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TECHNO-ECONOMIC, MS COMPATIBLE HPLC METHOD DEVELOPMENT FOR THE ESTIMATION OF EMPAGLIFLOZIN IN BULK AS WELL AS PHARMACEUTICAL FORMULATION: APPLICATION IN ROUTINE ANALYSIS OF EMPAGLIFLOZIN IN MANUFACTURING INDUSTRIES

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ABSTRACT

An efficient and simple HPLC method has been developed and validated for the determination of Empagliflozin in marketed formulations containing Empagliflozin. The mobile phase used for the chromatographic runs consisted of Water:ACN (55:45 v/v). The separation was achieved on a Symmetry, Waters C-18, 100 x 4.6 mm, 2.7 um, Poroshell 120 EC-C18, using isocratic mode. Drug peaks were well separated and were detected by a UV detector at 225 nm. The method was linear at the concentration range of 2–12 μg/ml for both the formulations. The method has been validated according to ICH guidelines concerning precision, accuracy, and forced degradation. Empagliflozin limit of detection (LOD) and limit of quantification (LOQ) were 0.005938 ng/ml and 0.017813 ng/ml respectively.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. It may be due to impaired insulin secretion, resistance to peripheral actions of insulin, or both [1]. According to the International Diabetes Federation (IDF), approximately 415 million adults between the ages of 20 to 79

years had diabetes mellitus in 2015. Disase is a chronic; the patients with T2DM have more risk of metabolic disorder in which the ability of the secondary complications like cardiovascular risk pancreatic cells to produce or respond to the insulin and hypertension [2]. The sodium-glucose co-transporter 2 (SGLT2) inhibitors have recently emerged as important new treatments for diabetes mellitus. It's a new class of oral hypoglycemic agents that lower blood glucose levels in patients with type 2 diabetes. SGLT2 inhibitors; E acts by blocking SGLT-2 reduces blood glucose. It is blocking glucose reabsorption in the kidney and thereby excreting glucose (i.e., blood sugar) via the urine[3],[4].Empagliflozin is an oral selective Sodium-Glucose co-transporter 2 (SGLT2) inhibitor used for the management of type 2 Diabetes Mellitus. Chemicallyit's (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3- yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane3,4,5-triol (C23H27CLO7) and its structure is shown in **Figure 1 [5]**.
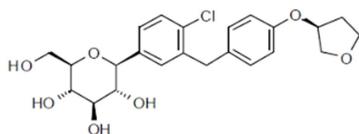
Figure 1: Structure of Empagliflozin

As per the Literature Survey, it is revealed that very few studies were done on the empagliflozin as a single drug assay (HPLC).It has been estimated by UV spectroscopy, High Performance Liquid Chromatography, High Performance Thin Layer Chromatography, Liquid Chromatography-Mass spectrometry but not innovating techno-economic, MS compatible HPLC Method development for the estimation of Empagliflozin in bulk as well as pharmaceutical formulation.

The aim and objective of the present work was to develop and validate a simple, precise, sensitive liquid chromatography method forEmpagliflozin in its bulk and tablet dosage form and validate as per International Conference on Harmonization (ICH) Q2 (R2) guidelines.

Thus, in the proposed work, stress studies and tablet analysis were performed using HPLC. Moreover, the proposed method is economic as less solvent will be consumed due to short running time.

Experimental

2.1 Materials, reagents and pharmaceutical products

Empagliflozin (99.91%) was received as a gift sample from Manus Aktteva Biopharma LLP, Ahmedabad, India. To assess the purity of the drug, melting point was determined which was found as per the literature. Thus, the drug was used without further purification. Methanol, acetonitrile and water of HPLC grade were obtained from Spectrochem Pvt. Ltd., Mumbai.

Film-coated tablet formulations were purchased by a local pharmacy.

2.2 Instrumentation

The HPLC system used for the method development and validation consisted of the Agilent LC1260 series, with VWD detector. Analysis and separation have been done on Symmetry, Waters C 18 (100 mm × 4.6 mm x 3.5μm) at 225 nm in an air-conditioned lab (temperature maintained at 25 °C throughout all chromatographic runs). The mobile phase used for the chromatographic runs consisted of water: acetonitrileat ratio (55:45, v/v), the flow rate was set at 0.5 ml/min in an isocratic mode and the injection volume was set at 1 μl for all samples.

