IJPSR (2016), Vol. 7, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 May, 2016; received in revised form, 13 July, 2016; accepted, 09 August, 2016; published 01 November, 2016

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

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Keywords:

Forced degradation, Empagliflozin, RP-HPLC, Stability –indicating, Validation

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ABSTRACT: A novel stability-indicative of RP-HPLC method was developed and validated for the quantitative estimation of Empagliflozin in bulk drugs and pharmaceutical dosage form in the presence of degradation products. Chromatographic separation was achieved on an Intersil C_{18} (150mmx4.6mm, 5 µm) analytical column using mobile phase composition of methanol and acetonitrile in ratio of (50: 50 v/v) that was set at a flow rate of 20µl/min with detection of 265 nm. The retention time of Empagliflozin was found to be 2.184min. The high correlation coefficient value indicated clear correlation and their peak area within the LOQ (Limit of quantification) to 150% level. The drug was analyzed by following the guidelines of International conference on Harmonization (ICH) underneath hydrolytic, photolytic, oxidative, and thermal stress conditions. The presentation of the method was validated according to the present ICH guidelines for accuracy, precision and robustness, Linearity, limit of quantification, limit of detection linearity.

INTRODUCTION: Empagliflozin,(1-chloro-4-[b-d-glucopyranos-1-yl]-2-[4-([s]-tetrahydrofuran-3-yl-oxy) benzyl]-benzene (**Fig.1**), is an orally carry on exacting sodium glucose co-transporter-2 (sglt-2) inhibitor, which lowers blood glucose in kinfolk amongst kind 2 diabetes by surpass the reabsorption of glucose in the kidneys and promoting excretion of excess glucose in the urine [1-4]. SGLT2, located in the bring together tubule of the nephron, is guestimated to facilitate-90% of this reabsorption [5-8].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(11).4523-30

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (11).4523-30

Empagliflozin is structurally waiting upon to phlorizin; despite prowl, to the fullest phlorizin is an o-glucoside and according lyreceptive to disrepute by b-glucosidase in the gastrointestinal garden plot, Empagliflozin is a c-glucoside and the carbon–carbon coalition between the glucose and aglycone moieties makes it resistant to gastrointestinal degradation, allowing oral administration. SGLT2 inhibitors in conflict back an insulin-independent agency for sanctioning bust glucose levels, in regard to the further urinary glucose excretion (uge) by inhibiting glucose reabsorption in the kidney [9-10].

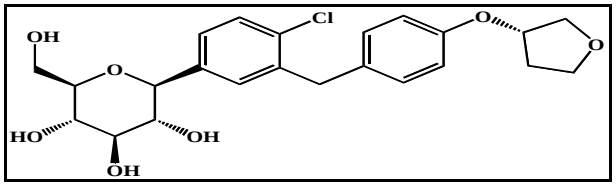

FIG. 1: CHEMICAL STRUCTURE OF EMPAGLIFLOZIN

Handful clinical trials were reported in the literature for Empagliflozin [11-15]. But scarcely reports have been found for RP-HPLC determination of Empagliflozin in pharmaceutical preparations. In this paper, we described validation of a method for accurate quantification of an Empagliflozin in bulk drugs and pharmaceutical dosage form along with validation as per ICH [16-18]. The plan of present study was to establish the inherent stability of Empagliflozin through stress studies under variety of conditions and develop a stability –indicating RP-HPLC method.

MATERIALS AND METHODS:

Chemicals and reagents:

Sample of Empagliflozin was received from MSN Laboratories Hyderabad, India. Jardiance 25 mg tablets were purchased from Indian pharma network, Noida, Delhi. HPLC grade acetonitrile and methanol were procured from Merck, Darmstadit Germany.

Instrumentation and Chromatographic Conditions:

The LC system, used was a waters 2695 binary pump plus auto sampler and a 2996 photo diode array detector. The output signal was monitored and processed using Empower software on Pentium computer (Digital Equipment Co). Separation was carried out on an Intersil C_{18} (150mmx4.6mm, 5 µm). The mobile phase contains mixture of methanol: acetonitrile in the ratio of (50:50 v/v). The mobile phase was pre-mixed, filtered through a 0.4µm nylon filter and degassed. The flow rate was kept at 1.2 mL. Throughout the LC column oven was maintained at 35ºC and detection was monitored at 265 nm. The injection volume was 20µL.Methanol was used as a diluent.

