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Stability Indicating Method Development and Validation of Empagliflozin in Bulk and Pharmaceutical Dosage form by using RP-HPLC

Devi NK* and Sarang SD

Department of Pharmaceutical Analysis, Teegala Krishna Reddy College of Pharmcy, India

***Corresponding author:** N Kalpana Devi, Department of Pharmaceutical Analysis, Teegala Krishna Reddy College Of Pharmcy, Medbowli, Meerpet, Hyderabad 500097, TS, India, Email: kalpanadevi12@gmail.com

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Abstract

An easy reverse stage HPLC approach was created for the determination of Empagliflozin existing wholesale as well as pharmaceutical dosage forms. A Kromasil 100-C18 (250 X 4.6 mm, loaded with 5µ) column in an isocratic mode with mobile stage Acetonitrile: Phosphate barrier [Pot.dihydrogen Orthophosphate 2.725 gm + Dipottasium hydrogen orthophosphate 0.525 gm, 50:50% V/V] effluent was monitored at 301nm. The retention times were 3.333 minutes (± 0.5). The flow rate was 1ml \ min and also injection quantity was 20µl. The run time offered was 10 mins. The recommended technique was likewise verified.

Conclusion: The proposed method was straightforward, delicate as well as dependable with excellent precision and accuracy. The recommended approach specifies while estimating the industrial formulations without disturbance of excipients and also other additives. Thus, this method can be used for the regular determination of Empagliflozin in pure examples and pharmaceutical formulas.

Keywords: Empagliflozin; RP-HPLC; Stability studies; Methanol

Introduction

High Performance Liquid Chromatography

HPLC is a form of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector.

Compounds are separated by injecting a sample mixture onto the column. The different component in the mixture pass through the column at differentiates due to differences in their partition behavior between the mobile phase and the stationary phase.

High performance liquid chromatography (HPLC) is basically a highly improved form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.

All chromatographic separations, including HPLC operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation [1-3].

Types of HPLC

In general there are five techniques of HPLC. They are as follows:

Based on Polarity

Normal Phase HPLC: NP-HPLC uses polar stationary

phase and non-polar mobile phase.
Reverse Phase HPPLC: The stationary phase is nonpolar (hydrophobic), while the mobile phase is a polar.

Based on Principle of Separation

- Adsorption chromatography
- Ion exchange chromatography
- Ion pair chromatography
- Size exclusion chromatography
- Chiral chromatography

Based on Elution Technique

- Isocratic
- Gradient

Based on Scale of Preparation

- Analytical HPLC
- Preparative HPLC

Based on Type of Analysis

- Qualitative
- Quantitative.

Figure 1: HPLC Equipment.

Retention Time

The time difference between the point of injection and appearance of peak maxima is called retention time. It is the time required for 50% of component to be eluted, measured in minutes or seconds.

Retention Volume

The volume of a carrier gas required to elute 50% of the component from the column is called retention volume.

Retention volume = Retention time X Flow rate

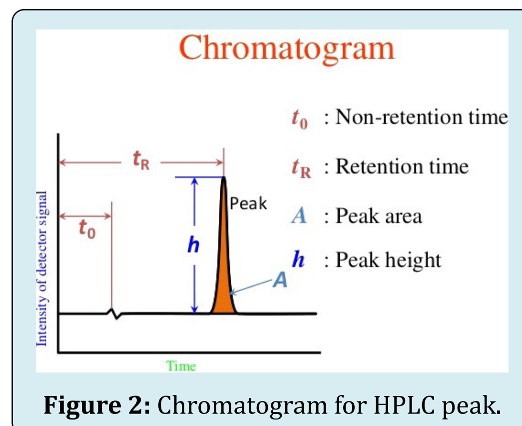

Figure 2: Chromatogram for HPLC peak.

