IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
October 2019 Vol.:16, Issue:3

Development and Validation of Simple, Novel RP-HPLC Method for Determination of Empagliflozin in Bulk and Its Marketed Tablet Formulation

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Submission: 23 September 2019
Accepted: 29 September 2019
Published: 30 October 2019

Keywords: Empagliflozin, RP-HPLC method, validation

ABSTRACT

The purpose of the proposed research work was to develop a simple, novel, rapid and accurate RP-HPLC method for estimation of Empagliflozin. Chromatographic separation was successfully done on Grace C18 column (250mm x 4.6mm) 5µ, with mobile phase Methanol: Water (90:10) at 0.8ml/min flow rate using UV detector at 227nm. The run-time per sample and retention time was found to be 6.56 min and 4.05 min respectively. The procedure was found to be linear (r^2 = 0.999) the concentration range of 10-50 µg/ml with acceptable percentage relative standard deviations for Intra-day (0.32%) and Inter-day (0.23%). The % recovery was found to be 99.80 %. LOD and LOQ value was 0.76µg/ml and 2.31µg/ml respectively. The mobile phase is simple to prepare so; the proposed estimated method was economical for routine analysis of Empagliflozin.

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INTRODUCTION

Empagliflozin is the gliflozin class of drugs used in the remedy of type 2 diabetes. The chemical name of empagliflozin is (empagliflozin; 1-chloro-4-[b-Dglucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran – 3 –yl -oxy) benzyl]-benzene (Fig no. 1). Empagliflozin is a sodium-glucose co-transporter-2 (SGLT-2) inhibitor, which is seen most particularly in the proximal tubules of the nephron in the kidneys [1-3]. The main function of SGLT-2 is re-absorption of approximate 90% of glucose into the blood. SGLT-2 inhibitor decreases blood glucose by preventing glucose re-absorption in the kidney and whereby excreting glucose through the urine. The side effects of this drug are a higher frequency of urinary tract infections and also increase the risk of diabetic ketoacidosis [4-6].

Various analytical methods were reported in the literature for the determination of empagliflozin individually and combination with another drug in pure drug, dosage forms and in biological samples using HPLC with complex mobile phase and other analytical techniques. The newer, robust, accurate and simple method developed and validated for Empagliflozin in its bulk and tablets using a simple mobile phase at early retention time for routine analysis and quality control test [1-8].

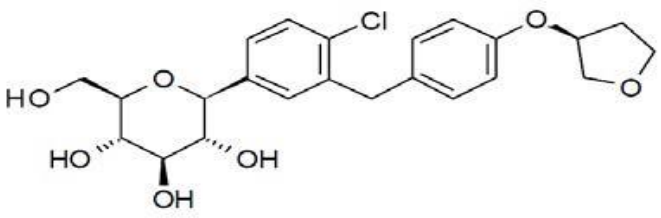

Figure No. 1: Chemical structure of Empagliflozin

MATERIALS AND METHODS

Chemicals and Reagents

The empagliflozin sample was kindly provided by RAP analytical Research and Training Center, Nashik, India. HPLC grade acetonitrile and methanol were procured from Fisher Scientific and Honeywell**.** O-phosphoric acid and Potassium di-hydrogen phosphate procured from Finnar and Fisher Scientific.

Instrumentation and chromatographic condition

UV-Spectrophotometer (Analytical Technologies Limited) and pH meter (VSI pH meter) are used for the analysis of the sample. The chromatography was performed on an HPLC Binary Gradient System, Model no.: HPLC 3000 Series with HPLC Workstation software. The analysis was carried using Grace C18 (250mm x 4.6mm internal diameter with particle size 5 micron) column at 0.8ml/min flow rate with mobile phase Methanol: Water (90:10) at 227nm detection wavelength.

