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Research Article

Development And Validation of High-Performance Liquid Chromatographic Method for Analysis of Empagliflozin in A Controlled Release Marketed Tablet Formulation

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ABSTRACT

A rapid, simple, and sensitive HPLC method of analysis for in Empagliflozin in a controlled release marketed table formulation. Effective chromatographic separation was achieved using C8 (Thermo Hypersil gold) /4.6 x 250 mm, 5 μ particle size with isocratic elution of the mobile phase consisting of 0.2% OPA and ACN (60:40) The wavelength of detection was set to be 225 nm (UV detector), and a flow rate of 1.0 ml/min. was employed, 20 μ l was used as injection volume and the column temperature was maintained at 25°C. Under chromatographic conditions, the Peak of Empagliflozine was obtained at a retention time of about 4.560 min. and run time of about 10 minutes. The developed method was validated according to ICH guidelines for the validation of analytical procedures and was successfully used.


INTRODUCTION

Empagliflozin 3 (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol"Figure 1" which is a, Sodium-glucose co-transporter-2 (SGLT-2) inhibitor used in type 2, it was approved in the USA market in August 1 2014 is used in the form Jardiance for the treatment of type 2 diabetes mellitus. The molecular formula is

$C_{23}H_{27}ClO_7$, and the molecular weight is equal to 450.9 g/mol According to the safety data sheet for EMPAGLIFLOZINE and toxicological information, The most reported side effects were urinary tract infections, genital mycotic infections, and dyslipidemia. Due to its diuretic properties related to volume depletion, there were also reports of dehydration and hypotension.

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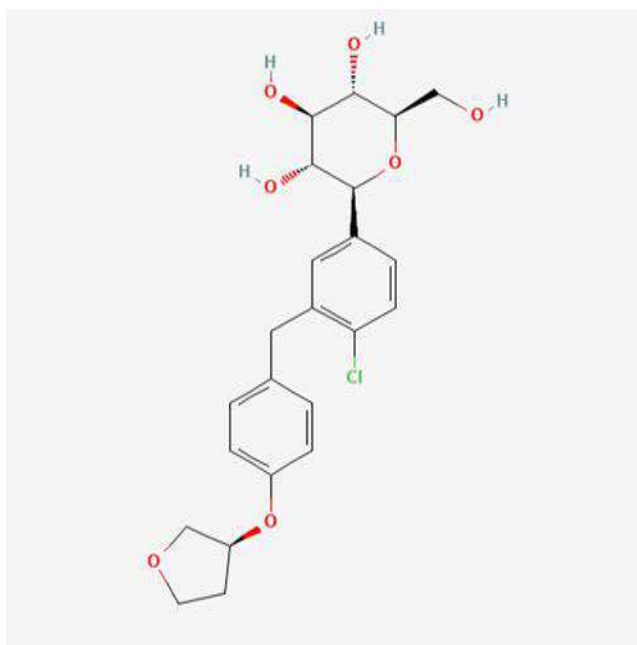


Figure 1. Chemical structure of Empagliflozine

Method development is still very much a trial-and-error approach, expedited by a logical sequence of generic scouting runs and fine-tuning steps to achieve the requisite resolution and method performance. Separation processes are used to decrease the complexity of material mixtures. The most utilized separation method is chromatography. Following types of techniques are used:

1. Gas chromatography (GC)
2. High performance liquid chromatography (HPLC)
3. Size- exclusion chromatography
4. High-performance thin layer chromatography (HPTLC)
5. Paper chromatography
6. Thin layer chromatography (TLC)
7. Affinity chromatography
8. Ion exchange chromatography

Chromatography

Chromatography is defined as a method of separating a mixture of components into individual

components through equilibrium distribution between two phases

Principle of Chromatographic Separation

Chromatographic techniques are dynamic process where in a mobile phase transports the sample mixture across or through a stationary phase medium. As the sample comes in contact with the stationary phase interaction occurs. A partitioning or separation of the component in the mixture results from the differential affinity of each component with the stationary phase. As the separated component emerges or elutes, a detector respond with a signal change that is plotted against time thus producing a chromatogram.