Preparation of solutions:

Preparation of standard solution:

Accurately weighed quantity of 10 mg of Empagliflozin was transferred into 10 mL volumetric flask, dissolved and diluted up to mark with methanol. This was a stock solution having strength of 1000 µg/mL of Empagliflozin. From this solution, 0.2 mL of solution was pipetted out and diluted up to 10 mL to get 20 µg/mL of Empagliflozin. Mix well and filter through 0.45µm filter.

Preparation of sample working solution:

20 Jardiance Tablets (25mg) are weighed and calculated the average weight. A quantity of tablet powder equivalent of 10 mg Empagliflozin was weighed specifically and transferred to a 100 mL volumetric flask. The tablet grind was dissolved in methanol prevalent the sanction of ultra-sonication, diluted up to mark with same and filtered through a whatman filter paper to make a test solution having 20 µg/mL Empagliflozin then analyzed for assay determination. It was mixed well and filtered through 0.45µm filter. 20 µl of the standard sample was injected into the chromatographic system.

Method validation:

The method was validated for System suitability, linearity, precision, accuracy, LOD, LOQ and robustness study.

System suitability:

System suitability test was carried out to verify that the analytical system is working properly and can give accurate and precise results. The overall system suitability was evaluated for the system suitability of the proposed method. Data from six injections (20µg/mL) were utilized for calculating parameters like theoretical plates, resolution, tailing factor and %RSD of 6 injections.

Linearity:

To establish the linearity of the method, calibration solutions were prepared from the stock solution at five concentration levels from 50 to 150% of analyte concentration. The correlation coefficient, Y-intercept and slope of the calibration curve were calculated.

Precision:

The precision of an analytical method expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample over the prescribed conditions. Intra-day and inter-day Precision were determined through repeatability analysis. The precision for drug was checked by injecting six individual preparations. The % RSD of Empagliflozin was calculated.

Accuracy: The accuracy of the assay method was evaluated in triplicate at three concentration levels

i.e., 25, 50, 75, ug/mL^{-1} (50,100,150% of the normal assay concentration) for bulk drug sample. The %recoveries were calculated. The study was carried out in triplicate (n=3). The solutions were injected into HPLC system and the mean peak area of analyte (Empagliflozin) peak was calculated for assays. Assay (%w/w) of test solution was determined against three injections (n=3) of qualified Empagliflozin reference or working standard.

Limit of detection (LOD) and Limit of quantification (LOQ):

LOD and LOQ for Empagliflozin was calculated as suggested by ICH guidelines using equations LOD = 3.3 σ/s and LOQ = 10 σ/s, respectively. Where, σ is the SD of the response and S is the slope of the calibration curve.

Robustness:

To determine the robustness of the method, system suitability parameters were verified by making deliberated changes in the chromatographic conditions, viz, changing flow rate by0.2 units from 1.0 to 1.4 mL^{-1}. The effect of pH variation was studied by varying from 2 to 2.4 in 0.2 pH units. The effect of column oven temperature on resolution was studied at 35 to 45^{0}C. In all the above varied conditions, the components of the mobile phase were held constant. To study the effect of change in mobile phase composition by changing the organic ratio, the organic component was changed by 10% from 90 to 110% keeping the buffer ratio constant.

Stability studies:

Selectivity was assessed by performing forced degradation studies. The ICH stress testing of the drug substance can help to demonstrate the basic stability of the molecule and validate the stability – indicating power of the analytical procedures used.

Acid degradation:

Forced degradation in acidic medium was performed by separately taking 1 mL stock solution of Empagliflozin; in to 10 mL volumetric flask then, the volumetric flask was kept at 60^{o}C for 48hrs after adding 1 mL of 1 M HCl, the solution was then neutralized with 1 N NaOH and diluted up to the mark with methanol and made to get concentration of 20 μg/mL.

