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Research Article

Novel Simplified Analytical Method for Stress Degradation Study of Empagliflozin an Oral Anti-diabetic Agent by RP-HPLC Method

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Abstract

The present study involved developing novel, simplified analytical methods for estimating Empagliflozin in API and finished formulation as per the guidelines of ICH. Meanwhile the study focused on conducting forced degradation studies for Empagliflozin to identify the degraded products and its percentage. The applied chromatographic separation method as follows RP-HPLC ZORBAxC18 (250 x 4.6mm, 5µm particle size) with a mobile phase consisting of Acetate buffer: Acetonitrile in a ratio of 60:40% v/v at a flow rate of 1.0 mL/min with an injection volume of 10µl with 6 minutes run time. The Retention time of Empagliflozin was found to be 2.57 ± 0.05min and detected at 232 nm UV wavelength. The method was found to be linear based on the Linear regression equation y = 61309 x- 8123 with correlation coefficient 0.9999. Validation parameters performed as per the prescribed protocol. Stress degradation experiments were performed by exposing the Empagliflozin into acidic, alkaline, oxidative, thermal, and photolytic conditions, withdrawing samples at different time intervals and injected into the system as per ICH guidelines to analyze the drug. The developed method was novel, precise, simple, and accurate with low consumption of organic solvents to estimate Empagliflozin in API and bulk formulation compared to previously reported studies.

Keywords: Empagliflozin; Stress degradation; Validation; ICH; Forced stability; SGLT2; RP-HPL

Introduction

Empagliflozin (EMP) gliflozin derivative, chemically called as (2S, 3R, 4R, 5S, 6R) -2- [4-chloro -3- [[4-[(3S)-oxolan-3-yl] oxy phenyl] methyl] phenyl]-6-(hydroxy methyl) oxane-3, 4, 5-triol [1]. Empagliflozin by its independent hypoglycemic mechanism protects the diabetic patients suffering with cardiac and kidney disorder. The structure of Empagliflozin is shown in figure 1.

As per 2017 report 6.28% largest number of the population suffered from Type 2 Diabetic mellitus which is a life threatening and cause remarkable changes in social and health care systems. Treating patients suffering with Type 2 diabetes mellitus need drug therapy with changes in lifestyle pattern like proper diet, exercise, and prescribed lifestyle [1].

In T2DM drug therapy treating patients with SGLT found to be recurrent and positive. Gliflozin derivatives reduce the glucose

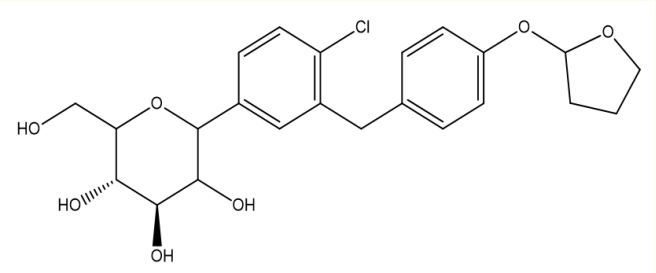

Figure 1: Structure of Empagliflozin.

reabsorption by kidney and improve urinary excretion of glucose thereby reducing the blood glucose level in patients suffering with Type 2 diabetic mellitus. In diabetic type 2 blood glucose level is found more and it is due to 90% absorption of glucose by SGLT, inhibiting them by gliflozin reduces the level which was highly recommended therapy nowadays. Empagliflozin by its independent hypoglycemic mechanism protects the diabetic patients suffering with cardiac and kidney disorder [2-4].

Empagliflozin was assayed by various methods and only a very limited number of single component analyses by HPLC [5-8] published by the researchers. Many combination reports of Empagliflozin with Metformin [9-11] and Linagliptin [12-15] analyzed by HPLC by utilizing various organic mobile phases. Other spectroscopic methods [16-18] adapted to assay by UV/Visible spectroscopy [19-22] and bioanalytical process [23, 24].

Materials and Methods

Chemicals and reagent

HPLC grade Acetonitrile, Sodium Acetate HPLC grade of Rankem Limited, India, HPLC grade water of SD fine-Chem ltd; Mumbai and Glacial Acetic acid HPLC grade Loba chemie Pvt Ltd; Mumbai procured to perform our research work. Empagliflozin API received as a gift sample from Supriya Life science Ltd, India. Jardiance 10 mg film coated tablets of Empagliflozin purchased from a local chemist shop.

