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STABILITY INDICATING HPTLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF EMPAGLIFLOZIN IN BULK DRUG AND TABLET DOSAGE FORM

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ABSTRACT

The present work describes development and validation of a new simple, accurate and precise stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Empagliflozin as bulk drug and in tablet formulation. As stability testing is major step in the development of new drug as well as formulation, stress degradation studies were carried out in accordance with ICH guidelines. Empagliflozin was found susceptible to all the analyzed stress conditions. HPTLC plates precoated with silica gel 60 F_{254} were used as the stationary phase and chromatographic separation was achieved by using Chloroform: Methanol: Glacial acetic acid (8.5: 1.5: 0.2, v/v/v) as mobile phase. Densitometric detection was carried out at 225 nm. The retardation factor (Rf) was found to be 0.47±0.03. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. The developed method was found to be linear in the concentration range of 200-1200 ng band^{-1}. The LOD and LOQ was found to be 59.62 ng band^{-1} and 180.68 ng band^{-1} respectively. The developed method has been successfully applied for the estimation of Empagliflozin in tablet dosage form.

KEYWORDS: Empagliflozin, Stability indicating method, HPTLC, Forced degradation studies.

INTRODUCTION

Empagliflozin, chemically, (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-[[4-[(3*S*)-oxolan-3-yl] oxyphenyl] methyl] phenyl]-6-(hydroxylmethyl) oxane-3, 4, 5-triol is antidiabetic drug which is used to improve glucose control in people with type 2 diabetes and also used to reduce the risk of cardiovascular death in adults with type 2 diabetes and established cardiovascular disease.[1,2]

An extensive literature survey revealed that a different analytical methods has been reported for quantitative analysis of empagliflozin. Analytical methods such as UPLC/DAD[3] and RP-HPLC[4] have been reported estimation of empagliflozin in human plasma. Analytical methods representing the estimation of empagliflozin either as single drug or in combination with other drugs in pharmaceutical formulations using UV spectrophotometry[5-7] and RP-HPLC[8-14] were also reported in the literature. One HPTLC method demonstrating simultaneous quantification of metformin hydrochloride and empagliflozin in bulk and marketed formulation using Box-Wilson experimental design approach has been also reported.[15]

To best of our information, no reports were found in the literature for determination of empagliflozin in pharmaceutical tablet dosage form by stability-indicating high performance thin layer chromatographic (HPTLC) method. Hence the present study was undertaken with the aim to develop and validate a simple, precise and accurate stability indicating HPTLC procedure for determination of empagliflozin as bulk drug and in tablet dosage form in accordance with International Conference on Harmonisation Guidelines.[16, 17]

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure Empagliflozin obtained as a gift sample from Aarti Industries Ltd. Mumbai, India. The pharmaceutical tablet dosage form Jardiance labelled to contain 25 mg of empagliflozin (Boehringer Ingelheim India Pvt Ltd) was procured from local pharmacy. All chemicals and reagents used for analysis were of analytical grade. Chemicals used viz. Methanol, chloroform, glacial acetic acid were purchased from Loba Chemicals Pvt. Ltd., India.

Instrumentation and chromatographic conditions

Chromatographic resolution of the drug was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm × 10 cm with 250 µm layer thickness) from E. MERCK, Darmstadt, Germany, using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with 8 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). Linear ascending development was carried out in 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using chloroform: methanol: glacial acetic acid (8.5: 1.5: 0.2, v/v/v) as mobile phase. The saturation of mobile phase was done for 20 min in the chamber at room temperature. The length of chromatogram run was 70 mm. Densitometric scanning was performed on a CAMAG TLC scanner III at 225 nm for all developments operated by win CATS software version 1.4.2.

Preparation of working standard solution

Working standard solution was prepared by dissolving accurately weighed 10 mg of the drug in 10 mL of methanol to get solution having concentration 1000 ng μL^{-1} which was diluted further using methanol to acquire final working standard concentration 100 ng μL^{-1}.

