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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF EMPAGLIFLOZIN

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ABSTRACT

A simple, Precised, Accurate method was developed for the estimation of Empagliflozin by RP-HPLC technique. Chromatographic conditions used are stationary phase YMC ODS A C-18 (150mm x 4.6mm),Mobile phase Acetonitrile : Water in the ratio of 50:50 % v/v and flow rate was maintained at 0.5 ml/min, detection wave length was 224 nm, column temperature was set to 35ºC and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 50 µg/ml to 150 µg/ml, R_2 value was found to be as 0.999. Accuracy was carried out on level 50 % to 150% and assay value found in range of 99.55 % to 100.56% .Precision was found to be 0.23 for repeatability, 0.40 to 0.59 for interday precision, 0.19 to 0.26 for intraday precision. LOD and LOQ are 1.24µg/ml and 3.75µg/ml respectively. By using above method assay of marketed formulation was carried out 99.40% was present. Empagliflozin was subject to stress condition including alkaline, acidic, oxidation, thermal degradation and photolysis. Empagliflozin is more sensitive towards acid and Alkali degradation. Also there was no interference of excipients and degradation product at retention time of Empagliflozin, indicating specificity of the method.

KEYWORDS: RP-HPLC, Stress Degradation, Method development, Validation, ICH Guideline.

INTRODUCTION

Type 1 and 2 Diabetes Mellitus is a serious and lifelong condition commonly characterized by abnormally elevated blood glucose levels due to a failure in insulin production or a decrease in insulin sensitivity and function.[1] In type 1 Diabetic mellitus body's immune system attacks the insulin-producing beta cells of the pancreas also called as insulin-dependent diabetes mellitus and type 2 is insulin resistance, a condition in which cells fail to respond to insulin properly and as the disease progresses, a lack of insulin may also develop also called as non insulin-dependent diabetes mellitus. Type 2 diabetes (T2D) and hypertension are two major and independent risk factors for the development of cardiovascular diseases including heart disease, stroke, peripheral arterial disease and chronic kidney disease.[2] There are many classes of drugs available to treat diabetic mellitus but now a day's most widely used class sodium glucose co-transporter-2 (SGLT-2) inhibitors in combination with others class of drugs. The kidneys work by filtering glucose out of the blood and then reabsorbing glucose back into the blood. The proteins that reabsorb glucose are called sodium-glucose transport proteins. SGLT 2 Inhibitors works in the proximal tubules of kidney to block reabsorption of glucose back in to the blood system, thus reducing blood glucose level.[3] Diabetes is the most frequent cause of chronic kidney disease (CKD) it become main reason for most of the deaths in patients with diabetes and cardiovascular disease (CVD). In clinical trials designed to demonstrate the CVD safety of SGLT2 inhibitors in type 2 diabetes mellitus (T2DM), consistent reductions in risks for secondary kidney disease end points (albuminuria and a composite of serum creatinine doubling or 40% estimated glomerular filtration rate decline, kidney failure, or death), along with reductions in CVD events, were observed.[4] Empagliflozin is sodium glucose co-transporter-2 (SGLT-2) inhibitor. Chemically it is known as (2S,3R,4R,5S,6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy]phenyl}methyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol 2,3.[5] The chemical structure of Empagliflozin was given in Fig.1.

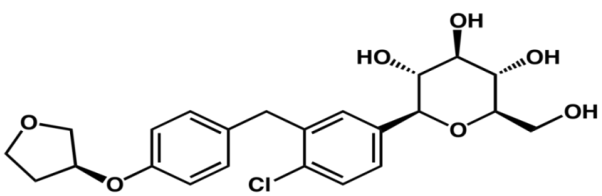

Figure 1: Structure of Empagliflozin.

Stability study is important to determine product's shelf life, optimal storage conditions, retest period, and assuring its overall quality. In this method name of marketed formulation is Jardianc in which Empagliflozin is 2.5 mg per tablet. As per the literature review, Empagliflozin was estimated individually by few methods like UV spectroscopy[6], and HPLC[3]and stability indicating RP-HPLC method in combination.[7,11] The aim of present work was to develop and validate a accurate, cost effective and precise stability indicating RP-HPLC method for determination Empagliflozin.