Preparation of standard stock solution

100 mg of standard Empagliflozin was accurately weighed and transferred into a 100 ml volumetric flask and 20 mL of the mobile phase mixture was added to it and sonicated for 10 min, the final volume was made up to 100 mL using the mobile phase mixture. This gave a standard stock solution of 1000 μg/ml. The standard stock solution was further diluted to get the desired concentrations.

2.3 Preparation of Pharmaceutical sample

Brand 1

20 tablets of Empagliflozin Jardiance™ were weighed and crushed. 10 mg powder equivalent to one tablet (10 mg Empagliflozin®) was placed in a 100 ml volumetric flask and sonicated for 10 min and the final volume was made up to the mark with mobile phase mixture followed by 5 min shaking. The solution was filtered and 10 ml of the filtrate was transferred into 20 ml volumetric flasks and the final volume was made to the mark with the mobile phase mixture. An aliquot of 2 ml from the above solution was transferred into a 20 ml volumetric flask and the mobile phase was added to the mark to produce a final concentration of 50 μg/ml Empagliflozin.

2.4 Method development and optimization

The suitability of the column and the mobile phase used in the optimized method has been decided based upon the basis of the selectivity, sensitivity as well as acceptable chromatographic parameters of the produced peaks. We used the mobile phase as a solvent for all samples to ensure minimum noise and to eliminate any unwanted solvent peaks.

2.4.1 Selection of UV wavelength

Empagliflozin has a λ_{max} at 225 nm in methanol [9]. An acceptable response was obtained upon the detection of both the brands of the drug at 225 nm **(Figure 2)**.

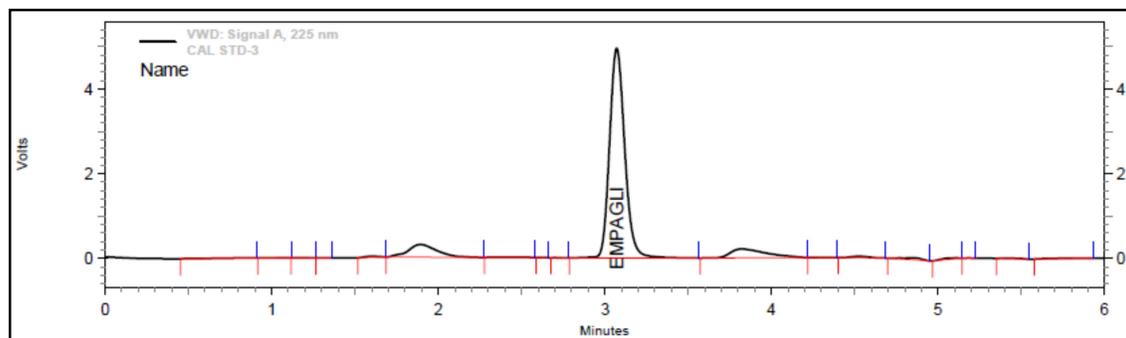The optimized HPLC condition is depicted in **Table 1**.

Figure 2: Empagliflozin λ_{max} at 225 nm in methanol

Table 1: HPLC conditions

System	Agilent 1260 Series
Mobile Phase	water: acetonitrile (55:45, v/v)
Flow rate	1 ml/min
Column	C-18, 100 x 4.6 mm, 3.5 µm (Symmetry, Waters)
Oven Temperature	40 degree Celsius
Wavelength of detection	225 nm
Back Pressure generated	134-136 bars
Total Run time	6 min
Average Retention time	3.0 min

2.4.2 Method validation

The method has been validated as per the International Conference of Harmonisation (ICH) guidelines Q2 (R1) [7] for evaluating system suitability, precision, accuracy, linearity, the limit of detection (LOD),the limit of quantitation (LOQ and forced degradation studies.

1. System suitability

System suitability parameters concerning tailing factor, number of theoretical plates, and retention time of Empagliflozin peak were assessed by injecting a blank mobile phase followed by six replicates of Empagliflozin (50 µg/ml).

