automatic applicator equipped with a sample micro-syringe from Hamilton, Switzerland, the capacity of which was 100 µL. The flow rate was set at 10 µLsec^{-1}, and a nitrogen aspirator was used. The twin trough chamber was used to develop the plate that was allowed to saturate for (20 min) with mobile phase Chloroform: Toluene: Methyl alcohol: Methanoic acid (8:4:2:0.1 v/v/v/v). The development distance was approximately 90 mm. Absorbance mode of CAMAG TLC scanner IV with a helium source and operated with Win CATS software (V1.4.2, CAMAG, and Switzerland) was used for scanning the density

of the separated bands at 235nm. The dimensions of the slit were set to 0.50 mm × 0.45 mm, while the speed of scanning was kept to be 20 mms^{1}, and the data resolution at 100 µm/step.

Validation of the HPTLC Method

International Conference on Harmonization (ICH) Q2 (R1) guidelines were employed to check if the developed HPTLC method was specific, linear, accurate, robust and precise. Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined for the method [35].

Specificity

The method's specificity was examined by comparing the plain mobile phase densitogram with the matching standard Empagliflozin densitogram at the LOQ level for any extraneous peak at the Empagliflozin R_f value.

Linearity

To prepare the calibration plot, 10 µL of each working standard solution, having concentrations of 10, 20, 30, 40, 50, 60 and 70 µgmL^{-1} were applied on the plate to achieve concentrations of 100, 200, 300, 400, 500, 600 and 700 ng per spot of Empagliflozin. These standards were analyzed three times and peak areas were recorded. A calibration curve was obtained wherein of drug concentration of each standard was taken on X axis and the average peak area for the respective concentration was taken on Y axis which was used to ascertain the method's linearity and to calculate the standard deviation (σ), correlation coefficient (R^2), slope, and intercept.

Accuracy

Standard solutions of Empagliflozin at 80 %, 100 %, and 120 % concentration levels were added to the samples to evaluate the accuracy of the method. This was done in triplicate after which recovery of drug by the developed method was evaluated.

Precision

Quality control samples at 3 levels, Lower Quality Control (LQC), Middle Quality Control (MQC) and Higher Quality Control (HQC) were used to check if the developed method was precise. The study was demonstrated by performing intra-day and inter-day analysis of LQC (100 ng per spot), MQC (400 ng per spot) and HQC (700 ng per spot) using triplicate analysis (3 concentrations × 3 replicates = 9 determinations). Determination of intra-day and inter-day precision was done by analyzing all the Quality Control samples for the drug on the same day and over three days respectively. Percentage of relative standard deviation of observed peak areas was calculated.

LOD and LOQ

Assessment of the method's sensitivity was done by determination of LOD and LOQ values. LOD and LOQ parameters were calculated using the regression equations of empagliflozin, and the formula given below:

$LOD = 3.3 \times \sigma / S$ $LOQ = 10 \times \sigma / S$

In these formulae "σ" stands for the standard deviation of the response wherein "S" indicates the slope of corresponding calibration graph.

Robustness

Small deliberate changes were made to the experimental conditions in order to determine their impact on robustness at LQC and HQC concentrations by calculating % relative standard deviation of peak areas. Conditions altered were wavelength of detection, saturation time, and solvent system composition. The samples were examined three times for each modification in the conditions. Optimal values were maintained for the other conditions while the impact of changing one set of conditions was examined.

The method's specificity was examined by comparing the plain mobile phase densitogram with the matching standard Empagliflozin densitogram at the LOQ level for any extraneous peak at the Empagliflozin R_f value.

Forced Degradation Studies

Evaluation of the natural stability of the drug Empagliflozin was done by subjecting it to various stressors according to the guidelines outlined in ICH Q1A (R2) for drug substance and product stability [36]. The impact of different forced degradation conditions on Empagliflozin was subsequently determined through HPTLC analysis.

Degradation in Acidic Condition

A quantity of 10 mg of Empagliflozin was made soluble in 10 mL of 0.1 N HCl solution which was then refluxed for 2 h. This solution was then diluted with methyl alcohol 10 times of its original concentration and analyzed.

Degradation in Alkaline Condition

A quantity of 10 mg of Empagliflozin was made soluble in 10 mL of 0.1 N NaOH solution which was then refluxed for 2 h. This solution was then diluted with methyl alcohol 10 times of its original concentration and analyzed.

H_2O_2 Induced Degradation

A quantity of 10 mL of a 6% v/v H_2O_2 solution was used to dissolve 10 mg of Empagliflozin. Then the solution was kept aside for 3 h. This solution was then diluted with methyl alcohol 10 times of its original concentration and analyzed.

Dry Heat Induced Degradation

Empagliflozin was subjected to heat in an oven at 105 °C for about 6 h. After 6 h, the drug was made soluble in 10 mL of methyl alcohol. This solution was diluted 10 times with methyl alcohol and then analyzed using chromatography.

Water Induced Degradation

A quantity of 10 mL of water were used to dissolve 10 mg of empagliflozin and this solution was refluxed at 8 h. Ten times dilution of this solution was carried out before analyzing the same using planar chromatography.