The specific components HPLC and their working roles are reported below.
Mobile phase and reservoir
Solvent degassing system
Pump
Injector
Column
Detector

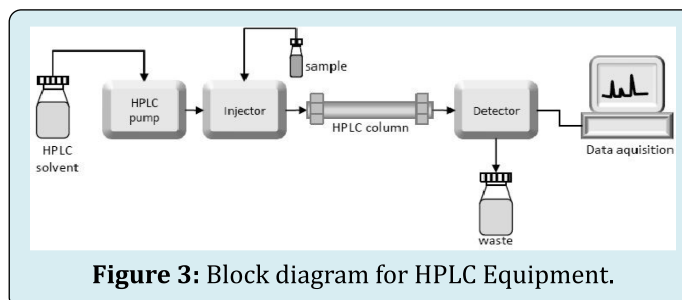

Figure 3: Block diagram for HPLC Equipment.

Principal involved in HPLC

HPLC principle is based on both Adsorption as well as Partition, depending upon the nature of stationary phase. If stationary phase is solid then it is adsorption and if stationary phase is liquid then it is partition. When a mixture of components are introduced into a HPLC Column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent, travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated [4,5].

Materials and Methods

Determination of λmax by UV spectrophotometer

Preparation of Primary stock solution: Stock solution is prepared by transferring accurately weighed 10 mg of

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Empagliflozin in 10 ml volumetric flask and dissolved in 5 ml acetonitrile and sonicate it for 15 min by using ultrasonicator. Then volume was make up to the 10 ml with acetonitrile to get the concentration of 1000 µg/ml.

Preparation of Secondary stock solution: Stock solution is prepared by transferring accurately 1ml of primary stock solution of Empagliflozin into 10 ml volumetric flask and dissolved in 5 ml acetonitrile and sonicate it for 15 min by using ultrasonicator. Then volume was make up to the 10 ml to get the concentration of 100 µg/ml.

Preparation of working standard: The standard solution of 10 µg/ ml was prepared by taking 1ml of solution from 100 µg/ ml stock solution and diluted up to the 10 ml. This solution was scanned between the range 200-400 nm in uv spectrophotometer against the acetonitrile as blank after base line correction. The optimum wavelength for Empagliflozin was found to be 301nm.

Preparation of calibration curve: Working solution was prepared from stock solution by further dilution with acetonitrile to obtained a concentration range 10, 12, 14, 16, 18, 20 µg/ml, respectively. These solutions were scanned from the range 301nm and calibration curve was obtained between concentrations of 10-20 µg/ml.

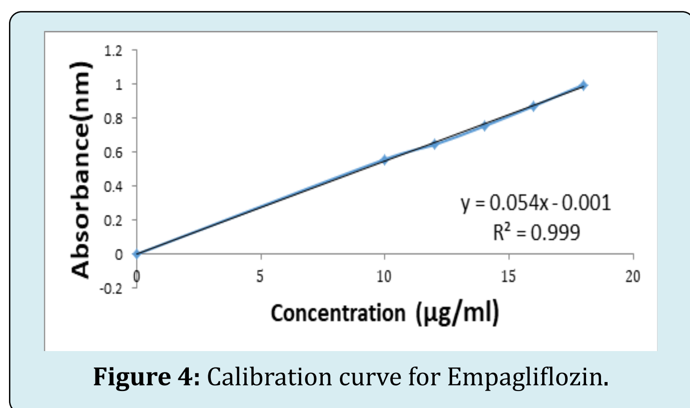

Figure 4: Calibration curve for Empagliflozin.

S.No	Concentration	Absorbance
1	0	0
2	10	0.559
3	12	0.645
4	14	0.754
5	16	0.873
6	18	0.998

Table 1: Calibration curve values for Empagliflozin.

Preparation of Mobile Phase

Accurately measured 700 ml (70%) of acetonitrile and 300 ml of potassium dihydrogen ortho phosphate (30%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µm filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water and Acetonitrile and water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and potassium dihydrogen ortho phosphate in proportion of 70:30 v/v respectively.

Optimization of Column

The method was performed with various C18 columns like ODS column, Xterra and kromasil column.) Kromasil 100-C18 (250 X 4.6 mm, loaded with 5µ) column was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow.