2. Linearity

Linear regression data over the range of 2 to 12 µg/mL for Empagliflozin with a correlation coefficient of 0.999 unfolds a good linear relationship between area and concentration in the calibration curve.

3. Precision, repeatability (intra-day precision) and intermediate (inter-day precision)

System and method precision were assessed by injecting 5 independent samples of Empagliflozin (50 µg/ml each) on the same day under the same operating conditions.

Intermediate or inter-day precision was assessed by comparing the results of 5 independent determinations on 3 different days.

4. Accuracy study and recovery

Accuracy of the method was resolved by standard addition method in which standard addition of pure API at three different concentration levels of 70%, 100%, and

130% was performed in triplicate. The accuracy of the method is calculated in the terms of % recovery of the API.

5. LOD and LOQ

LOD and LOQ for Empagliflozin were calculated from the linear regression equation based on the standard deviation of the intercept and the slope using the formula.

LOD = 3.3 Q/S and LOQ = 10 Q/S

where Q: the standard deviation of the intercept, S: the slope of the calibration curve.

6. Forced degradation studies

To assess the stability-indicating a property of the developed HPLC method stress studies were carried out under ICH recommended conditions. Forced degradation of Empagliflozin was carried out by exposing the bulk sample to acidic, alkaline, oxidative, photolytic, and neutral conditions. The aim was to study the ability of the proposed method to measure the analyte response in the presence of its degradation products.

Acid and alkali hydrolysis Aliquot of 1 ml of Empagliflozin solution (1 mg/ml) was transferred to a small round bottom flask. The solution was mixed with 9 ml of 0.1N hydrochloric acid or 0.1 N sodium hydroxide. The prepared solutions were subjected to reflux for 2 h in a boiling water bath. The samples were cooled to room temperature (25ºC), neutralized with an amount of acid or base equivalent to that of the previously added. From the resulting neutral solution, 20 µl of each was injected into the HPLC system.

Oxidation One milliliter of Empagliflozin solution (1 mg/ml) was transferred to a round bottom flask. The contents were then mixed with 9 ml of 30% hydrogen peroxide solution, and the reaction mixture was allowed to proceed at room temperature (25ºC) for 2 h with intermittent shaking. A volume of 20 µl was injected into the HPLC system.

Irradiation with ultraviolet light A sample powder of Empagliflozin (10 mg) was exposed to UV light (254 nm) for 48 h. The material was dissolved in 5 ml water. The solution was filtered with a syringe filtration disk claimed a concentration of 1 mg/ml. It was suitably diluted and a volume of 20 µl was injected into the HPLC system. As well, an aqueous solution of Empagliflozin (1 mg/ml) was exposed to UV light (254 nm) for 48 h, and after diluting 20 µl was injected into the HPLC system.

3. RESULTS AND DISCUSSION

3.1 Linearity studies (Table 2, 3)

3.2 Precision studies (Table 4, 5)

3.3 Accuracy and recovery studies (Table 6)

3.4 LOD and LOQ

The calculated LOD and LOQ were 0.002669 ng and 0.008007 ng for Empagliflozin.

3.5 Analysis of Pharmaceutical formulation

The developed method was validated and effectively applied to determine empagliflozin in different formulations. Three replicates determination was made for each observation as shown in **Table 6, 7**.

3.6 Forced degradation studies (Table 8)

3.6 DISCUSSION

Various mobile phases of different compositions were tested to develop an optimum mobile phase to achieve a satisfactory separation and good peak symmetry for Empagliflozin. A mobile phase consisting of was developed. The analysis was carried out based on peak area with UV detection at 225 nm **(Figure 2)**. The retention time obtained for Empagliflozin was at 3.00 min. The detector response was linear in the concentration range of 1-12 μg/ml.

Validation of the proposed method

A. System suitability

The obtained results of 6 replicate injections showed that the parameters tested were within the acceptable range. Empagliflozin was repeatedly retained at 3.00 min with RSD% of the recorded retention 2.02918 to indicate good repeatability of replicate injections on the integral HPLC system used, the tailing factor never exceeded 1.24 in all peaks indicating good peak symmetry (acceptance limit is < 2) and the number of theoretical plates was always >2000 in all chromatographic runs to ensure good column efficacy throughout the developed separation process.