High Performance Liquid Chromatography

HPLC is a modern form of liquid chromatography that uses small-particle column through which the mobile phase is pumped at high pressure. This is chromatographic process, where a mixture of analytes is separated into two distinct bands as they migrate down the column filled with stationary phase

Method Development in HPLC

Complex mixtures or samples required systematic method development involving accurate modeling of the retention behavior of the analyte. Among all, the liquid chromatographic methods, the reversed phase systems based on modified silica offers the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution “Best

column, best mobile phase, best detection wavelength, efforts in separation can make a world of difference while developing HPLC method for routine analysis. Determining the ideal combination of these factors assures faster delivery of desired results- a validated method of separation.”

Instrumentation of HPLC

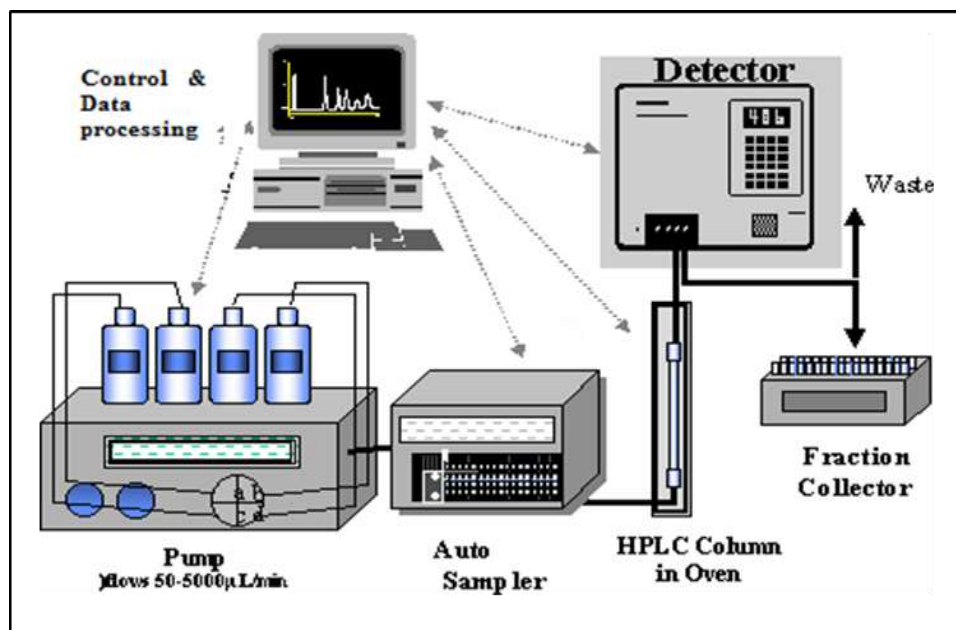


Fig. No. 2: Schematic diagram of HPLC system

2. Experimental.

2.1. Equipment

Sr. No	Instruments	Make	Model
1	UV-Visible Spectrophotometer	Shimadzu	UV 1900i
2	HPLC	Waters 600	996 PDA Detector
3	pH Meter	Hanna	-
4	Balance	Citizen	CY 104 (Micro Analytical Balance)
5	Ultra sonicator	-	1.5 L 50

2.2. Reagents and chemicals:

All reagents and chemicals used were of AR grade and HPLC grade. 1.5 L 50

1. Methanol (HPLC grade).
2. Acetonitrile (HPLC grade)
3. Disodium hydrogen phosphate (AR grade).
4. Distilled Water (HPLC grade).
5. Triethylamine (HPLC grade).

