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SIMULTANEOUS ESTIMATION AND VALIDATION OF EMPAGLIFLOZIN AND LINAGLIPTIN BULK DRUG AND IT'S DOSAGE FORM BY RP-HPLC

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ABSTRACT

A new method was established for simultaneous estimation of Empagliflozin and Linagliptin by RP-HPLC. The chromatographic conditions were successfully developed for the separation of Empagliflozin and Linagliptin by using Agilent C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid),detection wavelength was 254 nm.

The % purity of Empagliflozin and Linagliptin was found to be 99.87% and 100.27% respectively. The linearity study of Empagliflozin and Linagliptin was found in concentration range of 10µg-50µg and 20µg-100µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 1.2and 2.0, % RSD for intermediate precision was 1.1 and 1.1 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Empagliflozin and Linagliptin in API and Pharmaceutical dosage form.

Key Words: Empagliflozin, Linagliptin, RP-HPLC method, Linearity

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INTRODUCTION

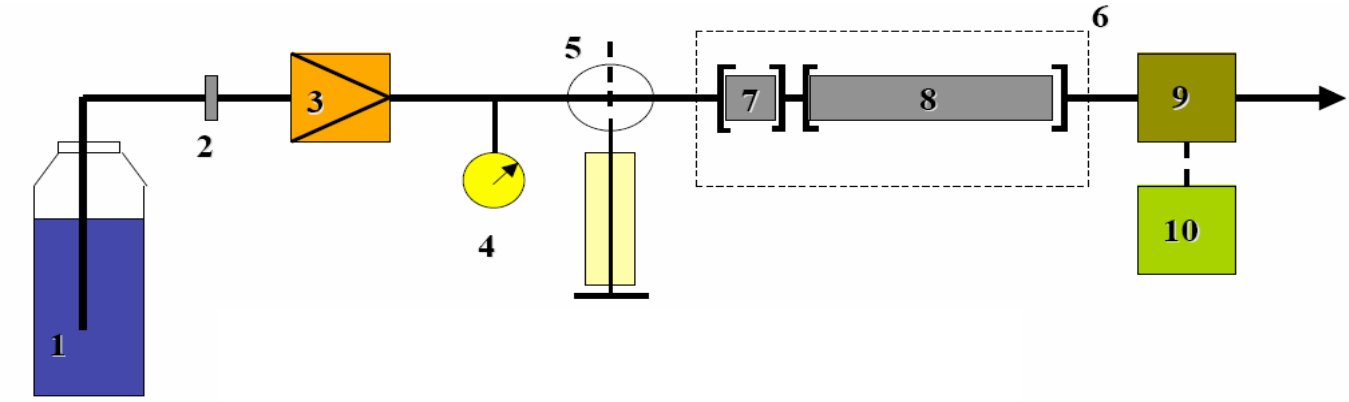
The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC.

The early 1970s saw a tremendous leap in Technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher Pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid.

Today, compounds in trace concentrations as low as *parts per trillion* (ppt) may easily be identified. HPLC (Fig-1) can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals.

System



1 = eluent reservoir
2 = filter
3 = high pressure pump with pulse dampener
4 = pressure gauge
5 = sample injection valve with syringe
6 = column oven
7 = guard column
8 = column
9 = detector
10 = recorder (integrator, PC etc.)

Figure-1 High-Performance Liquid Chromatography [HPLC]

Method validation can be defined as per ICH "Establishing documented evidence which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics (1-4).

Empagliflozin((2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl] methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol) is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), which is found almost exclusively in the proximal tubules of nephronic components in the kidneys. SGLT-2 accounts for about 90 percent of glucose reabsorption into the blood. Blocking SGLT-2 reduces blood glucose by blocking glucose reabsorptionin the kidney and thereby excreting glucose (i.e., blood sugar) via the urine

Linagliptin(8-[(3*R*)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1*H*-purine-2,6-dione) is an inhibitor of DPP-4, an enzyme that degrades the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP). Both GLP-1 and GIP increase insulin biosynthesis and secretion from pancreatic beta cells in the presence of normal and elevated blood glucose levels.

GLP-1 also reduces glucagon secretion from pancreatic alpha cells, resulting in a reduction in hepatic glucose output. Thus, linagliptin stimulates the release of insulin in a glucose-dependent manner and decreases the levels of glucagon in the circulation.