B. Linearity

A linear correlation was attained between peak area used absorbance vs concentration of Empagliflozin in the range of 2-12 mcg/ml. The linearity of the calibration curve was validated by the high value of the correlation coefficient of regression as shown in Figure(8) and the results are shown in **Table 2**.

C. Precision

The %RSD values of intra-day and inter-day for Empagliflozin are less than 2% which reveal that the proposed method is precise and is shown in **Table 4 and 5**.

D. Accuracy

The accuracy experiments were carried out by the standard addition method. The high value of recoveries obtained for Empagliflozin indicates that method is accurate as shown in **Table 6**.

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

The LOD and LOQ of Empagliflozin were found 0.002669 ng and 0.008007 ng, respectively.

The developed procedure was applied to two marketed formulations of these two compositions of empagliflozin 10 mg and excipients to q.s. The analysis obtained was in uniformity in the claimed amount in the marketed sample. Validation performed according to the ICH guidelines where the results are fast, accurate, robust, specific and linear.

STABILITY INDICATING STUDY

The ICH guideline characterized stability testing of drug substances and products require the stress testing to be carried out to enlighten the inherent stability characteristics of the active substance and also to produce a rapid identification of differences that might result from changes in the manufacturing processes or source sample **[10]**. Vulnerability to oxidation, hydrolytic, photolytic, and Neutral stabilities are the required tests. Ideal stability-indicating the method is one that not only evaluates the standard drug alone but also resolves its degradation products.In the degradation studies, the drug was stable up to 8 h in acidic medium, 24 h in basic medium, and 24 h under oxidative stress and there was no degradation in heat and light exposure **[7-9]**.

From the forced degradation, it was clear that there was no effect of photolytic degradation on the drug as it was completely recovered **(Figure 6)**. Moreover, the acid stability of Empagliflozin was also appreciable as it was degraded to a negligible amount **(Figure 2)**. However, in the case of alkaline hydrolysis, thermal and oxidation degradation, complete degradation of the drug was seen. In the case of acid hydrolysis, alkaline hydrolysis and oxidation degradation, neutral degradation and UV Light Degradation were observed and are shown in the respective chromatograms **(Figure 3-7)**. Nonetheless, the method was able to isolate completely the degradation products from the intact Empagliflozin.

This confirmed stability-indicating the property of the proposed method. The concentration of the produced degradation products analogous to the intact Empagliflozin was calculated and is shown in **Table 8**.

The HPLC chromatogram of standard Empagliflozin and that of standard and Phamaceutical formulation are shown in **Figure 8-10**.

Table 2: Linearity of Empagliflozin

Parameter	Result
Linearity range	2-12 mcg/ml
Slope	1.14412 x 10-5
Intercept	0.0463436
Coefficient of correlation	0.9994

Table 3: System suitability parameters

Parameter	Results
Retention time	3.00 min
Tailing factor	1.22
Theoretical plates	7834
% RSD	1.02918

Table 4: Intra-day precision studies of Empagliflozin

Conc	3 ng/ml	5ng/ml	11ng/ml
Mean Conc.	2.986267	4.9276	11.1774
SD	0.042023	0.028758	0.109132
SE	0.010859	0.007431	0.028199
CV	0.014072	0.005836	0.009764
% CV	1.407207	0.583617	0.976359

Table 5: Inter-day precision studies of Empagliflozin

Conc	3 ng/ml	5 ng/ml	11 ng/ml
Mean Conc.	2.995111	4.962111	11.25838
SD	0.071386	0.056114	0.113857
SE	0.010655	0.008375	0.016994
CV	0.023834	0.011308	0.010113
% CV	2.383408	1.130847	1.011312

Table 6: Accuracy Studies of Empagliflozin

Amount of sample taken (μg/ml)	5	5	5
Amount of standard added (μg/ml)	2.5	5	7.5
Percentage of Standard added	50	100	150
% Recovery	99.5	99.1	99.8
Relative Standard Deviation	1.13	1.01	1.45