Method Optimization

Preliminary chromatographic conditions:
Stationary stage: Kromasil 100-C18 (250 X 4.6 mm, loaded with 5µ) column
Mobile stage: Acetonitrile: Potassium dihydrogen orthophoshate (70:30 v/v).
Flow rate: 1 ml/ minutes.
Detector wavelength: 301 nm.
Column temperature: Ambient

Sample Blank

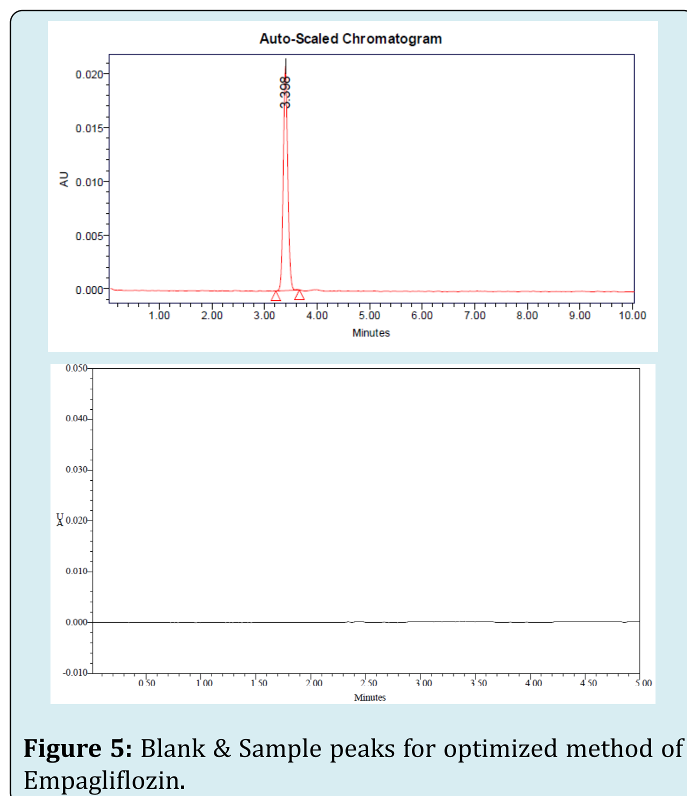

Figure 5: Blank & Sample peaks for optimized method of Empagliflozin.

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System Suitability

Selecting a proper system suitability testing mixture is essential to check the specifications of a chromatographic method. System suitability testing methods are the acceptance criteria that must be met prior to sample analysis. Some parameters that can be checked using system suitability are resolution, retention time , plate number etc.

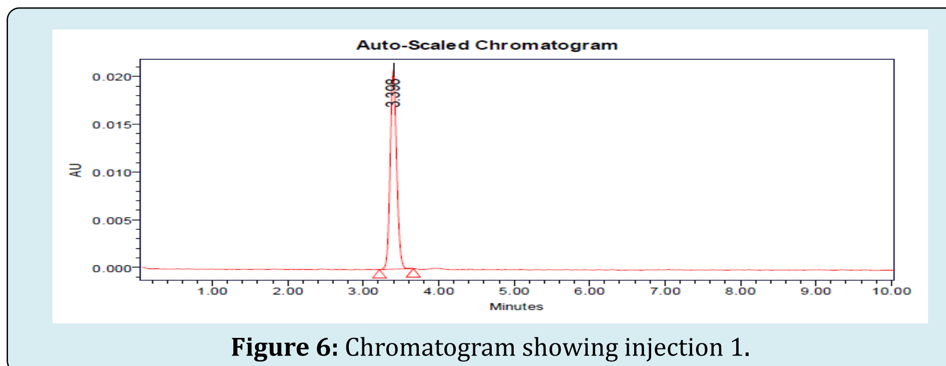

Figure 6: Chromatogram showing injection 1.