Preparation of Solutions

Preparation of Standard Stock Solution

Accurately weigh and transfer 0.01 g of Empagliflozin into a 10 ml clean dry volumetric flask, add 5ml of methanol: water (90:10) as diluents and sonicate to dissolve it completely. Make up the volume up to the mark with the same solvent. (Stock solution) Further dilution was carried out from the above stock solution within a 10-50 µg/ml range for analysis.

Preparation of Sample stock solution

Ten Tablets containing a label claim of 30 mg of Empagliflozin were weighed and finely powdered. Equivalent weight transferred into a 10 ml volumetric flask, dissolved in 10 ml methanol: water (90:10). The clear solution obtained was diluted to get an appropriate concentration in linearity ranges and absorbance was measured.

Procedure

Inject 20 µL of the standard, sample into the chromatographic system and measure the area for the Empagliflozin peak and calculate the %Assay by using the formulae.

Method development selection of wavelength

A stock solution of Empagliflozin was prepared in distilled water. 20µg/ml sample was taken from a stock solution for scanning in UV spectrometry between 200 to 400nm range. The maximum absorbance was recorded at 227nm which is selected as a λmax for the detection of Empagliflozin by RP-HPLC (Figure no 2).

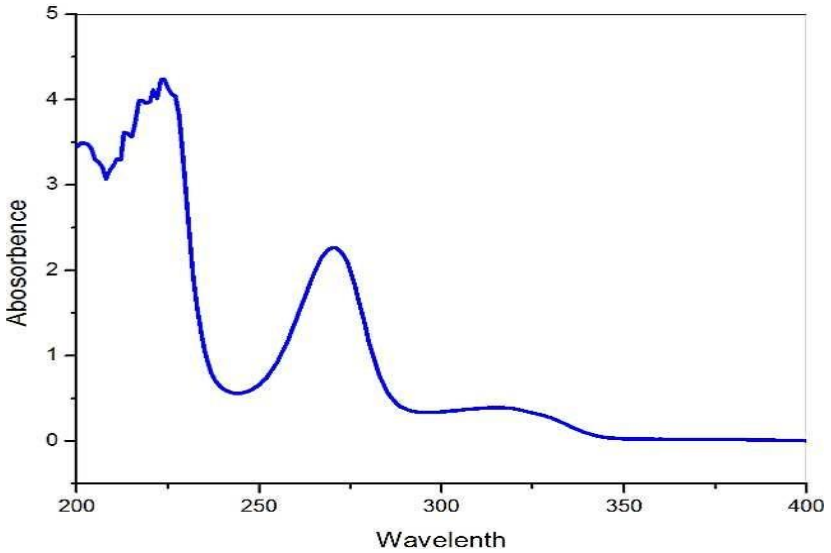

Figure no 2: UV spectrum of Empagliflozin

METHOD VALIDATION

The developed method was validated for linearity, precision, and accuracy, the limit of detection and limit of quantitation, robustness and system suitability parameters as per ICH guidelines.

Linearity and Range

From the stock solution, 10, 20, 30, 40, 50 µg/ml solutions were made within a range and their chromatograms were recorded. The regression coefficient was calculated from the linear plot of the mean peak area versus their concentration.

Accuracy

Recovery a study was performed by spiking the known amount of standard drug corresponding to 50,100,150% of label claim had been added to the marketed drug sample (standard addition method). Three determinations at each level were performed and the results achieved were compared with expected results.

Precision

The precision of the method was validated from repeatability and intermediate precision studies. Repeatability study was carried out by analysis of concentrations 30µg/ml of Empagliflozin for RP-HPLC on a similar day. Intermediate precision of the method was checked by repeating these studies on two different days.

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

The standard deviation and slope of the calibration curves were used to calculate the LOD and LOQ for the drugs used in the following formulae:

$$LOD=3.3(S)/s \ \&$$

$$LOQ = 10(S)/s$$

where,

S=Standard Deviation and

s= Slope of the line

Robustness

Robustness was calculated by deliberate variations in a few parameters of the RP-HPLC method. The parameters involved a variation of flow rate (±0.2%), a wavelength in the mobile phase and (±0.4%) pH of the mobile phase (±0.2ml).