*Average of three determinations (n=3)

Table 7: Pharmaceutical formulation studies of Empagliflozin

Marketed formulation	Recovered amount			Claimed amount (mg)
	Amount found (mg)	Recovery(%) ± SD*	RSD* (%)	
Formulation 1 (Empagliflozin 10 mg, excipients qs)	9.97	98.10 ± 0.584	0.258	10

Table 8: Forced degradation studies of Empagliflozin

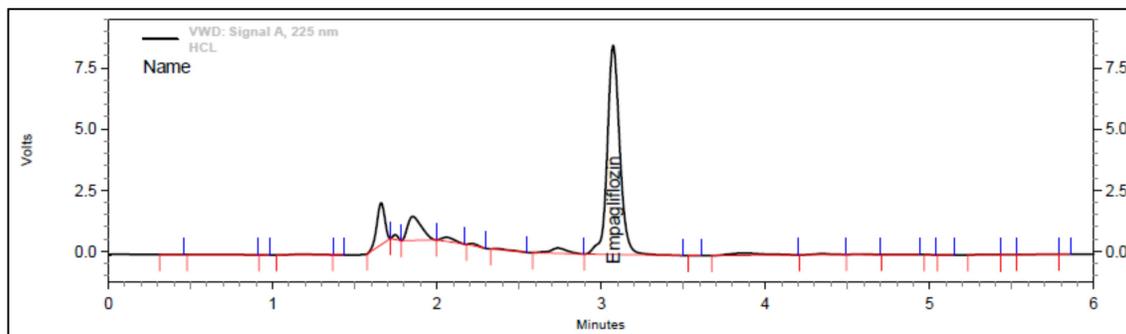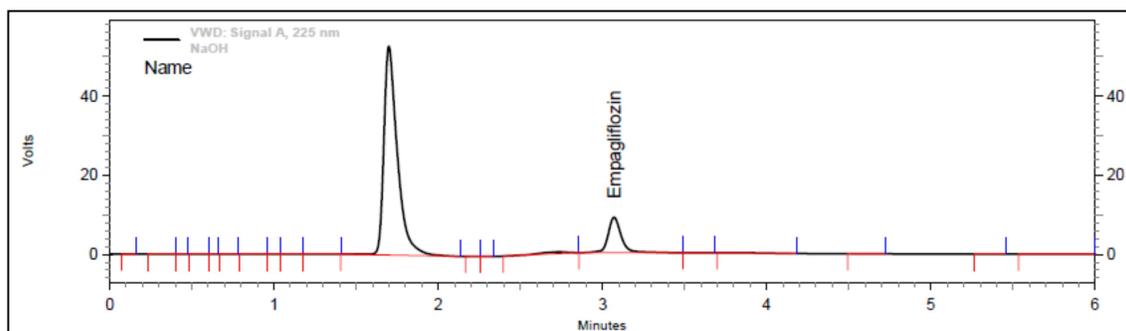| Sample	Drug (ng)	Concentration used (ng)	% Empagliflozin obtained	% degradation
Acid hydrolysis	6.715	12.5	53.72	46.28
Alkaline hydrolysis	7.376	12.5	59.008	40.992
Oxidation	6.75	12.5	54	46
Neutral	6.366	12.5	50.928	49.072
UV	11.628	12.5	93.024	6.976