Base degradation:

Forced degradation in base media was performed by separately taking 1 mL stock solution of Empagliflozin, in to 10 mL volumetric flask. Then, the volumetric flask was kept at 60^{o}C for 24 hrs after adding 1 mL of 1 N NaOH. The solution was then neutralized with 1 M HCl and diluted up to the mark with methanol and made to get a concentration of 20 μg/mL.

Oxidative degradation:

Forced degradation in base media was performed by separately taking 1 mL stock solution of Empagliflozin, in to 10 mL volumetric flask. Then, the volumetric flask was kept at 60^{o}C for 48 hrs after adding 1 mL of 30% v/v H_2O_2. The solution was diluted up to the mark with methanol and made to get a concentration of 20 μg/mL.

Photolytic degradation:

10 mg of Empagliflozin was weighed accurately and exposed to sunlight for 10-12 days. After this exposure, the drug powder was mixed and transferred in to 10 mL volumetric flask, dissolve in methanol and diluted up to mark with methanol and made to get a concentration of 20 μg/mL. The absorbance of this solution was measured at 265 nm.

Thermal degradation:

10 mg of Empagliflozin was weighed accurately and exposed to 60º C for 10 days. After this exposure, the drug powder was mixed and transferred in to 10 mL volumetric flask, dissolve in methanol and diluted up to mark with methanol and made to get a concentration of 20 μg/mL.

RESULTS AND DISCUSSION:

Method development and optimization of Chromatographic Conditions:

Stock solution of 100 mg/ml was prepared for Empagliflozin and further diluted to get the concentration of 20μg/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 265nm. Therefore 265nm was selected as the detection wavelength for the RP-HPLC investigation **Fig. 2**.

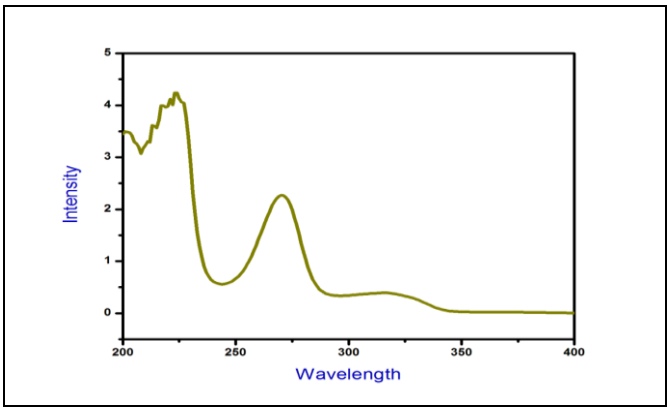

FIG. 2: UV SPECTRA OF EMPAGLIFLOZIN

Optimized Method:

In this work, simple, accurate and stability-indicative of RP-HPLC method was developed and validated for the quantitative estimation of Empagliflozin in bulk drugs and pharmaceutical dosage form using a150mmx4.6mm, i.d Intersil C_{18} 5 µm analytical column has been developed. The mobile phase was chosen after several trials to match the optimum stationary/mobile phase. The present method contains mobile phase composition of methanol and acetonitrile in ratio of (50: 50 v/v) which was found to be the most suitable, as the chromatographic peaks obtained were better defined, well resolved and almost free from tailing. The flow rate is 20µl/min with detection of 265 nm. The average retention times under the conditions described was 2.184min for Empagliflozin. The total run time is 10 minutes with which all the system suitability parameters are ideal for the mixture of standard solutions. **Fig.3** represent chromatogram of mixture of standard solutions, respectively **Table 1**.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Optimized	Chromatographic Conditions
Mode of separation	Isocratic
Mobile phase	Methanol and acetonitrile in ratio of (50: 50 v/v)
Column	IntersilC_{18} (150mmx4.6mm, 5 µm)
Column temperature	30^0C
Detector wave length	265nm
Runtime	10 min
Injection volume	20ul/ml
Flow rate	1.2 ml/min