Instrumentation

For this study, HPLC system Shimadzu 2010CHT with a gradient pump connected to a UV detector, ZorbaxC18 column was used. The cumulative Data acquired from Lab solution software (5.5.2 Version). An electronic analytical weighing balance Shimadzu 0.001gm sensitive and an Equibtron Ultra-sonicator were used in this entire study with I-Therm dry air oven, UV Chamber was utilized to conduct forced degradation studies of Empagliflozin in API and bulk formulation.

Chromatographic parameters

Equipment: SHIMADZU 2010CHT HPLC System

Wavelength: 232 nm

Injection volume : 10 µL.

Flow rate: 01 mL/minute.

Run time: 6 Minutes.

Column : ZORBAxC_{18} (250 x 4.6mm, 5µm particle size)

Mobile Phase: Acetate buffer (pH 3.4): Acetonitrile (60:40)

Oven Temperature: 28°C.

Preparation of sodium acetate buffer solution (pH 3.4)

Measured and dissolved 28.6 ml glacial acetic acid with 10 ml of 50% (w/v) NaOH in a 1000 ml volumetric flask, mixed it properly and made the volume with HPLC grade water.

Preparation of stock solution

Accurately weighed and transferred 10 mg of Empagliflozin into 10ml volumetric flask. Dissolved and mixed it properly then made the volume with diluent. Diluent is the solvent used as a mobile phase in this experiment. Final concentration of the Stock solution is 1000 µg/ml.

Method validation

Method validation parameters carried out for Empagliflozin as follows: Precision, Accuracy, Specificity, Linearity, Precision and Robustness as per guidelines of ICH Q2A and Q2B.

For Linearity, the series of solutions over the range of 10, 20, 30, 40, 50, 60, 100 and 120 µg/ml of Empagliflozin prepared in triplicate by diluting stock solution in mobile phase and injected into the HPLC system. Linearity graph was plotted by taking the

concentration on the x-axis against the corresponding peak area on the y-axis.

Intra and Interday Precision were performed with 5 µg/ml, 10 µg/ml, and 15 µg/ml concentration of Empagliflozin. Replicates of 3 injections with 5 µg/ml, 10 µg/ml and 15 µg/ml concentration injected into the HPLC system and its retention time, peak area were calculated for assuring its method precision.

Recovery studies conducted for Empagliflozin by adding 8 µg/ml, 10 µg/ml and 12 µg/ml samples into sample solution with known concentration 10 µg/ml. Addition of 8 µg/ml, 10 µg/ml and 12 µg/ml with 10 µg/ml sample stock solution gives 80%, 100% and 120% solution.

Robustness studies conducted by applying modified slight changes in the method parameters such as flow rate (± 0.2 ml/min), temperature (± 2%) and mobile phase (± 2%).

Stress degradation studies

Drug stability of Empagliflozin in stressed conditions is studied as per the guidelines of ICH by exposing it into various stress conditions such as acidic, alkali, oxidative, Thermal and Photolytic conditions. All solutions utilized for this study were diluted from the stock solution of 1000 µg/ml as per requirements.

Acidic degradation

Untreated samples of Empagliflozin solution withdrawn from sample stock solutions 1000 µg/ml. Reflux the solution for 1 hour in a thermostat maintained at 80°C. After 1hour, withdraw the solution cool and neutralize it with 0.1N NaOH.

Alkali degradation

100 µg/ml of sample stock solution refluxed in 80°C thermostat condition. After refluxing, the solution cooled and neutralized with 9 ml of 0.1N HCl. 10 µg/ml sample solution injected into HPLC system.

Oxidation

10 ml of stock solution transferred into a 250 ml round bottom flask and added 90 ml of oxidative agent 30% H_2O_2. Allow the reaction to proceed for 2hours at high temperature (80ºC) for at least 2hrs with continuous shaking.

Irradiation with ultraviolet light

The powder sample of Empagliflozin spread uniformly as exposed into UV light (254 nm) for 2 days on a neat and clean surface. Dilute and inject the 10 µl sample solution after exposure into the HPLC system.

Thermal degradation

The powder sample of Empagliflozin (0.1 mg/ml) was exposed to a temperature of 80ºC for 48hrs, a diluted sample of 10 µl was injected into the HPLC system after exposure to measure peak height, area and retention time.

Assay of marketed formulation (Tablets)

Weighed and transferred 100 mg equivalent Empagliflozin tablet powder, triturated and dissolved it in 100 ml solvent system. Filtered using 0.45 µm syringe filter and injected 10 µg/ml equivalent sample solution into HPLC system to measure its Retention time and peak area.