6. Ortho Phosphoric Acid (HPLC grade).

2.3. Pharmaceutical formulation

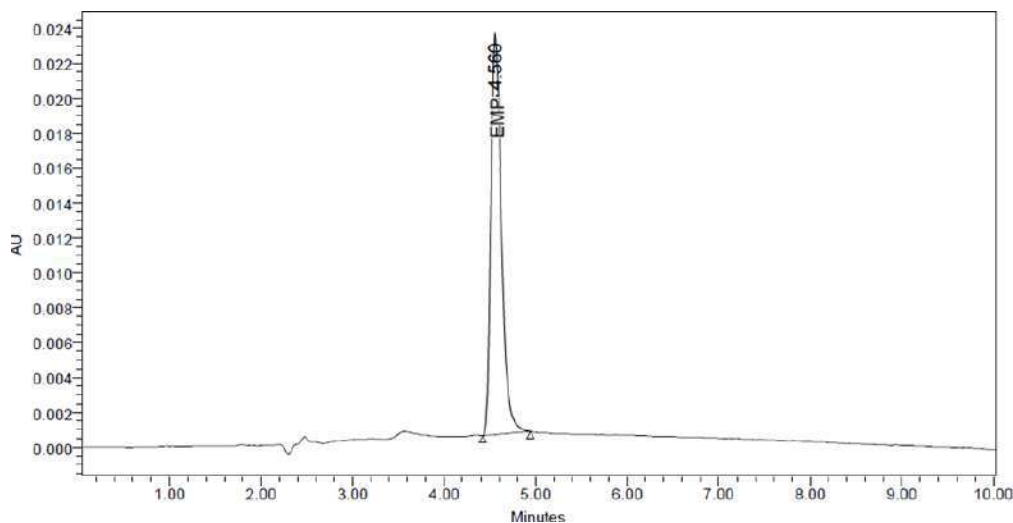
Brand Name	Mfd by	Content	Quantity
Ajardy-CR	Einzig Pharmaceutical Pvt Ltd	Empagliflozin-25 mg	10 tablets

2.4. Method development.**2.4.1. Preparation of mobile phase**

Dilute 2 ml ortho phosphoric acid in 1000 ml of volumetric flask and make up the volume upto the mark with HPLC water

Chromatographic conditions

Chromatographic separation was achieved using C8 (Thermo Hypersil gold) /4.6 x 250 mm, 5 μ particle size The wavelength of detection was set to be 225 nm detector), and a flow rate of 1.0 ml/min. was employed, 20 μ l was used as injection volume and the column temperature was maintained at 25°C. Under these chromatographic conditions, Peak of Empagliflozine was obtained at Retention time about 4.560 min. (**Figure 1**) and Run time of about 10 minutes.

**2.4.3. Preparation of standard solution.****Empagliflozin standard stock solution:**

Accurately weighed quantity of 25 mg EMP was dissolved in ACN and volume was made up to 100 ml mark by same to obtain 250 μ g/ml stock solution.

Empagliflozin standard working solution:

Pipette out 1 ml from standard stock solution and dilute it with 10 ml ACN to obtain 25g/ml of EMP.

Sample solution preparation:

Entire content of Ajardy-CR® Controlled release tablet (25 mg) was transferred to a 100 ml volumetric flask, the volume was made upto the mark with ACN, the resultant concentration was 250 μ g/ml. The whole content was centrifuged at 5000 rpm for 10 min followed by passing through 0.45 μ membrane filter. 1 ml of resultant was transferred to a 10 ml volumetric flask and the volume was made upto the mark with ACN, the concentration of working sample solution was 25 μ g/ml.

2.5. Organic impurities.

To determine any percentages of unknown impurities resulting from some degradations of the active substance in pharmaceutical preparations and compare their ratio to the ratio of the principal substance apply the method of assay regarding: chromatographic conditions, mobile phase, and diluent, except run time is 10 minutes.

2.5.1. Preparation of sample.

An accurately weighed quantity of Empagliflozin (EMP) 5 mg was transferred to the 10 ml volumetric flask and dissolved in HPLC grade ACN. The volume was made up to the mark with the same to make (500 µg/ml).

2.5.2. Preparation of standard solution

Accurately weighted EMP 1.5 mg was dissolved in 100ml ACN. This solution was used as standard stock solution 3.