Daylight Induced Degradation

A quantity of 10 mg of Empagliflozin was exposed to daylight for about 8 h. After 8 h, the drug was solubilized in 10 mL of

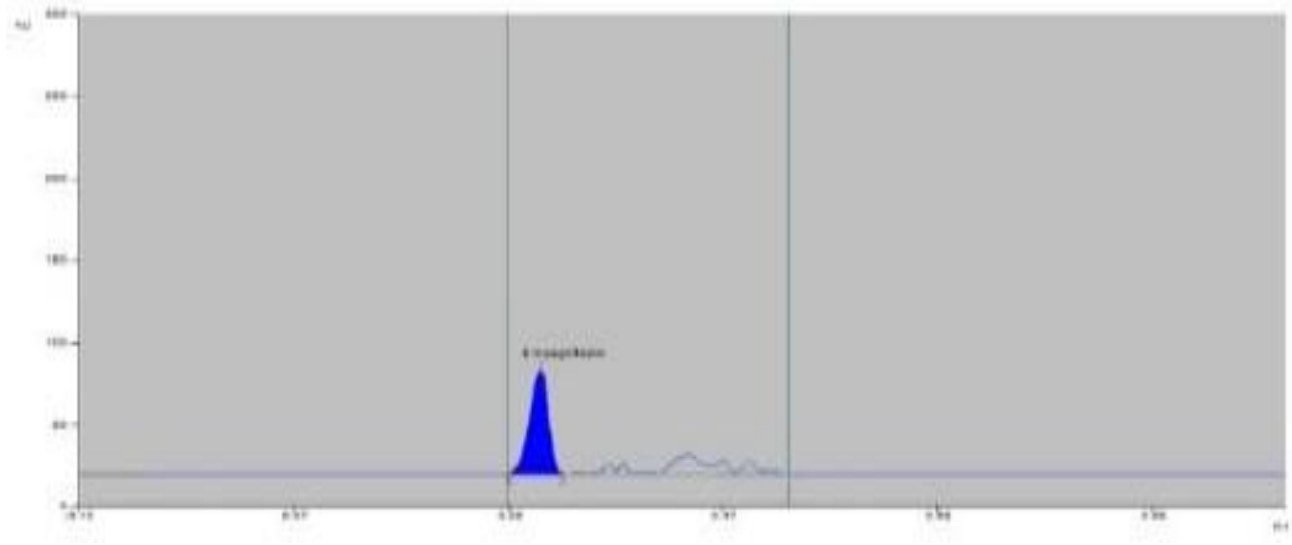
methyl alcohol. The solution was diluted 10 times with methyl alcohol and then analyzed using chromatography.

RESULT

Chromatographic separation

An HPTLC method was developed for Empagliflozin using silica gel $60F_{254}$ (10.0 × 10.0 cm) as stationary phase. A solvent mixture containing Chloroform: Toluene: Methyl alcohol: Methanoic acid (8:4:2:0.1 v/v/v/v) was used as the mobile phase. Empagliflozin gave a resolved peak at the R_f of 0.33±0.02 (Figure 2)

Figure 2: HPTLC chromatogram of Empagliflozin



Validation of HPTLC Method

Validation of developed HPTLC method was carried out by following the ICH Q2 (R1) guidelines for following parameters.

Specificity

Specificity was investigated by running plain mobile phase at the optimized chromatographic condition along with standard Empagliflozin at its lowest concentration. A clean baseline free from any extra peak was observed at the R_f values of drug. This proved the specificity of the method for empagliflozin.

Linearity

The proposed method showed linear response over 100-700 ng per band concentration range. The linearity equation (y= 6.5318x+481.42) and coefficient of correlation (R^2 = 0.9959) achieved by graphing concentrations versus average peak area, reflected the linear relationship of the method.

Accuracy

Accuracy was performed by spiking a predetermined amount of standard to the sample solution. Percentage recovery studied by analyzing samples spiked at three different levels of 80%, 100% and 120% at three different times was found to be 99.51%, 100.49 % and 100.51% respectively.

Precision

Inter-day and Intra-day precision were analyzed for 3 different concentrations (100, 400, 700 ng per spot), each in triplicate. The % Relative Standard Deviation values achieved for the interday precision at 100, 400, 700 ng per spot level, obtained were 1.314, 0.709 and 0.1646 respectively. The % Relative Standard Deviation values achieved for the intraday precision at 100, 400, 700 ng per spot level, obtained were 0.7117, 0.3992 and 0.2055 respectively.

Limit of detection and Limit of quantification

The linear regression equation's slope and the response standard deviation were used to calculate the LOD and LOQ values. Empagliflozin was determined to have LOD and LOQ values of 22.45 ng per spot and 88. 64 ng per spot, respectively.

Robustness

Method's robustness is indicated by the % relative standard deviation values of peak areas observed after making small deliberate changes in the experimental conditions. The % relative standard deviation values of peak areas determined for every condition set employed in robustness study were less than 2 %, as shown in Table 1.