Results and Discussion

Simplified method designed to estimate this weakly ionizable Empagliflozin drug, adopting the right method after various trials using different mobile phase composition, column, column temperature, flow rate and pH of solvent system ensured that the weakly ionizable drug is very sensitive towards its pH.

The method was developed by varying different chromatographic conditions such as detecting wavelength, mobile phase composition, and polarity of mobile phase, temperature, column, and injection volume to obtain shorter retention time and good separation.

Various solvents such as Acetonitrile, Methanol, HPLC grade water and buffers of phosphate, Acetate were used in different ratios to determine the right mobile composition. The mobile phase Acetate buffer pH 3.4 with Acetonitrile eluted the drug within 3 minutes and it consumed very low organic solvent compared to reported methods [5-8]. Improved tailing factor observed with mobile phase acetate buffer with Acetonitrile (60:40% v/v).

The proposed method showed high linearity in its peak area with concentration range of 10-120 µg/ml. The linearity results were confirmed by its value y = 61039x- 8123 (r^2 = 0.9999). Re-

covery results were found to be more accurate (99.55%, 99.08%, 98.41%), No deliberate variations observed in the results obtained in deliberate variation method (% RSD less than 02), % RSD for Intra-day and Inter-day precision was found to be more precise (0.25 to 0.34).

Stress degradation methods used to study the specificity of the developed method for Empagliflozin in solution and solid-state form. Solution state method involved in analyzing the analyte in solution form such as Acid, Alkaline and oxidative condition, whereas solid method involved in analyzing the drug in solid form such as Thermal and Photolytic condition. Oxidative degradation, small reduction in peak area observed indicates possible degradation compared to Acidic, Alkaline, Thermal and Photolytic conditions figure 4.

Results of various analytical validating parameters found promising and precise for our entire experimental procedure as per ICH criteria [25,26]. The peak area and value obtained for various analytical methods experimented for Empagliflozin was summarized below in Hierarchy.

The chromatogram of Blank, Sample and Standard Empagliflozin summarized in figure 2.

Figure 2: Chromatogram of Empagliflozin A-Blank, B-Standard, C-Sample in Optimized Condition.

Method validation

Linearity

The calibration curve was found to be linear for Empagliflozin with concentration range 10 to 120 µg/ml. The correlation coefficient of regression value, concentration and intercept value figure. 3, was calculated using the formula y = 61039x- 8123 (r^2 = 0.9999) respectively and summarized in table 1.

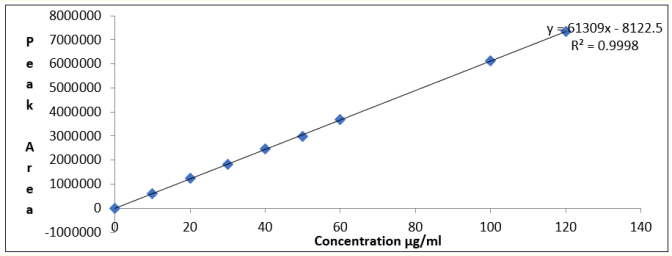

Figure 3: Calibration Curve of Empagliflozin.

S. No	Injection no	RT	Concentration (µg/ml)	*Mean Peak area
1	Injection 1	0	0	0
2	Injection 2	2.57	10	613832
3	Injection 3	2.57	20	1235638
4	Injection 4	2.57	30	1826487
5	Injection 5	2.58	40	2452463
6	Injection 6	2.58	50	2977747
7	Injection 7	2.58	60	3694700
8	Injection 8	2.57	100	6131007
9	Injection 9	2.58	120	7357830
			Slope	61309
			Intercept	-8122.450829
			Coefficient Correlation value	0.9999

Table 1: Linearity Results.

* Mean of three replicates.

Accuracy

The % Mean recovery for Empagliflozin is 99.55, 99.08 and 98.41 for 80%, 100% and 120% and these results are within acceptable limits of Empagliflozin 98-102. The % RSD for Empagliflozin was 0.43, 0.57 and 0.45 is within limit of ≤ 2 and its high value of recoveries at 80%, 100% and 120% concentrations indicate the performed method is accurate. The accuracy data of the proposed method is summarized in table 2A.