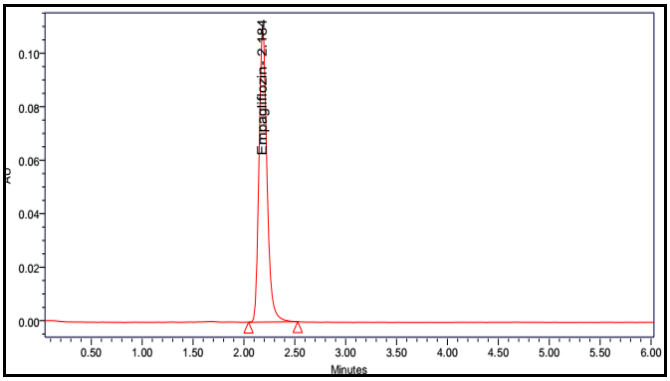

FIG. 3: CHROMATOGRAM UNDER OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Method validation:

System suitability:

A RP-HPLC method was developed by monitoring the system suitability parameters i.e. tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. System suitability method acceptance criteria set in each validation run were: tailing factor ≤ 2.0 and theoretical plates > 2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution was presented. System suitability parameters are tabulated in **Table 2.**

TABLE 2: SYSTEM SUITABILITY PARAMETERS

Name	Retention Time	USP Tailing	USP count
Empagliflozin	2.184	1.20	4331

Linearity:

A linear calibration plot for the assay method was obtained over the calibration ranges tested, i.e., 50-150 µg/mL with a correlation coefficient greater than 0.999 which indicates that the concentration had given good linearity as shown in **Fig. 4**. The slope and Y-intercept of calibration curve were 17384 and 7490 respectively Linearity results are tabulated in **Table 3**.

TABLE 3: LINEARITY RESULTS OF EMPAGLIFLOZIN

Concentration (in ug/mL)	Average area
50	201932
75	385071
100	577759
125	760654
150	950396
Correlation coefficient	0.999
Slope	17384
Y-Intercept	7490

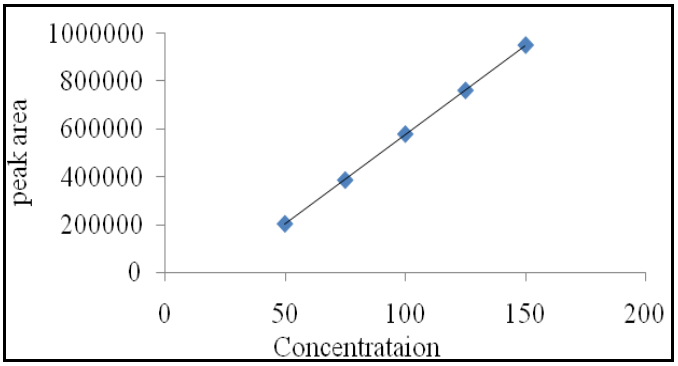

FIG. 4: CALIBARATION CURVE OF EMPAGLIFLOZIN

Precision:

The % RSD for the peak area of Empagliflozin in assay method was within the limits, confirming the good precision of the method. The data of repeatability, inter day precision and reproducibility (n=3) are shown in **Table 4**. The % RSD for repeatability was found to be 0.1-0.11%, inter day precision was found to be 0.1-0.12%, and reproducibility was found to be 0.1% respectively.

TABLE 4: RESULTS OF PRECISION STUDIES

Parameter	Concentration(µg/mL)	Mean peak area± SD	% RSD
Intra - day precision	60	1508773±1188.6	0.1
	80	1866573.4±2134.2	0.11
	100	1942321.5±1073.6	0.05
Inter-day precision	60	1507512±1341.3	0.1
	80	1868280±2414.1	0.12
	100	1944321±2828	0.14
Reproducibility	60	1508773±1188.6	0.1
	80	1867930±1919	0.1
	100	1944822±2122	0.1

Accuracy: Percentage recovery of Empagliflozin in bulk and pharmaceutical dosage form ranges from 99.56-100.12 % as shown in **Table 5.**

TABLE 5: % RECOVERY OF THE ASSAY METHOD FOR EMPAGLIFLOZIN

S.no	Concentration (%)	Mean recovery(n=3)	% Recovery	% RSD
1	50	1508773.6	99.97	0.1
2	100	1866573	99.56	0.1
3	150	1942321.5	100.12	0.1

Limit of detection and limit of quantification: Limit of detection and limit of quantification were found to be 1.9 µg and 0.655 µg respectively. The low values of LOD and LOQ indicate high sensitivity of the method.