MATERIALS AND METHODS

Instruments

Chromatographic analysis was carried out on automatic liquid chromatography Model LC-2010 (Shimadzu, Japan), Pump-single pump systems using UV-VIS Detector with Software-LC Solution to acquire and process the data. Reversed-Phase YMC ODS A C-18 (150mm x 4.6mm) column was used as stationary phase, Semi micro analytical balance (Sartorius CD2250, Germany), pH tutor (313927, Eutech Instruments), Ultrasonic cleaner (D 120/1H, Trans-O- Sonic) and Nylon membrane filters (0.22 µm, 47 mm D) were used in the study.

Chemicals and Reagents

Empagliflozin was purchase from Simson Pharma,Mumbai. Acetonitrile and water were used of HPLC grade.

Instrumentation

A Simadzu HPLC system with LC Solution software, fitted with UV detector and a YMC ODS A C-18 (150mm x 4.6mm) column was used for the analysis.

Chromatographic conditions

An HPLC system (make: Simadzu, model- LC-2010) which is operated using a software, LC Solution, fitted with YMC ODS A C-18 (150mm x 4.6mm) Column and UV Detector (at 224nm) was used for the analysis. The mode was isocratic. The mobile phase was filtered through a 0.22 µm nylon membrane filter and degassed prior to use.

Mobile Phase Preparation

A mixture (50:50) of Acetonitrile and Water was used as mobile phase.

Preparation of standard stock solution

Standard stock solutions (1000 µg/ml) of Empagliflozin were prepared by dissolving 10 mg of drug in 100 ml Methanol.

Preparation of working standard solutions

working standard solution of Empagliflozin (100 µg/ml) were prepared by take 1 ml from Stock Solution in 10 ml volumetric flask and add methanol up to the mark.

Sample Preparation

10 tablets were weighed and calculate the average weight of each tablet then Crush all the tablets with mortal and Postal then weight powder equivalent to 100 mg of Empagliflozin on basis of label claim. Powder was transferred into a 100 ml volumetric flask, 50 ml of diluent added and sonicated for 15 min, further the volume made up with diluent and filtered. From the filtered solution 1 ml was pipette out into a 10 ml volumetric flask and made up to 10 ml with diluents.

Method Validation

The method was validated according to ICH guidelines for validation of analytical procedures in order to determine linearity, sensitivity, accuracy and precision for each analytes.

System suitability

The system suitability was evaluated by five replicate analysis of drug at specific concentration. The column efficiency, resolution, and peak asymmetry were calculated for the standard solutions.

Linearity

Appropriate dilutions of working standard solution of Empagliflozin were prepared in the concentration range of 50-150µg/ml and analyzed as per the developed method. Calibration curve was generated and the linearity was evaluated by the least square regression method.

Accuracy (Recovery studies)

To ascertain the accuracy of the proposed method, recovery study was carried out by standard addition method at three different levels according to ICH guidelines. A series of solution Empagliflozin at 50%, 100%, and 150% of the standard preparation in the ratio of the formulation were prepared. To a fixed concentration of the formulation, varying concentrations of pure drug solution was added and percentage recoveries calculated.

Precision

Precision is the degree of repeatability of analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intraday) and intermediate (interday) and reported as %RSD for a statistically significant number of replicate measurement. The intermediate precision was studied by comparing the assays on three different days and the results documented as standard deviation and %RSD. Precision studies were performed in triplicate at three different concentration levels covering the entire linearity range for Empagliflozin.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ are estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as

LOD = 3.3 × SD/ Slope

The LOQ may be calculated as

LOQ = 10 × SD/ Slope

Where, SD = ten replicates of absorbance

Slope = the mean slope of the 6 calibration curves

Robustness

Robustness and Ruggedness of the method was determined by subjecting the method to slight change in the method condition like pump flow rate, wavelength and Mobile Phase. Three replicates were made for the same concentration (100 µg/ml of Empagliflozin), % RSD was calculated.

Degradation studies

Forced degradation experiments were carried out on Empagliflozin under various conditions explained in ICH guideline Q1A (R2), namely, acid, alkali, wet heat, dry heat, and oxidative and photolytic conditions.