Analysis of tablet dosage form

Commercial brand of tablets Jardiance containing 25 mg of drug was used to estimate the amount of Empagliflozin in existing tablet formulation. For this, 20 tablets were weighed accurately and powdered. Powder quantity equivalent to 10 mg of was weighed and transferred to the 10 mL volumetric flask and 5 mL methanol was added and sonicated for 10 min. The solution was filtered using Whatman filter paper No. 41, and the volume was made up to the mark with methanol. The resulting solution was diluted further with methanol to get final concentration 100 ng μL^{-1}. Four micro-liter volume of this solution was applied to a TLC plate to provide final concentration of 400 ng $band^{-1}$. After chromatographic development the peak areas of the bands were measured at 225 nm and the amount of drug present in sample was estimated from the respective calibration curve. Procedure was repeated six times for the analysis of homogenous sample. The % drug content was found to be 100.27 ± 0.64.

Stress degradation studies

Stress degradation studies were carried out to confirm the stability by exposing the bulk drug to different physical stress conditions recommended by ICH guidelines. The study was carried out at concentration of 1000 ng μL^{-1}. The acid and base hydrolytic studies were performed by treating standard drug solution separately with 0.1 N HCl and 0.1 N NaOH at room temperature for 30 min. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to provide the final concentration of 800 ng $band^{-1}$. Standard drug solution was treated with 3 % H_2O_2 at room temperature 1 h to perform the oxidative degradation and was diluted with methanol to obtain 800 ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping drug in oven at 100ºC for period of 1 h. The solid drug powder was exposed UV light up to 200-watt hour square $meter^{-1}$ to check photolytic degradation. Thermal and photolytic samples were diluted with methanol to get concentration of 800 ng $band^{-1}$.

RESULTS AND DISCUSSION

Method optimization

The TLC procedure was optimized with a view to develop a stability indicating method for empagliflozin which would be proficient to give the satisfactory resolution. Varied solvent systems comprising different ratios of benzene, chloroform, toluene, methanol, ethyl acetate and glacial acetic acid were examined (data not shown) to achieve better separation and to resolve spot of empagliflozin. Finally, the mobile phase comprising of chloroform: methanol: glacial acetic acid (8.5: 1.5: 0.2, v/v/v) was chosen as optimum which gave acceptable resolution of drug with symmetrical peak shape. Densitometry detection was carried out at 225 nm. The retardation factor (Rf) was found to be 0.47±0.03. Representative densitogram of standard solution of empagliflozin is represented in Figure 1.

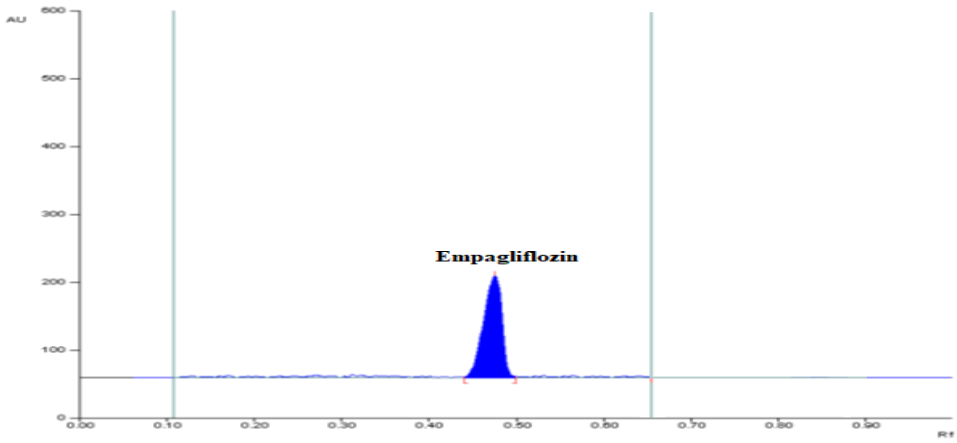

Figure 1: Representative densitogram of standard solution of empagliflozin. (600 ng $band^{-1}$, Rf= 0.47±0.03)

The stress degradation studies demonstrated susceptibility of empagliflozin to all the analyzed stress conditions. The product was found to degrade significantly in oxidative, acid, and base hydrolysis degradation conditions. The degradation of drug was observed without appearance of degradation product. The findings of stress degradation studies along with % degradation and % recovery is summarized in Table 1. The chromatograms of acid, alkali and oxidative degradation are shown in 2, 3 and 4.