Ruggedness

Ruggedness was performed by a change in analysts and was found to be reproducible.

RESULTS AND DISCUSSION

System suitability Parameter

In System suitability parameter, tailing factor ≤ 2.0 and theoretical plates > 2000 should be within defined criteria. From obtained data, it was found that all parameters fall within the defined criteria and results are tabulated in Table no 1. Chromatogram of standard Empagliflozin shown in Figure 3.

Table No. 1: System suitability parameters for the developed method (AZM)

Parameters	Values
Chromatographic Column	Grace C18
Mobile phase	Methanol: Water (90:10)
Detection Wavelength	227 nm
Flow Rate	0.8 ml/min
Injection Volume	20 µl
Temperature	Ambient
Run Time	6.56 min
Retention time	4.05 min
NTP	9101
Asymmetric factor	1.24

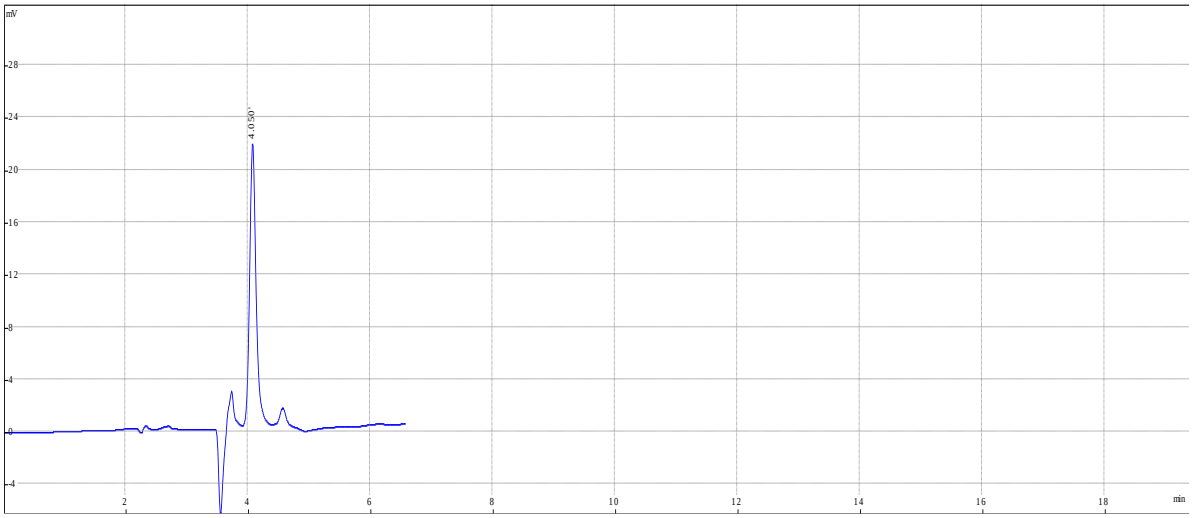

Figure No. 3: Chromatogram for standard Empagliflozin with 4.05min Retention time

Method Validation

Linearity and Range

The given method was obtained in a range of 10-50µg/ml for empagliflozin. The standard Calibration curve was acquired by plotting the absorbance against its concentration measured at 227 nm shown in fig 4. The regression coefficient and line equation were found to be 0.99 and y = 15689x – 19523 respectively.

