A NOVEL RP-HPLC METHOD FOR THE ESTIMATION OF EMPAGLIFLOZIN AND LINAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORMULATIONS

Devika.G S.[1]*, Manokar .P.[2] Rameshpetchi Rajendran, [3] T. Delphina[4], A.Kavidha[5]

ADDRESS: [1,2] Department of Pharmaceutical Analysis
Cherraan's college of Pharmacy, 521,
Siruvani main road, Coimbatore-641039, Tamilnadu, India.

[3,4]Department of Pharmacology, Cherraan's college of Pharmacy, 521,
Siruvani main road, Coimbatore-641039, Tamilnadu, India.

[5]Department of Pharmacognosy, Cherraan's college of Pharmacy, 521,
Siruvani main road, Coimbatore-641039, Tamilnadu, India.

***For correspondence**
Tel: 7207718315, 08143802251
E-mail address: devikasubramaniyan@gmail.com

ABSTRACT
A selective and sensitive reverse phase high performance liquid chromatography (RP-HPLC) has been developed for the separation and quantification of and Empagliflozin and Linagliptin in tablet dosage form and validated. Chromatographic separation of the two drugs was performed on a Purospher BDS C18 column (250 mm× 4.6 mm id, 5µm particle size). The mobile phase used was a mixture of Methanol: Water 47:53 % V/V (pH adjusted to 4.5 using Ortho phosphoric acid and Triethylamine. Detection was performed at 247nm and sharp peaks were obtained for Empagliflozin and Linagliptin retention times of 2.8±0.01 min and.6.3±0.01 min respectively. The calibration curve was linear in the concentration range 10-60µg/ml for Empagliflozin 5-30µg/ml for Linagliptin; the correlation coefficients were 0.9999 and 0.9998, respectively. The optimized method showed good performance in terms of specificity, linearity, detection and quantitation limits, precision and accuracy in accordance with the International Conference on Harmonization (ICH) Q2 (R1) guidelines. This assay was demonstrated to be applicable for routine quantitation of Empagliflozin and Linagliptin in tablet dosage form.

Key Words: Empagliflozin, Linagliptin RP-HPLC and ICH Guidelines.

1.INTRODUCTION

Empagliflozin (EMP) Figure.1 is used as a sodium glucose co-transporter-2 (SGLT-2) inhibitor to improve glycemic control in adult patients with type 2 diabetes. This drug selectively blocks the sodium–glucose reabsorption cotransporter 2 (SGLT2) in addition to reducing cardiovascular risks associated with type 2 diabetes[1-6] .Chemically EMPA is 1-chloro-4-(glucopyranos-1-yl)-2-(4-(tetrahydrofuran-3-yloxy)benzyl)benzene and having empirical formula is $C_{23}H_{27}ClO_7$ with molecular weight 450.91 g/mole .

Linagliptin (LIN) Figure 1. is having competitive, reversible DPP-4 inhibitory action which is responsible for DPP-4 breakdown reduction of GLP-1 and glucose-dependant insulinotropic polypeptide (GIP). From beta cells of the pancreas, GLP-1 and GIP stimulate the release of insulin during inhibiting release of glucagon from pancreatic beta cells.[7-10] These effects together reduce the breakdown of glycogen in the liver and increase insulin release in response to glucose. Chemically LINA is (R)-8-(3-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione and having empirical formula is $C_{25}H_{28}N_8O_2$ with molecular weight 472.5422 g/mole. Empagliflozin and linagliptin combination is used together with proper diet and exercise to treat high blood sugar levels caused by type 2 diabetes. It is also used to lower the risk of death in patients with type 2 diabetes and heart or blood vessel disease. Empagliflozin works in the kidneys to prevent the absorption of glucose (blood sugar). Linagliptin helps to control blood sugar levels by increasing substances in the body that make the pancreas release more insulin. It also signals the liver to stop producing sugar (glucose) when there is too much sugar in the blood.[3-6]

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17-21

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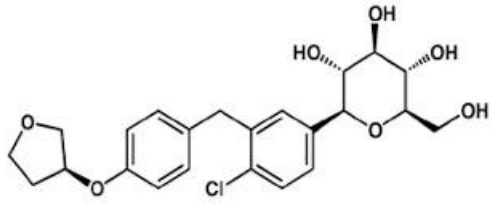
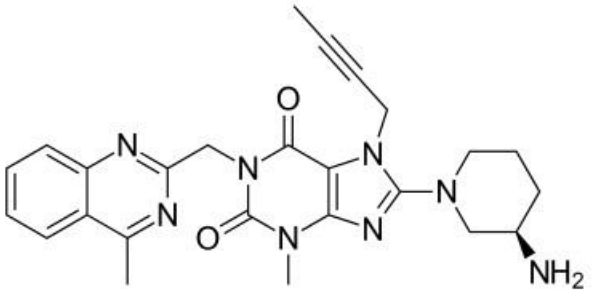
HPLC methods are available with the combination of above-cited drugs17-21, with lower linearity range and or having longer retention times. Therefore, an attempt was made to develop a novel, simple, accurate and precise method for the simultaneous estimation of Empagliflozin and Linagliptin in combined pharmaceutical dosage form. This manuscript describes the development and validation of RP-HPLC method for simultaneous estimation of these drugs as per ICH guidelines.