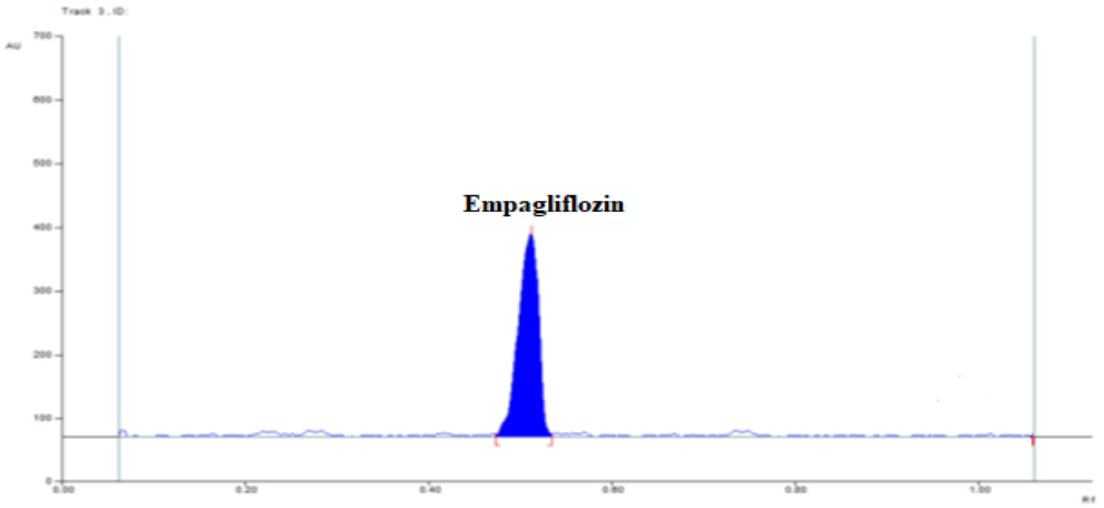

Figure 2: Densitogram after treatment with 0.1 N HCl kept at RT for 30 min.

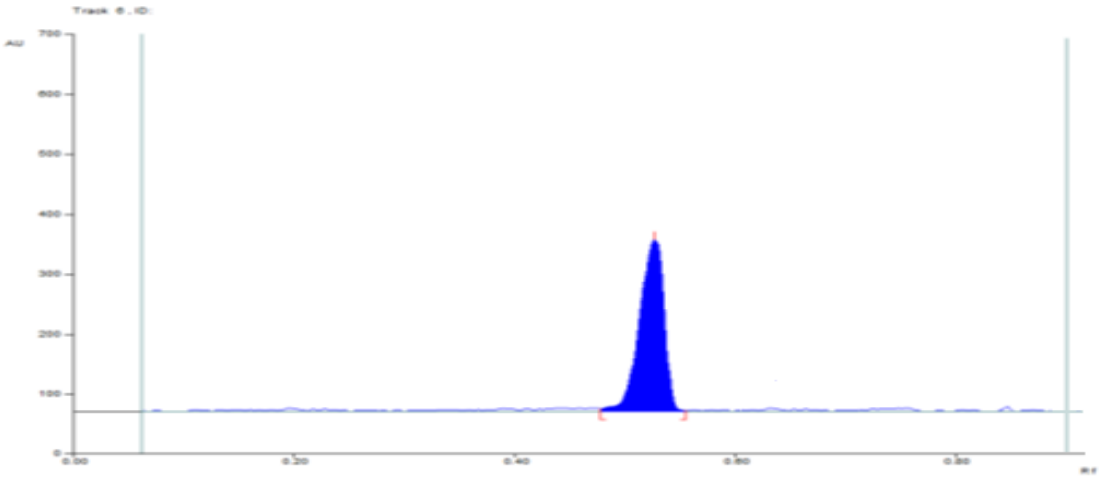

Figure 3: Densitogram after treatment with 0.1 N NaOH kept at RT for 30 min.