Acid degradation

To 1 ml of stock solution of Empagliflozine, 1 ml of 2 N Hydrochloric acid was added and refluxed for 24 hr at 60ºC. Add 1 ml 2 N Sodium Hydroxide and Dilute up to 10 ml with

Mobile Phase to make final concentration 100µg/ml and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample. The Chromatogram of acid degradation was given in figure 4.

Alkali degradation studies

To 1 ml of stock solution of Empagliflozine, 1 ml of 2 N Sodium Hydroxide was added and refluxed for 24 hr at 60ºC. Add 1 ml 2 N Hydrochloric acid and Dilute up to 10 ml with Mobile Phase to make final concentration 100 µg/ml and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample. The Chromatogram of alkali degradation was given in figure 5.

Oxidation studies

To 1 ml of stock solution of Empagliflozine, 1 ml of 30% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 24 hours at 60ºC From the above stress solution, 1 ml was pipeted out in to a 10 ml volumetric flask and then make up to the final volume with mobile phase 100 µg/ml solution and 10µL were injected into the system and the chromatograms were recorded to assess the stability of the sample**.** The Chromatogram of alkali degradation was given in figure 6.

Thermal degradation studies

In a petriplate drug powder form was placed in oven at 60ºC for 24 hr to study thermal degradation. For HPLC study, the resultant drug was weigh 10 mg accurately and transfer into 10 ml clean dry volumetric flask, add few ml of methanol, sonicated for 15 minutes and make up to 10 ml with methanol. From the stress solution, 0.1 ml was pipeted out in to a 10 ml volumetric flask and then make up to the final volume with mobile phase 10µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.** The Chromatogram of alkali degradation was given in figure 7.

Photo Stability studies

In a petri plate drug powder form was directly expose to sunlight for 7 days for photo stability study. For HPLC study, the resultant drug was weigh 10 mg accurately and transfer into 10 ml clean dry volumetric flask, add few ml of methanol, sonicated for 15 minutes and make up to 10 ml with methanol. From the stress solution, 1 ml was pipeted out in to a 10 mL volumetric flask and then make up to the final volume with mobile phase 100 µg/ml solution

and 10µL were injected into the system and the chromatograms were recorded to assess the stability of the sample**.** The Chromatogram of alkali degradation was given in figure 8.

RESULT AND DISCUSSION

Proper selection of method depends upon the nature of the sample, its molecular weight and solubility. Empagliflozin were dissolved in polar solvent, so the developed method of estimation was called as reverse phase high performance liquid chromatography. To develop a rugged and suitable HPLC method for the quantitative determination of Empagliflozin the analytical condition was selected after consideration of different parameters such as diluent, solvents for mobile phase and mobile phase composition and other chromatographic conditions. Preliminary trials were taken with different composition of Acetonitrile and water. The column selection has been done by backpressure, resolution, peak shape, theoretical plates and day-to-day reproducibility of the retention time and resolution. After evaluating all these factors, YMC ODS A C-18 (150mm x 4.6mm) column was found to be giving satisfactory results. The selection of Acetonitrile and water were based on chemical structure of both the drugs. Best results were obtained with, Acetonitrile: Water. For the selection of organic constituent of mobile phase, Acetonitrile: water was chosen to reduce the longer retention time and to attain good peak shape. Therefore, final mobile phase composition consisting of a mixture of Acetonitrile: Water (50:50), set at a flow rate 1 ml/min was selected for the chromatographic analysis (figure 2). Empagliflozin was subject to stress condition including acidic (figure 4), alkaline (figure 5), oxidation (figure 6), thermal degradation (figure 7) and photolysis (figure 8). Empagliflozin is more sensitive towards acid (48.54%) and Alkali degradation (38.79%).