Figure1.Structure of Empagliflozin and Linagliptin



EMPAGLIFLOZIN



LINAGLIPTIN

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2. EXPERIMENTATION:

2.1 Equipment:

Chromatographic separation was performed on Waters HPLC system consist of model 2695 having PDA detector and Rheodyne injector with 20µl loop volume. Waters Empower software was applied for data collecting and processing.

2.2. Reagents and chemicals:

Acetonitrile, Methanol and water of HPLC grade were procured from Rankem lab ltd. working standard of Empagliflozin was provided by Orchid pharmaceuticals Chennai and Empagliflozin and Linagliptin was provided by Emcure Pharmaceutical Ltd, Pune. Tri ethyl amine and orthophosphoricacid were A.R grade from Merck chemicals Mumbai, India. Tablets Glyxambi were purchased from Indian market, containing a 10mg of Empagliflozin and 5 mg of Linagliptin per tablet.

2.3 Optimized chromatographic Condition:

A Purospher BDS C18 column (250 mm× 4.6 mm id, 5µm particle size) column was used as the stationary phase. The mobile phase used was a mixture of Methanol: Water 47:53 % V/V (pH adjusted to 4.5 using Ortho phosphoric acid and Triethylamine. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1 ml/min. Detection was performed at 247nm and sharp peaks were obtained for Empagliflozin and Linagliptin retention times of 2.8±0.01 min and.6.3±0.01 min respectively. The injection volumes of samples and standard were 10µl.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 2695 model.
Temperature	:	25ºC
Column	:	Purospher BDS C18 (4.6×250mm, 5µ)
pH	:	4.5
Mobile phase	:	Methanol: Water 47:53 % V/V (pH adjusted to 4.5 using Ortho phosphoric acid and Triethylamine
Flow rate	:	1ml/min
Wavelength	:	247 nm
Injection volume	:	10 µl
Run time	:	11 min

Standard preparation:

2.4 Preparation of standard solution:

10 mg of Empagliflozin and 10 mg of Linagliptin were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicated to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml. (Stock solution) . The working standard solutions were prepared and further diluted in mobile phase to and Empagliflozin and Linagliptin contain a mixture of in over the linearity ranges from 10-60 µg/ml and 5-30 µg/ml.

2.5 Analysis of Tablet formulation:

Twenty tablets Glyxambi ^{Rx} were weighed and finely powdered. A quantity of powder equivalent to 10 mg of Empagliflozin and 5mg of Linagliptin was weighed and transferred to a 10 ml volumetric standard flask and added 10 ml of mobile phase. The sample was kept in an ultrasonic bath for 20 min and further diluted using mobile phase to get 1000µg/ml of Empagliflozin and 500µg/ml of Linagliptin. Then it is filtered through 0.22µ membrane filter paper. Then this primary sample solution were further diluted to get the concentration of 30 µg/ml Empagliflozin 15 µg/ml of Linagliptin sample solutions.

20µl of this solution was injected in to HPLC system and chromatograms were recorded. Concentrations of Empagliflozin and Linagliptin in the tablet formulation were calculated by

comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in Table1.

Table1: Table for Assay

Drug Name	Amount present mg	Amount found* (mg/tab)	% label claim*
Empagliflozin	10	9.99	99.99
Linagliptin	5	4.99	99.99

3. RESULTS AND DISCUSSION:

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in Pharmaceutical industries. The chromatograms sample and standard solution of Empagliflozin and Linagliptin were shown in (Figure.1) and (Figure.2). There was clear resolution between Empagliflozin and Linagliptin with retention time of 2.358 and 4.542 minutes respectively.