Table No. 2: Linearity Data of Empagliflozin for RP-HPLC

Sr. No.	Conc.(µg/ml)	Peak Area	Regression coefficient
1	10	146985	0.999
2	20	286606	
3	30	444974	
4	40	604939	
5	50	772288	

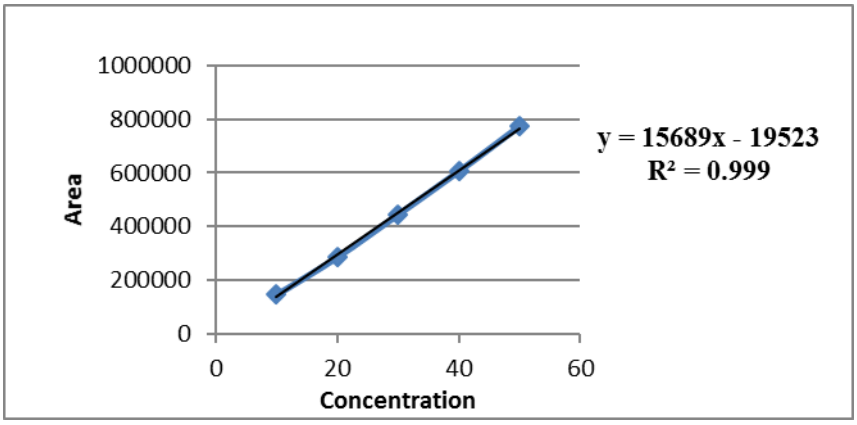

Figure No. 4: Calibration curve of Empagliflozin by RP-HPLC.

Accuracy

The accuracy of the RP-HPLC method was determined as % recovery and is shown in table 3. Good recoveries (99.59–100.01 %) of the spiked drug were obtained at each concentration level. The % RSD (0.16- 0.45%) of the method was found to be very low which indicated the accuracy of the proposed method. The higher values of % recovery which are near to 100% indicate that the proposed mobile phase and method are accurate.

Table No. 3: Recovery studies of Empagliflozin by RP-HPLC.

Sr. No.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50% Recovery	444974	445031	100.0128097
2	100% Recovery	604939	603753	99.80394717
3	150% Recovery	772288	769170	99.59626461

Precision

Intraday and Inter-day precision were determined by analyses of 30 µg/ml of the Empagliflozin on the same day and two different days. %RSD of both was found to be within a range indicating that the proposed method is precise.

Table No. 4: Intra-day and Inter-day precision of Empagliflozin

Sr. No.	Conc.	Intraday Precision		Inter-day precision	
		Morning	Evening	Day 1	Day 2
1	30	444974	446902	444974	445973
2	30	445236	443757	445236	445573
3	30	443253	446339	443253	445991
Mean		**445076.833**		**445991**	
%RSD		**0.32%**		**0.23%**	

LOD and LOQ

The LOD 0.76 µg/ml and LOQ 2.31 µg/ml ensures that the method is more sensitive and selective.

Robustness

Robustness was studied by a change in flow rate. One factor at the time was changed to estimate the effect. Variation in flow rate did not affect the results. Peak Area and Tailing factors of both the drugs at different levels of variations were similar only the small significant differences observed in retention times. Hence, the method was seen to be robust.

Table No. 5: Robustness studies for Empagliflozin

Sr. No.	Conc.	At 0.8ml/min flow rate			At 0.9ml/min flow rate		
		Area	SD	%RSD	Area	SD	%RSD
1	20	286606	672.203	0.235	286606	217.054	0.076
2	20	286576			286859		
3	20	285427			286427		

CONCLUSION

The drug was found to degrade under basic, thermal and photolytic conditions, whereas it was found to be stable under neutral hydrolysis and acidic conditions. The proposed estimated method was found to be simple, precise, accurate and rapid for the determination of Empagliflozin from Tablet forms, the mobile phase is simple to prepare and economical. The sample recoveries in all the formulations were in good agreement with their respective label claim and their method can be conveniently adopted for routine analysis of Empagliflozin in the pharmaceutical dosage form.

ACKNOWLEDGMENT

We extend sincere thanks to RAP analytical Research and Training center, Nashik for giving drug sample and Pravara Rural Education Society's College of Pharmacy, Chincholi, Nashik for necessary facility required for accomplishing this research work**.**

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