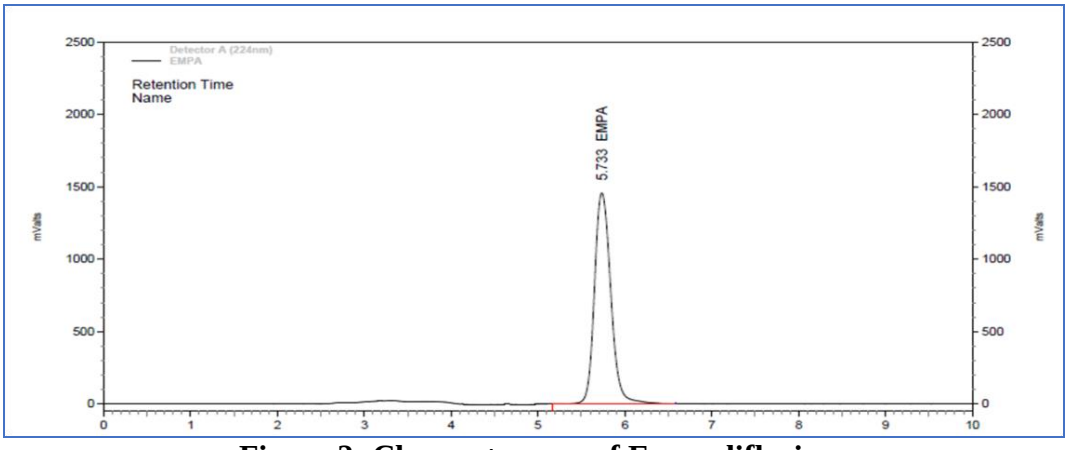

Figure 2: Chromatogram of Empagliflozin.

System suitability

The system suitability was evaluated by five replicate analysis Empagliflozin at specific concentration (Table 1).

Table 1: System suitability.

Sr. no.	Parameter	Value
1	Wavelength	224 nm
2	Regression equation	y = 22299X
3	Linearity range(µg/ml)	50-150
4	Slope (m)	22299
5	Intercept (c)	0
6	Correlation coefficient (r^2)	0.999

Linearity and Range

Linearity in the concentration range was 50-150 µg/ml for Empagliflozin. (Figure 3).

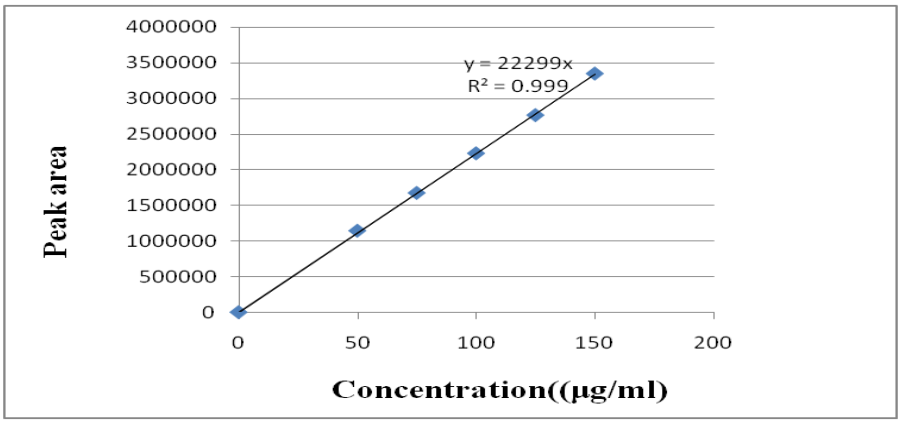

Figure 3: Calibration curve for Empagliflozin.

Correlation coefficient (r^2) for calibration curve of Empagliflozin was found to be 0.999. (Table 2).

Table 2: Calibration Data for Empagliflozin.

Sr. No.	Empagliflozin Concentration (µg/ml)	Peak Area ± SD	% RSD
1	0	0	0
2	50	1143819 ± 4306.53	0.3765
3	75	1674571± 7764.28	0.4637
4	100	2230669 ± 8360.96	0.3748
5	125	2667003± 7992.84	0.2889
6	150	3350642 ± 8799.88	0.2626

Precision

The data for intraday precision and interday precision for standard solution of Empagliflozin is presented in (Table 3, Table 4). These % RSD value were found to be less than 1.0 indicated that the method is precise.

Table 3: Intraday precision data.

Concentration (µg/ml)	Peak Area	Peak Area ± SD	%RSD
50	1146724	1147043 ± 3032.27	0.2643
	1143752		
	1147652		
100	2236574	2238621 ± 4373.52	0.1954
	2243643		
	2235647		
150	3350642	3357335 ± 7020.68	0.2091
	3356721		
	3364643		

Table 4: Interday precision data.