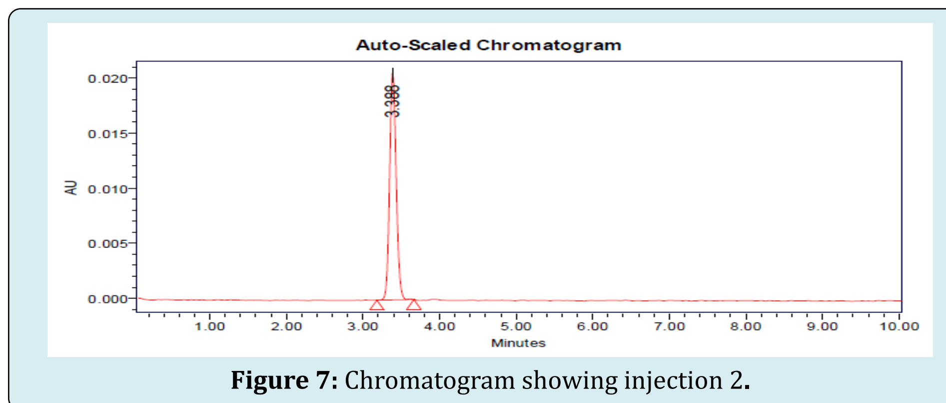

Figure 7: Chromatogram showing injection 2**.**

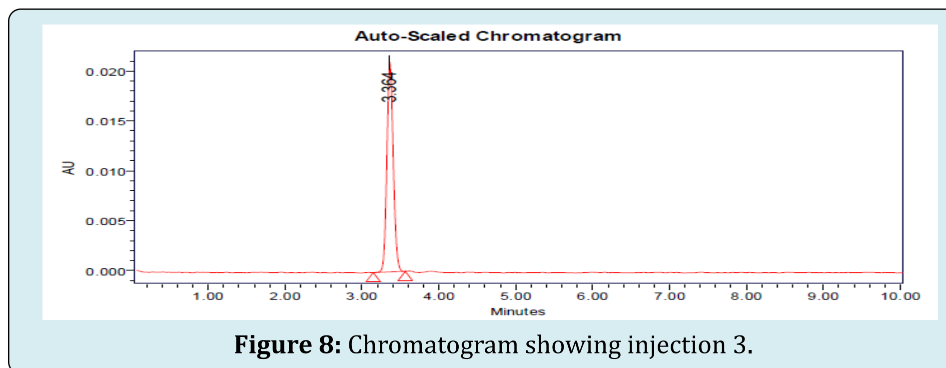

Figure 8: Chromatogram showing injection 3.

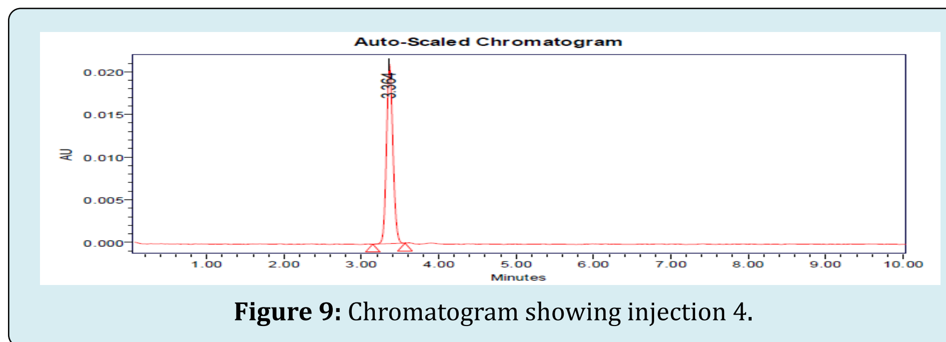

Figure 9: Chromatogram showing injection 4.

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S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Empagliflozin	3.398	145965	32653	8475	1.78
2	Empagliflozin	3.324	146857	32785	8495	1.79
3	Empagliflozin	3.349	145985	32598	8492	1.80
4	Empagliflozin	3.388	146697	32695	8463	1.76
5	Empagliflozin	3.364	145982	32675	8458	1.77
Mean			146380.25			
Std. Dev.			462.762			
% RSD			0.316137			

Table 2: Showing values for Empagliflozin AUC.

Assay (Standard)

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	EMPAGLIFLOZIN	3.379	145857	32654	8546	1.76
2	EMPAGLIFLOZIN	3.303	145874	32587	8574	1.77
3	EMPAGLIFLOZIN	3.322	145685	32564	8759	1.76
4	EMPAGLIFLOZIN	3.327	145876	32854	8598	1.76

Table 3: Peak results for Assay standard.