Drug	Spiked Conc (µg/ml)	Total Conc (µg/ml)	*Mean peak area ± SD (%RSD)	Drug found	% Recovery
Empagliflozin	8 (80%)	18	1099971.33 ± 4775.08 (0.43)	17.92	99.55
	10 (100%)	20	1216401 ± 6937.52 (0.57)	19.82	99.08
	12 (120%)	22	1328985.33 ± 6009.46 (0.45)	21.65	98.41

Table 2A: Recovery studies values of Empagliflozin.

*Mean of three replicates.

Drug	Concentration (µg/ml)	Intra-day		Inter-day	
		*Mean peak area ± SD	% RSD	*Mean peak area ± SD	% RSD
Empagliflozin	5	217594 ± 747.14	0.34	218970 ± 544	0.25
	10	613159 ± 2025	0.33	611914 ± 1735	0.28
	15	823856 ± 2026.11	0.25	825106 ± 2186.40	0.26

Table 2B: Intermediate and Method Precision studies of Empagliflozin.

*Mean of three replicates.

Precision

Intra-day Precision and Inter-day precision of Empagliflozin calculated by injecting 5, 10 and 15 µg/ml samples of three replicates into HPLC system, it was found to be more precise. The % RSD of both the methods was found 0.25 to 0.34 and 0.25 to 0.28 indicates that the method was precise and reproducible.

Robustness

Deliberate changes in validation parameters didn't alter the robustness of the developed method. Robustness value for different parameters like Flow rate, Temperature and Mobile phase were summarized in table 3. The % RSD was found to be less than 02% for Empagliflozin and no significant changes in the entire procedure which indicates the method is Robust.

Analysis of marketed formulation

The percentage purity of the six replicate samples found to be within the limit and the amount recovered for the assay method found to be 9.94 mg and % purity is 99.37. Reported % purity of Empagliflozin found within the limit as per pharmacopeia.

S. No	Parameters	Condition	*Mean peak area ± SD (%RSD)
1	Flow rate	(0.8 ml/min)	612896 ± 6913 (1.13)
2	Flow rate	(1.0 ml/min)	
3	Flow rate	(1.2 ml/min)	
4	Mobile phase	(58:42)	613086 ± 2097 (0.34)
5	Mobile phase	(60:40)	
6	Mobile phase	(62:38)	
7	Temperature	26°C	608960 ± 5371 (0.88)
8	Temperature	28°C	
9	Temperature	30°C	

Table 3: Result of robustness method.

*Mean of three replicates.

Formulation	Labeled claim (mg)	Amount found * (mg)	Recovery * (%)
Brand 1	10	9.94	99.37

Table 4: Assay estimation of Empagliflozin.
*Mean of Six replicates.

Stress degradation studies

Stress degradation studies conducted as per the ICH guidelines and the drug Empagliflozin doesn't degrade in acidic, thermal, and photolytic method whereas the drug degrades in presence of basic and oxidative hydrolysis process figure 4. Amount of degraded drug products recovered in acidic, basic, oxidative, Thermal and Photolytic conditions (0.84%, 2.73%, 5.05%, 1.44% and 0.93%) summarized and reported in table 5.

Stress Condition	*Mean peak area	Drug recovered (%)	Drug decomposed (%)
Standard drug (untreated)	613872	100	-
Acidic degradation	608741	99.16	0.84
Alkaline degradation	597100	97.27	2.73
Oxidative degradation	582856	94.95	5.05
Thermal degradation	605037	98.56	1.44
Photolytic degradation	608170	99.07	0.93

Table 5: Data of Stress degradation studies Empagliflozin.
* Mean of three replicates.

Figure 5: Chromatogram of Empagliflozin in Photolytic degradation A- Blank, B-Standard, C-Acidic, D-Alkaline, E-Oxidative, F- Thermal, G-Photolytic degradation.

Conclusion

This research work found promising in all parameters in eluting the analyte in less time by consuming low organic solvents. Intraday precision and Interday precision % RSD values indicates the designed method has less than 2% RSD value without any significant variable changes. In Linearity regression method the peak area increases with the increasing concentration of sample. Our method can perform estimation of Empagliflozin in different laboratory conditions. Degradation studies practically proved that weakly ionizable flozin derivatives can also alter its nature in stress conditions such as basic and oxidative conditions. Altering forced degradation studies may be useful in identifying the various degraded products and can be analyzed by the LCMS method. This simplified method consumes less organic solvent and at the same time it elutes the analyte within a short period of time.

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Conflict of Interest

Author declares No conflict of interest for this research work.

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