Concentration (µg/ml)	Peak Area	Peak Area ± SD	%RSD
50	1147656	1149356 ± 6807.32	0.5922
	1156853		
	1143561		
100	2257854	2248009 ± 9194.24	0.4090
	2239645		
	2246527		
150	3348742	3364304 ± 15032.12	0.4468
	3365424		
	3378745		

Accuracy

Accuracy of the method was determined by recovery study from synthetic mixture at three levels (50%, 100% and 150%) of standard addition. bThe % recovery values are tabulated in (Table 5).

Table 5: Recovery data.

Spiking Level	Conc. of Formulation (µg/ml)	Conc. of Standard Spiking (µg/ml)	Total Conc. (µg/ml)	Total Conc. of Empagliflozin found (µg/ml) Mean ± SD	% Recovery
50 %	50	25	75	74.84 ± 0.3927	99.76
100 %	50	50	100	100.20 ± 0.3579	100.56
150 %	50	75	125	124.60 ± 0.2510	99.55

Limit of Detection and Quantitation

The LOD for Empagliflozin was found to be 1.24 µg/ml.

The LOQ for Empagliflozin was found to be 3.75 µg/ml. (Table 6).

Table 6: LOD and LOQ data.

Regression equation (y = mx+c)	y = 22299X
Slope (m)	22299
Response standard deviation (SD)	8360.96
LOD(µg/ml)	1.24
LOQ(µg/ml)	3.75

Robustness and Ruggedness

The obtained Ruggedness and Robustness results are presented in (Table 7). These %RSD value was found to be less than ± 1.0 indicated that the method is precise. No significant changes in the Peak area were observed, proving that the developed method is rugged and robust.

Table 7: Robustness and Ruggedness data.

Standard Mobile phase composition Acetonitrile :Water (50:50 v/v)				
Sr. No.	**Factor**	**Level**	**Peak area ± SD**	**%RSD**
Ruggedness data of Empagliflozin				
1	Change in Analyst	Analyst-1	2233997 ± 5285.32	0.2365
		Analyst-2	2237712 ± 10989.48	0.4911
Robustness data of Empagliflozin				
3	Change in Wavelength (224 ± 2nm)	222	2234195 ± 4984.43	0.2231
		226	2231778 ± 5919.12	0.2652
4	Change in flow rate (0.5 ± 0.2 ml/min.)	0.3	2239896 ± 7535.32	0.3363
		0.7	2235248 ± 5637.54	0.2523
5	Change in Mobile Phase (± 5 ml)	45: 55	2236522 ± 11808.24	0.5279
		55: 45	2237946 ± 12281.37	0.5487

Assay

The present assay shows that there is no interference from excipients and the proposed method can successfully applied to analysis of commercial formulation containing Empagliflozin. The % assay values are tabulated in (Table 8).

Table 8: Analysis data of Marketed Formulation.

Sr. No	**Label claim**	**Amount of drug found in mg**	**%Assay**
1.	25	24.78	99.12
2.	25	24.87	99.48
3.	25	24.9	99.6
Mean ± SD		24.79 ± 0.06	99.40 ±

(n=3)		0.20
% RSD	0.2513	0.2051

Table 5: Degradation Data for Empagliflozin.

Sr. No.	Condition	Temperature (ºC)	Time duration for degradation	% Degradation observed
1	2 N HCL	60	24 hr	48.54
2	2 N NaOH	60	24 hr	31.79
3	30 % H_2O_2	60	24 hr	No degradation
4	Thermal	60	24 hr	No degradation
5	Photolytic	Sunlight	7 days	No degradation