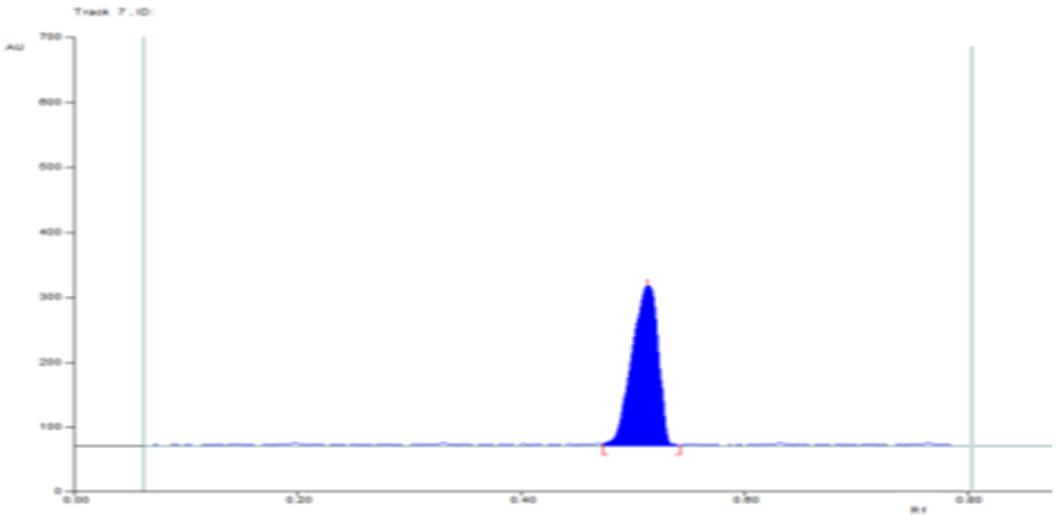

Figure 4: Densitogram after treatment with 3% H_2O_2 kept at RT for 1 h.

Table 1: Summary of stress degradation studies.

Stress conditions	% Recovery	% Degradation
Acid (0.1 N HCl, Kept at RT for 30 min)	78.82	21.18
Base (0.1 N NaOH, Kept at RT for 30 min)	72.52	27.48
Oxidative (3 % H_2O_2, Kept at RT for 1h)	70.05	29.95
Thermal (100º C for 1 h)	89.79	10.21
Photolytic degradation	75.35	24.65

Method validation

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation and robustness, in accordance with ICH Q2 (R1) guidelines.

Linearity

The linearity of proposed method was checked by spotting volumes 2, 4, 6, 8, 10 and 12 µL of standard solution of empagliflozin (100 ng μL^{-1}) onto the TLC plates, developed and scanned under optimized chromatographic conditions. The method was found to be linear in the concentration range 200-1200 ng $band^{-1}$ with high correlation coefficient. The linear regression equation was found to be with correlation coefficient y = 1.7677x + 364.31 with correlation coefficient (R^2) value of 0.997. The calibration curve was obtained by plot of concentration vs peak area of drug is shown in Figure 5. A 3D densitogram obtained in the concentration range 200-1200 ng $band^{-1}$ is shown in Figure 6.

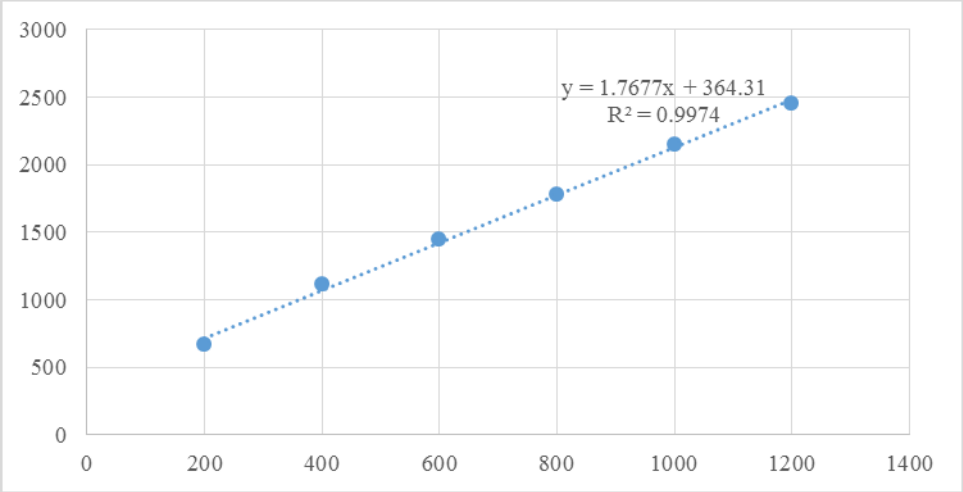

Figure 5: Calibration curve for empagliflozin.