RESULTS AND DISCUSSION

1. The aim during development of any method that achieve good resolution between analytes and peaks with acceptable peak symmetry, sharp peak, and in a reasonable analysis time

2. HPLC Column Selected:

HPLC Waters 600 system with C18 (Thermo Hypersil gold) /4.6 x 250mm, 5µ particle size column and PDA detector were used for the study.

The standard and sample solution of EMP were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

2.1.1. Mobile Phase selected:

Mobile phase composed of water (0.2 % OPA) and ACN (60:40 % v/v). An isocratic program was developed contributing a total run time of 20 min. The wavelength 225 nm was selected for the evaluation of the chromatogram of drugs.

Validation of the proposed method

Validation of these methods was performed as per the USP guidelines for these following parameters:

6.1.4.1. Precision:

System Precision

Prepared the standard solution as per test method and injected into the HPLC system in three replicates. It was found that all system suitability parameters are well within the limits.

Method Precision

Replicate estimation of tablet analysed by the proposed method has yielded quite consistent result indicating repeatability of method. Study showed R.S.D. less than 2.

Table No. 28: Data Showing System Precision

	Parameter	Observations	Limits
1	The % RSD of peak area response for three replicate injections of standard	1.117	NMT 2.0
2	Theoretical plates	6557.53	NLT 2000
3	Tailing factor	1.478	NMT 2.0

Table No.29: Method Precision Studies Set – I

Sr.no.	EMP	
	Assay (mg)	Assay (mg)
1	24.97	99.88



2	24.95	99.80
3	24.99	99.96
Average	24.97	99.98
SD	0.02	0.08
% RSD	0.08	0.08

Linearity & Range:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. Linearity was carried out for five levels in the range of 80% to 150%. A graph was plotted with concentration on X axis and mean peak areas on Y- axis. The R² value was found to be 0.999 for EMP. The result shows that an excellent correlation exists between concentration and mean peak areas within the concentration range. Thus the method

developed is accurate, precise, specific, & linear. Hence it can be said that, RP-HPLC is the most accurate, precise and reproducible among all methods.

Accuracy:

Accuracy of the proposed method was ascertained from the recovery studies by standard addition method. Recovery results were well within the range **99.95-99.96%**. Thus the method was found to be accurate.

Table No. 30: Result of Accuracy Studies

	EMP		
	Levels		
	80%	100%	120%
Amt added (µg/ml)	20	25	30
	20	25	30
	20	25	30
Amt taken (µg/ml)	20	25	30
	20	25	30
	20	25	30
Amt recovered (µg/ml)	19.99	24.99	29.98
	19.98	24.99	29.99
	19.99	24.98	29.99
% Recovery	99.95	99.96	99.93
	99.90	99.96	99.96
	99.99	99.92	99.96
Mean % recovery	99.95	99.96	99.95
% RSD	0.04	0.023	0.018

Robustness:

Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters, the standard solution and test solutions were injected

for each of the changes made to assess the Robustness of proposed analytical method.

Following Parameters were covered under robustness parameter.



1. Effect of variation in flow rate of mobile phase by $\pm 10\%$
2. Organic phase composition ($\pm 10\%$)
3. Change in Wavelength by ± 2 units

The results suggested all the system suitability parameters were within limits.

Specificity:

Is the ability to assess unequivocally the analyte in the presence of impurities, degradants, matrix etc. It is evaluated by injecting the blank, placebo and the control sample solution prepared as per the proposed method to check for the interference if any peak at the retention time of EMP. Thus, no interference was found at the Retention time of EMP.

CONCLUSION

From the results of the study, it can be concluded that the present RP-HPLC technique was successfully used for the estimation of the EMP in the controlled release tablet formulation. The method showed good reproducibility, it was accurate, precise, specific, reproducible and sensitive. The analysis of controlled release tablet formulation of EMP was done by the developed and validated RP-HPLC method. The RP-HPLC method was also simple, accurate, precise, reproducible and economical too. It may be adopted for routine control analysis of EMP alone in tablet formulation. No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies.

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