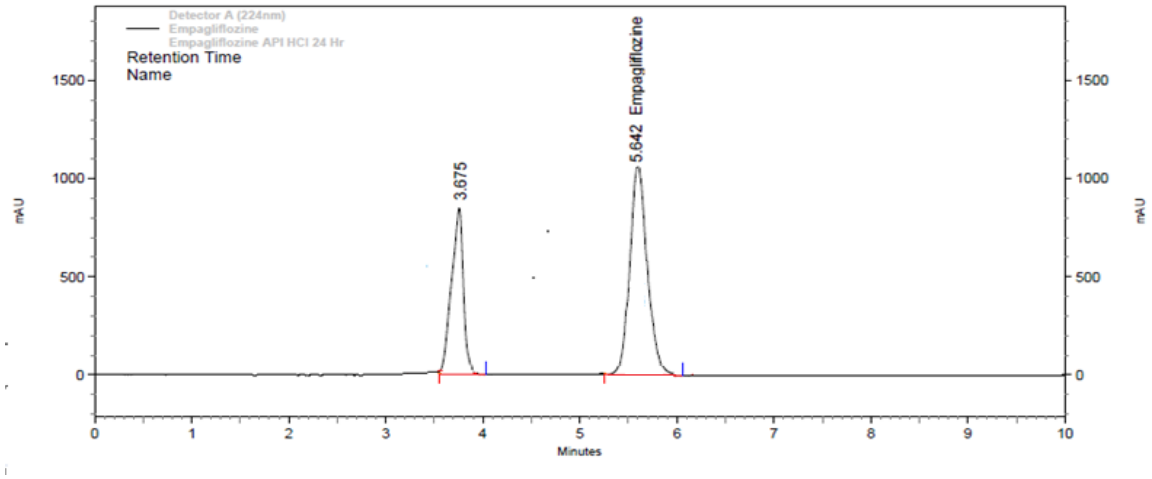

Figure 4: Chromatogram of Acid Degradation of Empagliflozin.

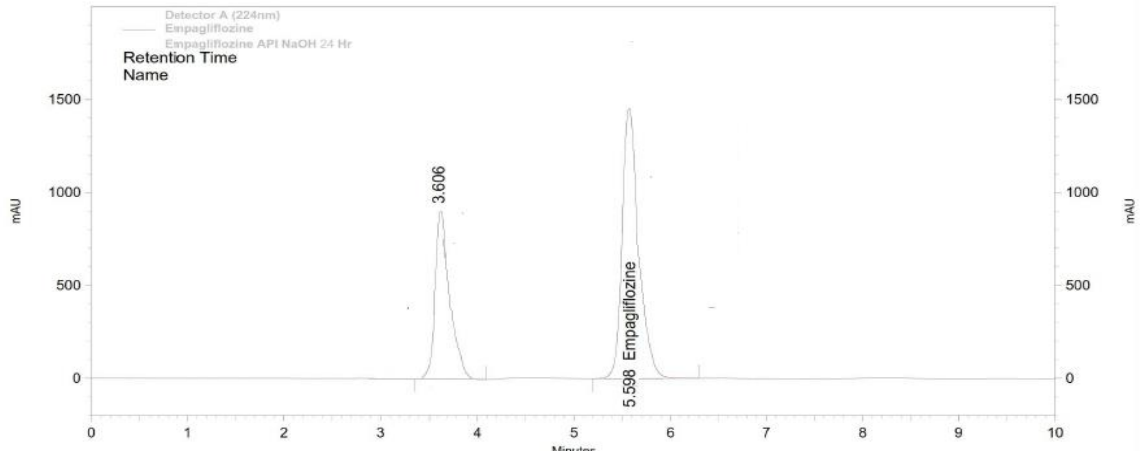

Figure 5: Chromatogram of Alkali Degradation of Empagliflozin.

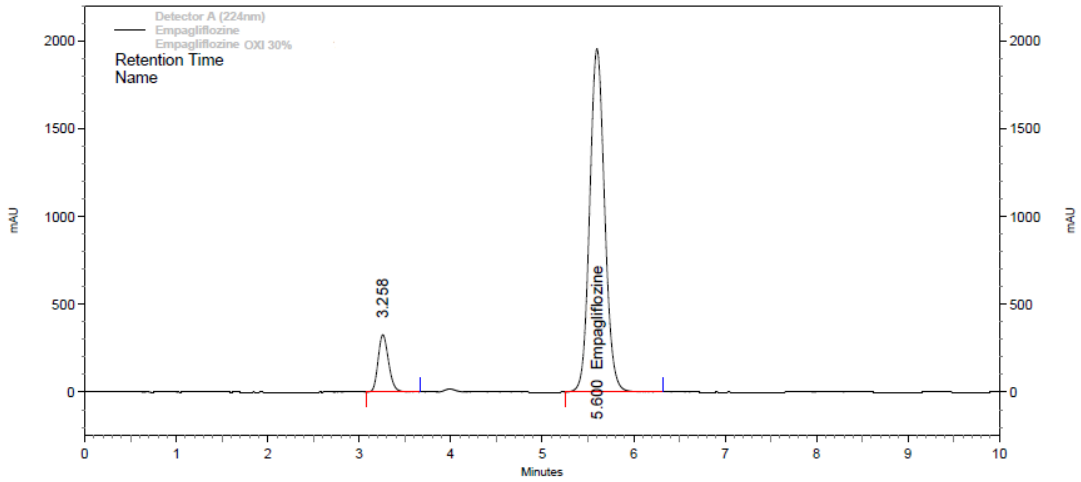

Figure 6: Chromatogram of Oxidative Degradation of Empagliflozin.