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Empagliflozin	3.310	145	32658	1.78	8457	1
2	Empagliflozin	3.398	146874	32547	1.77	8495	2
3	Empagliflozin	3.388	146524	32658	1.78	8475	3

Table 4: Peak results for Assay sample.

The % purity of Empagliflozin pharmaceutical dosage form was found to be 99.57%

Linearity

Concentration □g/ml	Average Peak Area
60	85784
80	112564
100	139867
120	165248
140	189586

Table 5: Linearity values for Empagliflozin.

Linearity Plot

The plot of Concentration (x) versus the Average Peak Area (y) data of DRUG is a straight Line. Y = mx + c (Slope (m) = 1358k, Intercept (c) = 2288, Correlation Coefficient (r) = 0.99)

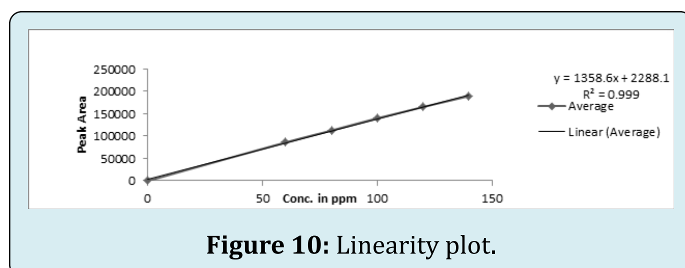

Figure 10: Linearity plot.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD

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S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Empagliflozin	3.397	145865	32652	8547	1.78
2	Empagliflozin	3.390	145874	32541	8498	1.78
3	Empagliflozin	3.384	145842	32564	8547	1.77
4	Empagliflozin	3.378	145869	32548	8572	1.77
5	Empagliflozin	3.364	145265	32569	8569	1.78
Mean			145743			
Std.dev			267.4911			
%RSD			0.183536			

Table 6: Precision values for Empagliflozin.

Limit of Detection for Empagliflozin

The detection limit of an individual analytical procedure is the lowest amount of analyte In a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result

LOD =1.5µg/ml

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

Where
σ = Standard deviation of the response
S = Slope of the calibration curve

Result

LOQ = 4.5µg/ml

Stability Studies

Acid Degradation Studies

To 1 ml of Empagliflozin stock, 1 ml of 2N HCl was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N NaOH and makeup to final volume to obtain (100µg/mL) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 10µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation Studies

To 1 ml of stock solution of Empagliflozin 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N HCl and makeup to final volume to obtain (100µg/mL) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. The sample of 10µl was injected into the system, and the chromatograms were recorded to an assessment of sample stability.

Oxidation Degradation Studies

To 1 ml of stock solution of Empagliflozin 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solution was kept for 30 min at 60ºC. For HPLC study, the resultant solution was diluted to obtain (100µg/mL) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 10µl solution was injected into the system, and the chromatograms were recorded to assess the stability of the sample [6-10].

Photo Degradation Studies

The photo stability of the empagliflozin was studied by exposing the stock solution to UV light for 1day or 200Watt-hours/m2 in photo stability chamber [11-18]. For HPLC study, the resultant solution was diluted to obtain (100µg/mL) solution and filtered with 0.45µm membrane filter. A sample of 10µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.

$$\%\ Degradation = \frac{Sample\ Area}{Standard\ Area} \times 100$$

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S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	145867.00	0	100%	100%
2	Acidic	112259.24	0.345	99.65	100%
3	Basic	124687.11	0.684	99.31	100%
4	Oxidative	133803.79	0.489	99.51	100%
5	Thermal	136341.88	0.765	99.23	100%
6	Photolytic	134956.14	0.233	99.76	100%

Table 7: Consolidate values for Stability studies of Empagliflozin.

Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Empagliflozin in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Empagliflozin was found to be soluble in organic solvents such as ethanol, DMSO, and acetonitrile, dimethyl formaldehyde and soluble in water and it is freely soluble in dichloromethane, sparingly soluble in ethyl alcohol. Acetonitrile and potassium dihydrogen ortho phosphate (70:30 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of empagliflozin in bulk drug and in Pharmaceutical dosage forms

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