Figure 3: HCl mediated degradation

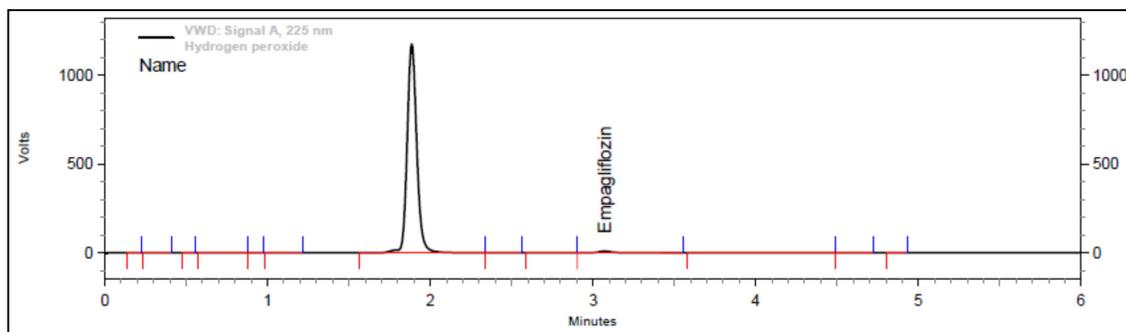**Figure 4: NaOH mediated degradation**
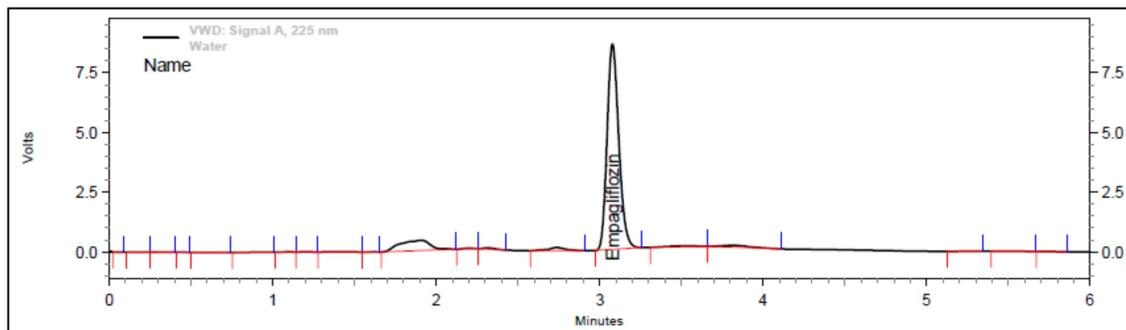

Figure 5: Hydrogen peroxide mediated degradation

Figure 6: Neutral degradation
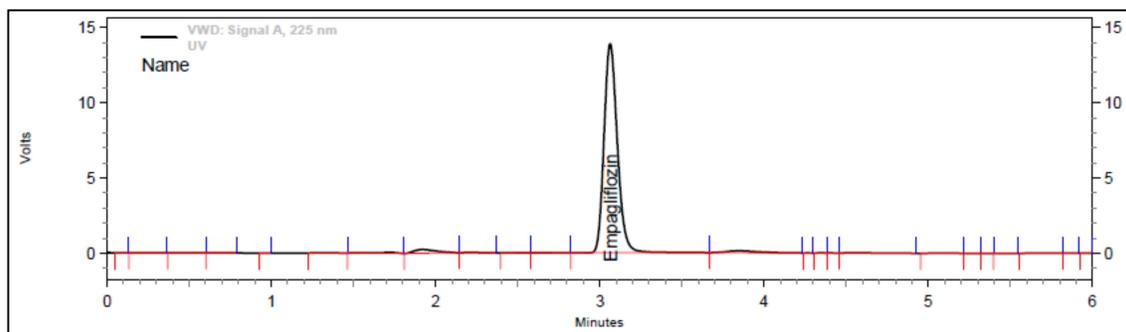
Figure 7: UV Light degradation
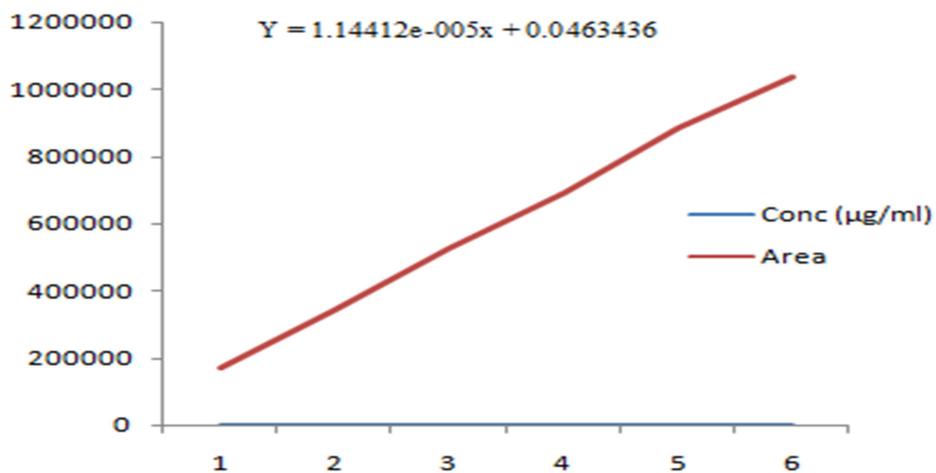