Robustness: In all the varied chromatographic conditions (flow rate, temperature and mobile phase composition) the % RSD was less than 2.0 and no significant change in assay value was observed, which confirms the robustness of the developed method, data are shown in **Table 6**.

TABLE 6: RESULTS OF ROBUSTNESS STUDY

Parameters	Conditions	Mean peak area	S.D	% RSD
Flow rate	1.1 mL. min^{-1}	910020	8895	0.9
	1.9 mL. min^{-1}	920363	6331	0.65
Column temperature	38^0C	915886	6463	0.70
	40^0C	917754	6950	0.75
Mobile phase composition	Methanol:ACN ; 40:60	919194	7035	0.75
	Methanol:ACN ; 60:40	919504	6596	0.71

Forced degradation studies:

Degradation conditions were given in **Table 7.** Degradation was not observed in Empagliflozin samples subjected to UV light, heat and acid hydrolysis. Degradation of the drug was observed under base hydrolysis and peroxide oxidation and the results are given in **Table 8**. The assay of Empagliflozin is unaffected in the presence of degradation products confirming the stability-indicating power of the method.

TABLE 7: DEGRADATION CONDITIONS

Acid degradation	Drug solution in 1M HCl was maintained at 60^0C for 48 hrs.
Base degradation	Drug solution in 1N NaOH was maintained at 60^0C for 24 hrs.
Oxidative degradation	Drug solution in 30% v/v H_2O_2 was maintained at 60^0C for 48 hrs.
Photo degradation	Drug substance in UV energy of not less than 200 W/h about 10-11 days
Thermal degradation	The drug substance was subjected to dry heat at 60^0C for 10 days.

TABLE 8: SUMMARY OF FORCED DEGRADATION RESULTS

Stressed condition	Assay(%w/w)	Degradation%	Peak purity results	
			Purity angle	Purity peak
Acid degradation	92.03	No degradation	0.250	1.339
Base degradation	93.09	20 %	0.250	0.923
Oxidative degradation	92.03	6.%	0.180	0.255
Photo degradation	93.44	No degradation	0.180	0.255
Thermal degradation	92.58	No degradation	0.253	0.268