Figure 1: Typical Chromatogram of standard solution of Empagliflozin and Linagliptin

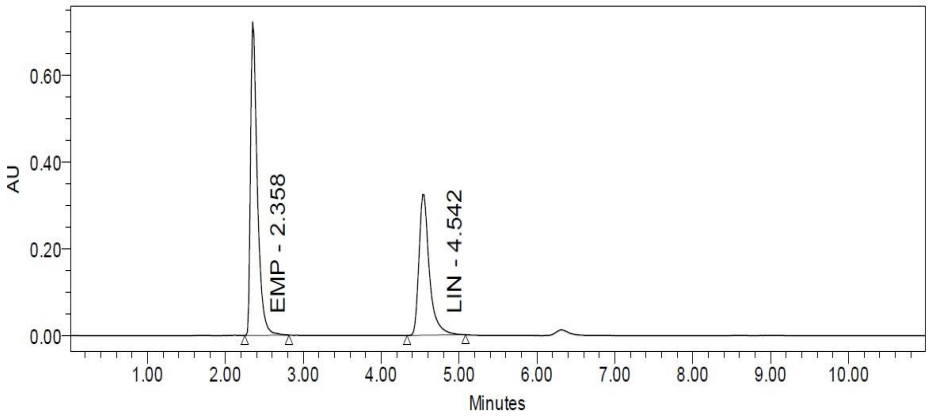
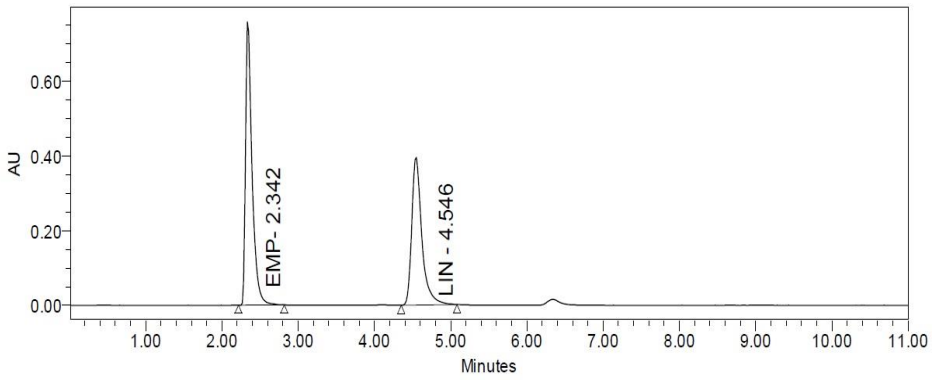


Figure 2: Typical Chromatogram of sample solution of Empagliflozin and Linagliptin



3.1. VALIDATION OF THE METHOD:

3.1.1. System suitability:

The column efficiency, resolution and peak symmetry were calculated for the standard solutions. Table.2. The RSD of system suitability factors was satisfactory that is less than 2% and resolution was satisfactory. The peaks obtained for EMP and LIN were sharp and have clear base line separation.

Table 2: System suitability parameters for EMP and LIN

Parameter	**Empagliflozin** ±RSD,%	**Linagliptin** ±RSD,%
Retention time,min	2.3±0.21	4.5±0.74
Tailing factor	0.73 ±0.15	1.20±0.81
Theoretical plates	7256.13±0.94	9156.21±0.45
Capacity factor	0.25±0.32	0.96±0.12
Resolution	7.23±0.55	

3.1.2. Linearity:

The response for the detector was determined to be linear over the range of 10-60µg/ml for Empagliflozin (10,20,30,40,50,60) and 5-30µg/ml (5,10,15,20,25,30) for Linagliptin. Each of this concentration was injected in six times to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level.(Figure 3& Figure 4.) The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. The results show that an excellent correlation exits between response factor and concentration of drugs within the concentration range indicated above. (Table 3)