Y = 1.14412e-005x + 0.0463436

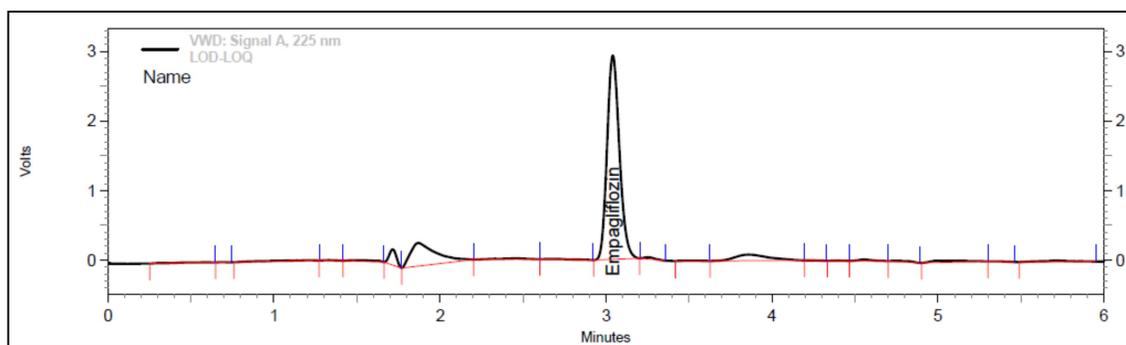**Figure 8: Calibration curve of Empagliflozin**

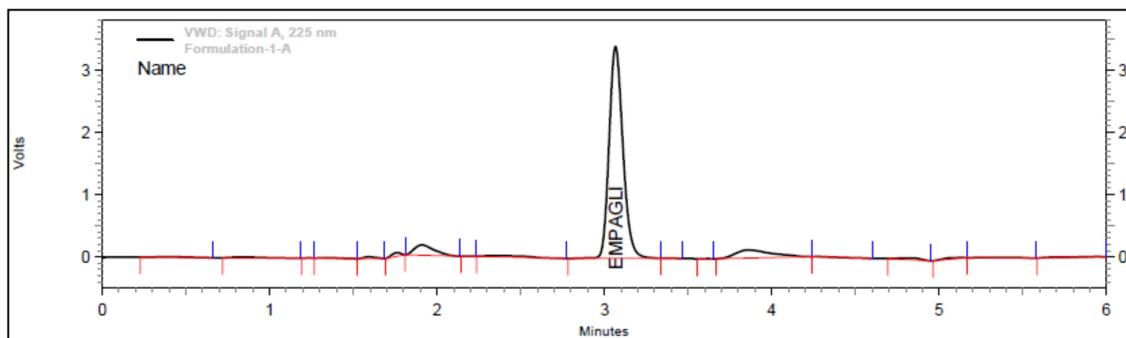**Figure 9: HPLC Chromatogram of standard Empagliflozin**

Figure 10: HPLC Chromatogram of Brand 1

4. CONCLUSION

The current research epitomizes the report that deals with the development of a stability-indicating HPLC method for determination of Empagliflozin. The values of accuracy, precision, LOD, and LOQ were within the limits. Empagliflozin is very sensitive so it is unstable in alkaline, oxidative and thermal conditions but stable in UV light or acid conditions. Statistical analysis for the results demonstrates that the method is suitable for the determination of Empagliflozin in different marketed drugs without any interference from the degradation products, and it is endorsed for routine use in quality control industry laboratories.

Author information

Contributions

Experimental work was done by SP. Both authors equally contributed in framing and writing of manuscript. All authors have read and approved the manuscript.

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