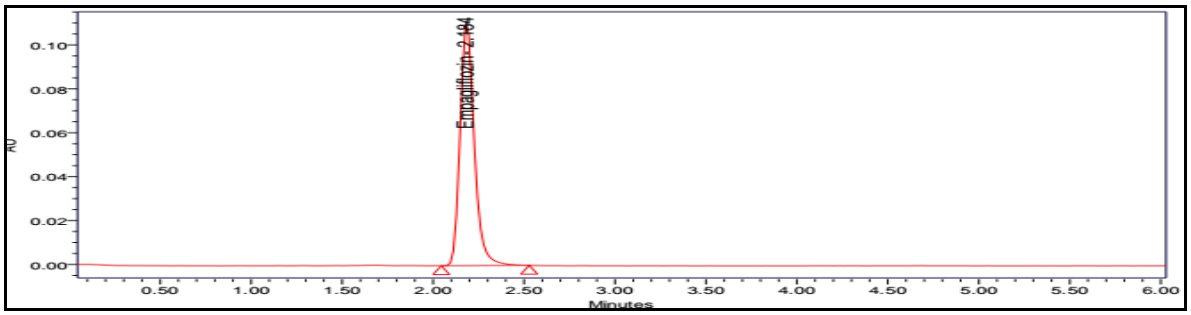

FIG. 5(A): TYPICAL CHROMATOGRAM OFEMPAGLIFLOZIN IN DRUG SAMPLE

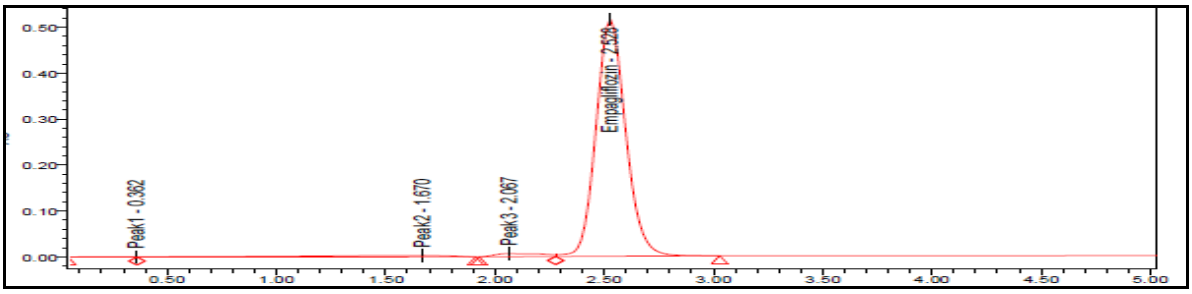

FIG. 5(B): TYPICAL CHROMATOGRAM OFACID DEGREDATION PATTERN OF EMPAGLIFLOZIN

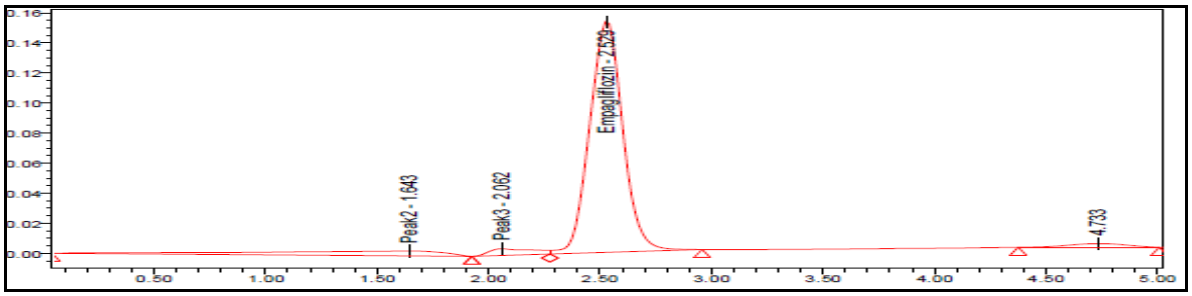

FIG. 5(C): TYPICAL CHROMATOGRAM OF BASE DEGREDATION PATTERN OF EMPAGLIFLOZIN

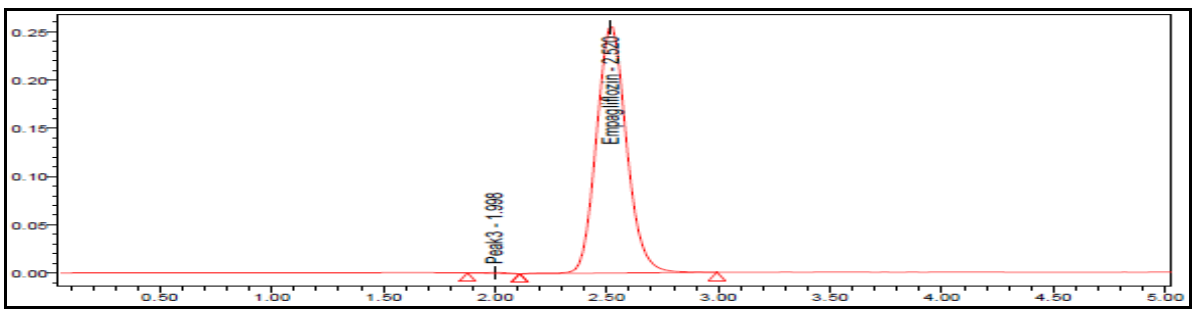

FIG. 5(D): TYPICAL CHROMATOGRAM OFOXIDATIVE DEGREDATION PATTERN OF EMPAGLIFLOZIN

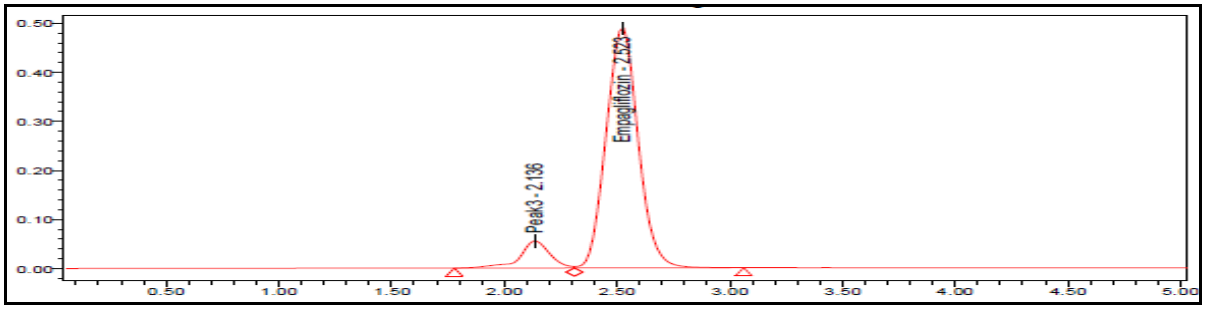

FIG. 5(E): TYPICAL CHROMATOGRAM OFPHOTO DEGREDATION PATTERN OF EMPAGLIFLOZIN

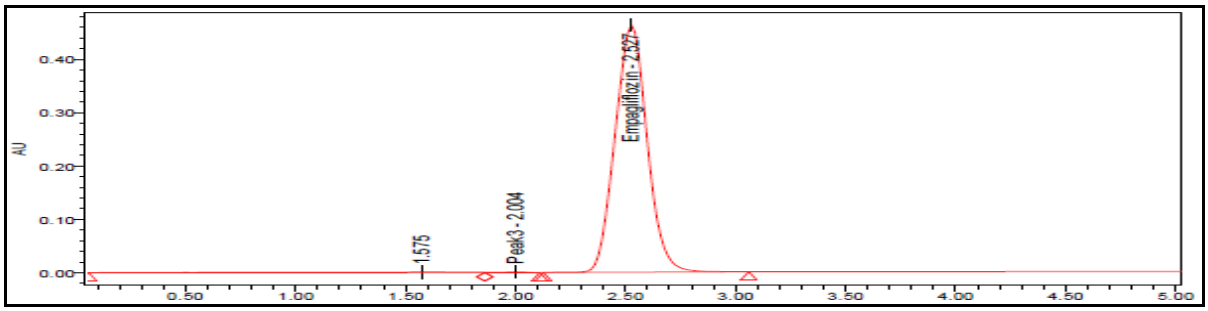

FIG. 5(F): TYPICAL CHROMATOGRAM OFTHERMAL DEGREDATION PATTERN OF EMPAGLIFLOZIN