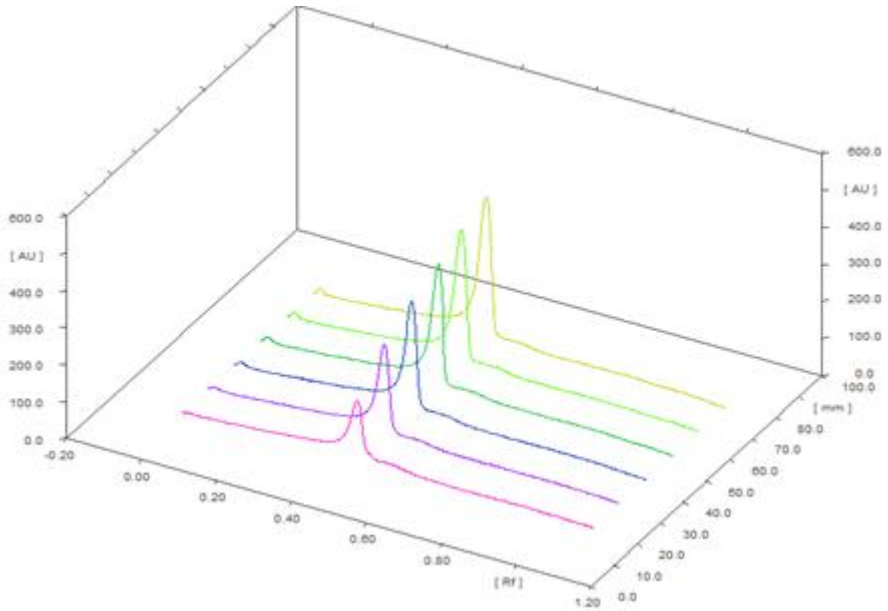

Figure 6: 3D densitogram in concentration range 200-1200 ng $band^{-1}$

Precision

The precision of the method was demonstrated by intraday and interday variation studies in which three replicates of three concentrations within the linearity range were analyzed on the same day and on three consecutive days, respectively and percentage R.S.D. was calculated. The % R.S.D. values obtained for intraday and interday variations were found to be < 2 which indicated that method is precise. The results obtained for intraday and inter-day precision studies are shown in Table 2 and 3, respectively.

Table 2: Intraday precision studies

Spotted concentration (ng band^{-1})	Recovered concentration (ng band^{-1})	% Recovery	% R.S.D.*
400	396.75	99.19	0.80
600	592.86	98.81	0.57
800	795.58	99.44	0.92

*Average of three determinations

Table 3: Interday precision studies.

Spotted concentration (ng band^{-1})	Recovered concentration (ng band^{-1})	% Recovery	% R.S.D.*
400	399.02	99.75	0.19
600	594.75	99.12	1.06
800	795.01	99.37	1.16

*Average of three determinations

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 59.62 ng band^{-1} and 180.68 ng band^{-1}, respectively.

Accuracy

Accuracy of developed method was checked by performing recovery studies by standard addition method. It involved addition standard drug solution to pre-analyzed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 400 ng band^{-1} from tablet solution. The drug concentrations were calculated from linear regression equation. The results of the recovery studies demonstrated the accuracy of developed method for estimation of drug in tablet dosage form.

Table 4: Accuracy studies.

Drug	Basic sample concentration (ng band^{-1})	Concentration added (ng band^{-1})	Concentration found (ng band^{-1})	% Recovery±R.S.D.*
Empagliflozin	400	320	719.59	99.94 ± 0.73
	400	400	799.73	99.96 ± 0.99
	400	480	883.08	100.34 ± 0.74

*Average of three determinations

Robustness

By introducing deliberate variation in the method parameters, the effects on the peak areas of drug were examined to check the robustness of the method. The parameters varied were mobile phase composition (± 1 % methanol), wavelength (± 1 nm) and saturation time and the effect on the area of drug was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters which demonstrated robustness of the method.

CONCLUSIONS

Stability-indicating HPTLC-densitometric method for determination of empagliflozin in bulk drug and tablet formulation has been developed and validated. The developed method is simple, sensitive, precise, accurate, and reproducible. The drug was found to be susceptible all analyzed stress conditions including heat and light. The developed method can be used for quantitative analysis of drug in pharmaceutical dosage form. As the method is stability indicating, it may be extended to study the degradation kinetics of drug.

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