Figure.3. calibration graph for Empagliflozin

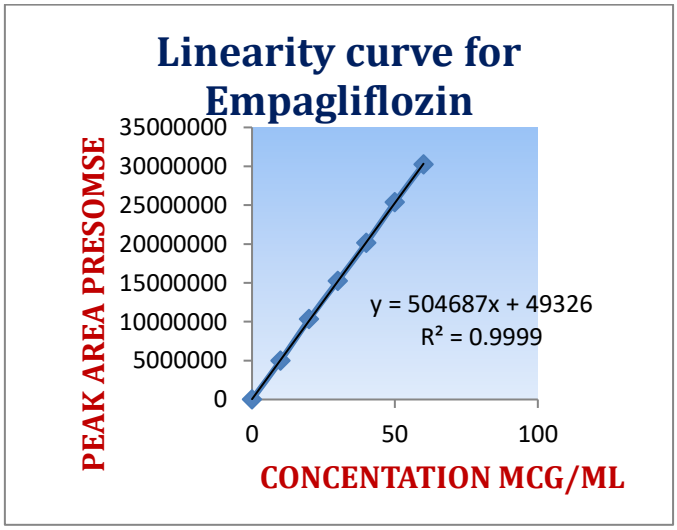


Figure 4. Calibration graph for Linagliptin

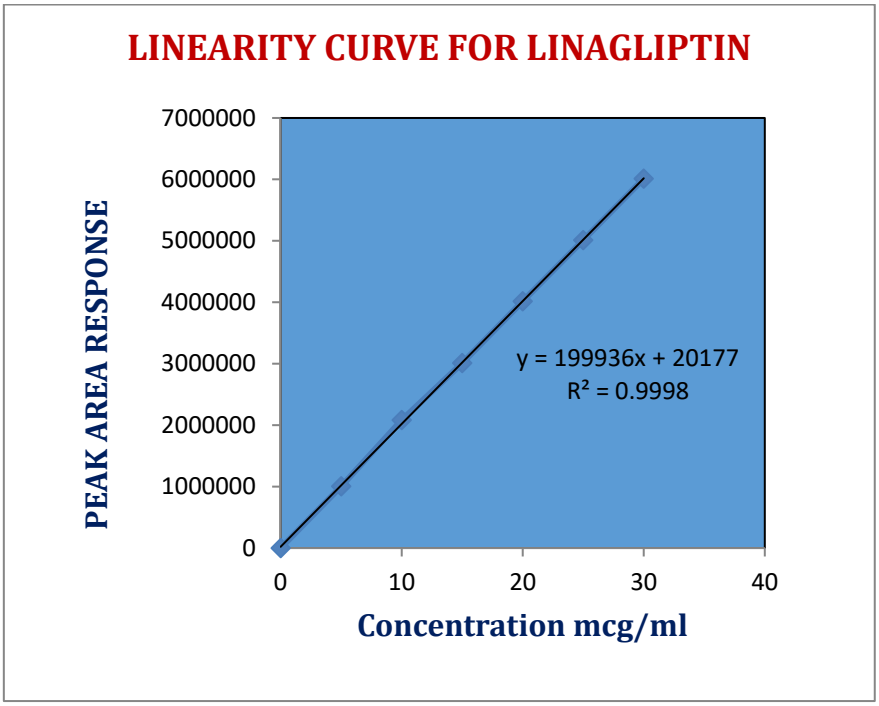


Table3: Summary of analytical method validation

S.No	Parameters	Acceptance criteria	Empagliflozin	Linagliptin
1	Linearity	r^2=0.995 to1.0	0.9998	0.9999
2	Specificity	No interference with placebo	specific	specific
3	Accuracy(Recovery studies)	Recovery 98.0-101.0%	99.98%	100.01%
4	**Precision**			
	Intraday	RSD NMT 2.0%	0.123	0.452
	Interday	RSD NMT 2.0%	0.952	0.215
5	**Robustness**			
	Change inflow rate	NMT±1%	0.3%	0.3%
	Change in mobile phase ratio	NMT±1%	0.2%	0.2%
	Change in p^H	NMT±1%	0.2%	0.%
6	Limit of detection µg/ml	----------	0.3µg/ml	0.5µg/ml
	Limit of Quantification µg/ml	----------	0.9µg/ml	1.5µg/ml

3.1.3. Precision and Accuracy:

Recovery studies were carried out by applying the standard addition method. A known amount of standard Empagliflozin and Linagliptin corresponding to 80%, 100%, and 120% of the label claim was added to pre analyze sample of tablet dosage form separately. The recovery studies were carried out six times at each level of recovery. From the data obtained, recoveries of standard drugs were found to be accurate (Table.3). The %RSD of interday and intraday precision obtained was less than2%for both the drugs. The intraday and interday precision of Empagliflozin was 0.123 and 0.952 and Linagliptin was 0.452and 0.215 respectively. From the data obtained, the developed HPLC method was found to be precise and accurate.

3.1.4 Specificity of the method:

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 10 µg/ml was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Empagliflozin and Linagliptin from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

3.1.5 LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 ∂/S and 10 ∂/S, respectively as per ICH guidelines, where ∂ is the standard deviation of the response (*y*-intercept) and *S* is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).The LOD for Empagliflozin and Linagliptin was found to be 0.3µg/ml and 0.5µg/ml, respectively. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10).The LOQ was 0.9µg/ml and 1.5µg/ml for Empagliflozin and Linagliptin respectively.(Table3)

3.1.6 Ruggedness:

The ruggedness of an analytical method is the degree of reproducibility of the test results obtained by the samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, and different days. The %RSD of below 2% indicated that the method was accurate with high precision.

3.1.7 **Robustness:**

Robustness is a measure of the performance of a method when small deliberate changes are made to the conditions of the method. The results of the robustness study are summarized in Table 3**.**

4.0 Conclusion:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Empagliflozin and Linagliptin 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. Methanol: Water 47:53 % V/V (pH adjusted to 4.5 using Ortho phosphoric acid and Triethylamine)was chosen as the mobile phase. The solvent system used in this method was economical. High percentage recovery of drug shows the method is free from inference of excipients present in the formulation

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectro photometric methods. This method can be used for the routine determination of Empagliflozin and Linagliptin in bulk drug and in Pharmaceutical dosage forms.

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