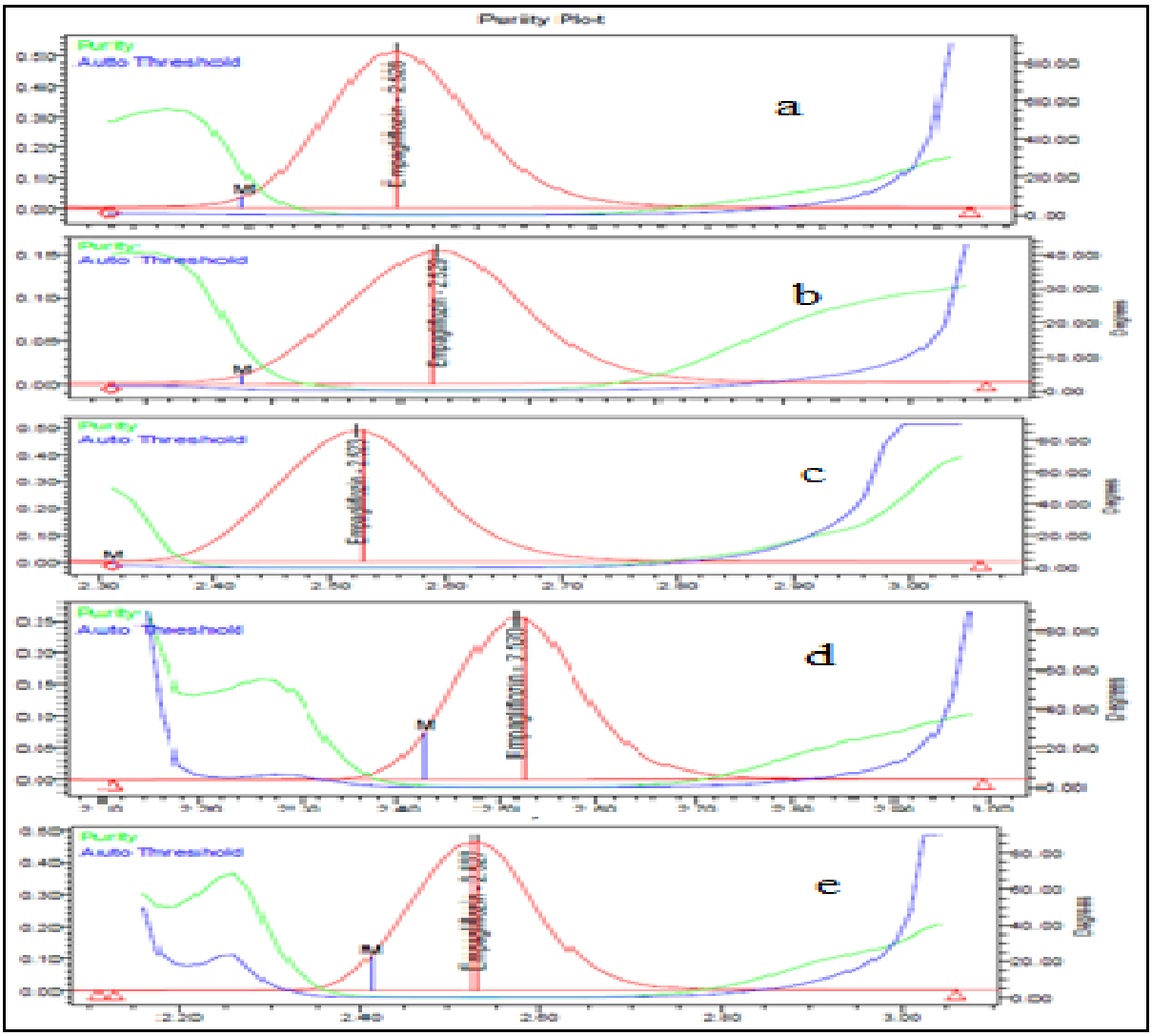

FIG.6: PEAK PURITY PLOTS FOR DEGRADATION PRODUCTS: A) ACID DEGRADATION; B) BASE DEGRADATION; C) OXIDATIVE DEGRADATION; D) PHOTO DEGRADATION; E) THERMAL DEGRADATION.

CONCLUSION: In present work precise, accurate and stability indicating RP-HPLC method for the determination of Empagliflozin in the presence of degradation products was developed and validated. The stability of Empagliflozin under various stress conditions were investigated using a forced degradation study. All of the degradation products were well resolved from the target analytes demonstrating the stability indicating power of the method. The information presented in this study could be used for quality control studies of pharmaceutical dosage forms of this combination.

ACKNOWLEDGMENT: One of the authors (Padmaja) is thankful to RGNF for awarding financial assistance in the form of SRF and Head Department of chemistry for providing necessary facilities.

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How to cite this article:
Padmaja N and Veerabhadram G: Development and validation of a novel stability-indicating RP-HPLC method for the determination of empagliflozin in bulk and pharmaceutical dosage form. Int J Pharm Sci Res 2016; 7(11): 4523-30.doi: 10.13040/IJPSR.0975-8232.7(11).4523-30.