Table 1: Results of the Robustness Study of Empagliflozin

Method parameter	Level of variation	Percentage Relative Standard Deviation	
		LQC (ng/spot)	HQC (ng/spot)
A] Mobile phase composition Chloroform: Toluene: Methyl alcohol: Methanoic acid (8: 4: 2: 0.1 v/v/v/v)	+0.2 (mL of chloroform)	0.61	0.19
	-0.2 (mL of chloroform)	1.66	1.43
B] Time taken for saturation of chamber	+2 min	1.59	1.48
	-2 min	0.75	1.23
C] Detection wavelength 235 nm	+5 nm	1.01	0.95
	-5 nm	1.23	1.22

Min: minutes; nm: nanometer; v/v: volume by volume; mL: milliliter; ng: nano gram; LQC: Lower Quality Control; HQC: High Quality Control.

Table 2: Summary of forced degradation study of Empagliflozin

Degradation conditions	Time (h)	Percentage Recovery	R_f of degradation product
Refluxing in presence of Acid (0.1 N HCl)	2	76.84	0.02, 0.03, 0.04, 0.93
Refluxing in presence of Base (0.1 N NaOH)	2	45.98	0.38, 0.68, 0.95
Exposure to H_2O_2 (6 % v/v)	3	54.02	0.95
Exposure to Dry heat (105 ºC)	8	79.03	0.69, 0.95
Refluxing in presence of Water	8	52.35	0.09, 0.58
Exposure to day light	8	85.42	0.12, 0.14

H: hour; R_f: retardation factor; ºC: degree centigrade; v/v: volume by volume.

Forced degradation studies

Different stress conditions including acidity, alkalinity, oxidation, heat, hydrolysis, and light were applied to Empagliflozin, and the developed HPTLC method was employed for sample analysis. The HPTLC method developed for quantifying Empagliflozin successfully separated the drug degradant in all forced degradation conditions. The concentration of unaffected Empagliflozin under different forced degradation conditions was based on the determination of peak area of Empagliflozin in the obtained densitogram which is tabulated in Table 2.

DISCUSSION

Today HPTLC has become a preferred method for analysis due to its ease of use and cost effectiveness associated with the lesser consumption of mobile phase. As this technique allows simultaneous estimation of number of samples giving decreased analysis time per sample. HPTLC method was developed for the estimation of Empagliflozin with UV detection at 235 nm. Trial runs were conducted employing a variety of solvents at various ratios through the linear ascending development technique using silica gel $60F_{254}$ (10.0 × 10.0 cm) as stationary phase. Mobile phase containing Chloroform: Toluene: Methyl alcohol: Methanolic acid (8:4:2:0.1 v/v/v/v) gave a good symmetrical peak for Empagliflozin and was selected for further studies. The developed method was validated using ICH Q2 (R1) guidelines. Comparison of densitogram obtained with plain mobile phase gave a clean baseline free from any extra peak at R_f of Empagliflozin which indicated the specificity of the method. R^2 value of 0.9959 for the calibration curve standard over 100-700 ng per band reflected the linearity of the method. Average percentage recovery between 99.5 to 100.5% indicated that the method was accurate to evaluate the standard drug spiked in the sample. The ability of an analytical method to give reproducible results for multiple evaluations is assessed by precision of the method. Average area for Empagliflozin obtained in the densitogram of the three quality control samples analyzed in triplicate on the same day and on three different days gave percentage relative standard deviation value between 0.1 to 1.3. All these values below 2% indicated that the method is able to produce reproducible and reliable results. LOD and LOQ values of less than 100 ng per spot indicate the sensitivity of the proposed method. Success of the analytical method is proved when the results remain unaffected if the chromatographic set of conditions is varied. Results obtained in the robustness study indicated that minute and purposeful changes in the composition of the mobile phase, time for saturation of the chamber and wavelength for detection are changed. This method was employed for the assessment of the samples generated by subjecting Empagliflozin to acidic, alkaline, oxidative, heat induced, water induced and light induced stress conditions. The developed method was able to resolve the degradant obtained from the unaffected Empagliflozin. Empagliflozin was found to be highly susceptible to alkaline and oxidative stress as indicated by 45.98 and 54.02 % recovery respectively. As the HPTLC method could detect the presence of the degradant formed in each stress condition, the method can be said to be stability indicating.

CONCLUSION

An analytical method which can indicate the stability of Empagliflozin was developed and validated as per ICH guidelines. Various ICH-recommended stress conditions were employed to determine the intrinsic stability of Empagliflozin in this research. The developed HPTLC method could efficiently resolve degradation product of Empagliflozin under all degradation conditions. Empagliflozin should be stored cautiously under alkaline, hydrolytic, or oxidative conditions as it shows fast degradation under these conditions. Observations of these experiments on the stability of Empagliflozin could provide assistance in storage and in both new and traditional drug product aspects. Thus, this method which is validated after the development could be employed for analyzing the quality in bulk drug production, testing pharmaceutical dosage forms, cross validation and carrying out in-process testing.

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