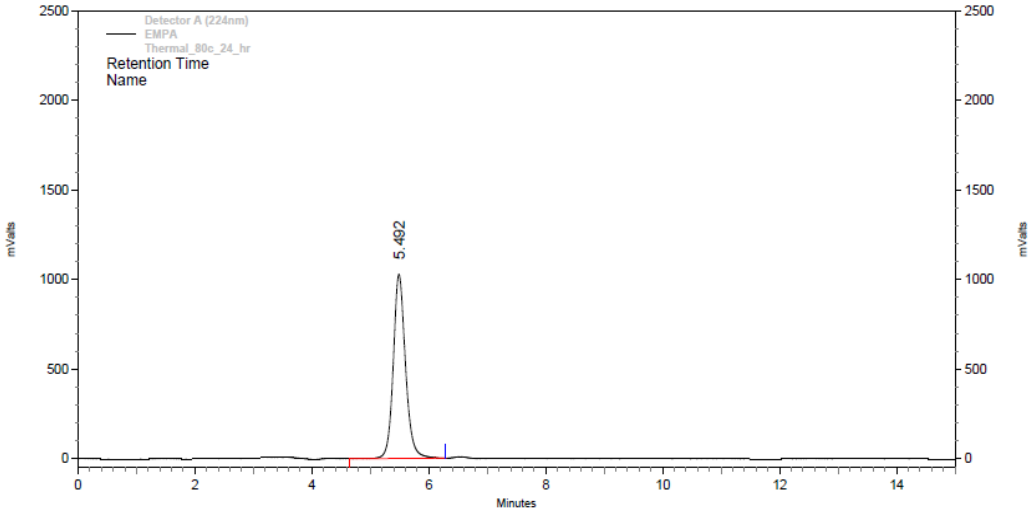

Figure 7: Chromatogram of Thermal Degradation of Empagliflozin.

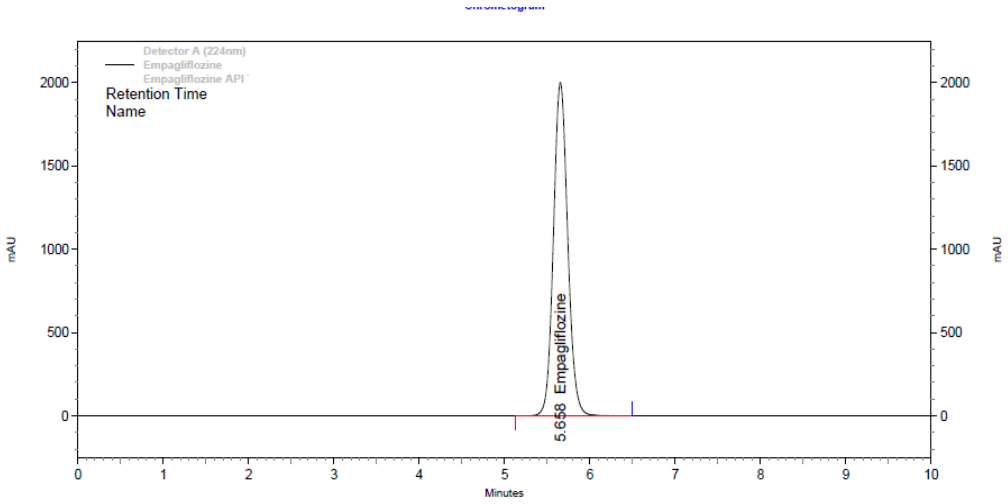

Figure 8: Chromatogram of Photo Degradation of Empagliflozin.

CONCLUSION

From the above experimental results and parameters it was concluded that, the developed method for the for estimation of Empagliflozin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institution, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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