Literature review reveals that there is no analytical method reported for the analysis of Empagliflozin and Linagliptin by simultaneous estimation by RP-HPLC. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form.

Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Empagliflozin and Linagliptin in pharmaceutical dosage form (5-10).

MATERIALS AND METHOD

Assay

Preparation of phosphate buffer

2.95 grams of $KH_2PO_4$and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation

Mobile phase was used as the diluent.

Preparation of the individual Empagliflozin standard preparation

10 mg of Empagliflozin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 0.2 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. Final concentration is 20µg/ml.

Preparation of the individual Linagliptin standard preparation

10 mg of Linagliptin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 0.4 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent. Final concentration is 40 µg/ml.

Preparation of the Empagliflozin and Linagliptin standard and sample solution

Sample solution preparation:

An equivalent tablet power such that 10 mg of Empagliflozin and 20 mg Linagliptin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10 mg Empagliflozin and 20 mg Linagliptin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluents.

Procedure

10µL of the blank, standard and sample were injected into the chromatographic system and areas for the Empagliflozin and Linagliptin the peaks were used for calculating the % assay by using the formulae.

Assay calculation

$$Assay\ \% = \frac{sample\ area}{Standard\ area} \times \frac{dilution\ sample}{dilution\ of\ standard} \times \frac{P}{100} \times \frac{Avg.wt}{Lc} \times 100$$

Where:

Avg.wt = average weight of tablets

P= Percentage purity of working standard

LC= Label Claim of Empagliflozin mg/ml.

Analytical method validation

Validation parameters such as Specificity, Linearity, Range, Accuracy, Precision, Repeatability, Intermediate Precision, Detection Limit, Quantization Limit and Robustness were studied (11).

RESULTS AND DISCUSSION

The present investigation reported in the thesis was aimed to develop a new method development and validation for the simultaneous estimation of Empagliflozin and Linagliptin by RP-HPLC method. Literature reveals that there are no analytical methods reported for the simultaneous estimation Empagliflozin and Linagliptin by RP-HPLC method. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Empagliflozin and Linagliptin in pharmaceutical dosage form.

Method Development

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The overlay spectrum of Empagliflozin and Linagliptin was obtained and the isobestic point of Empagliflozin and Linagliptin showed absorbance's maxima at 254 nm.

Assay calculation for Empagliflozin and Linagliptin

The assay study was performed for the Empagliflozin and Linagliptin. Each three injections of sample and standard were injected into chromatographic system. The chromatograms are shown in Fig-2 and 3.

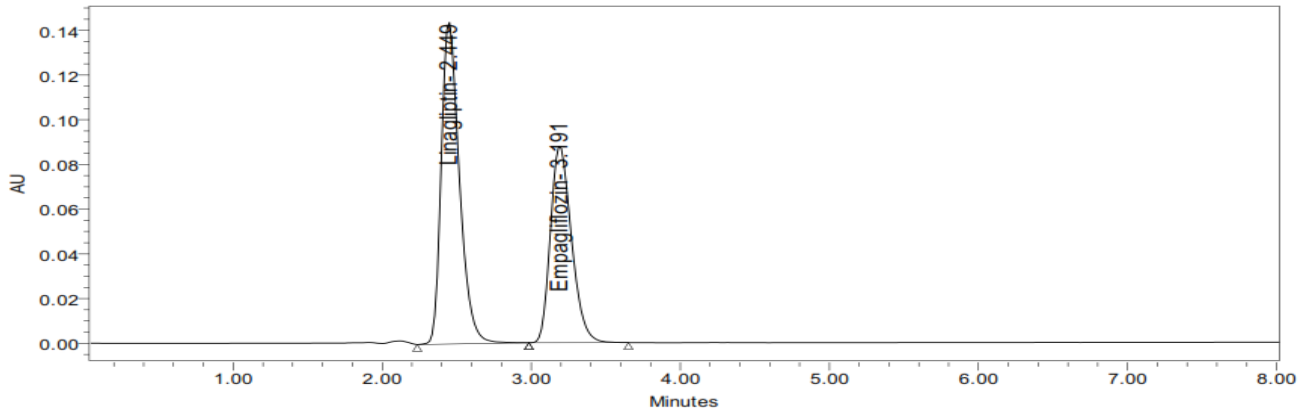


Figure- 2 Chromatogram showing standard injection

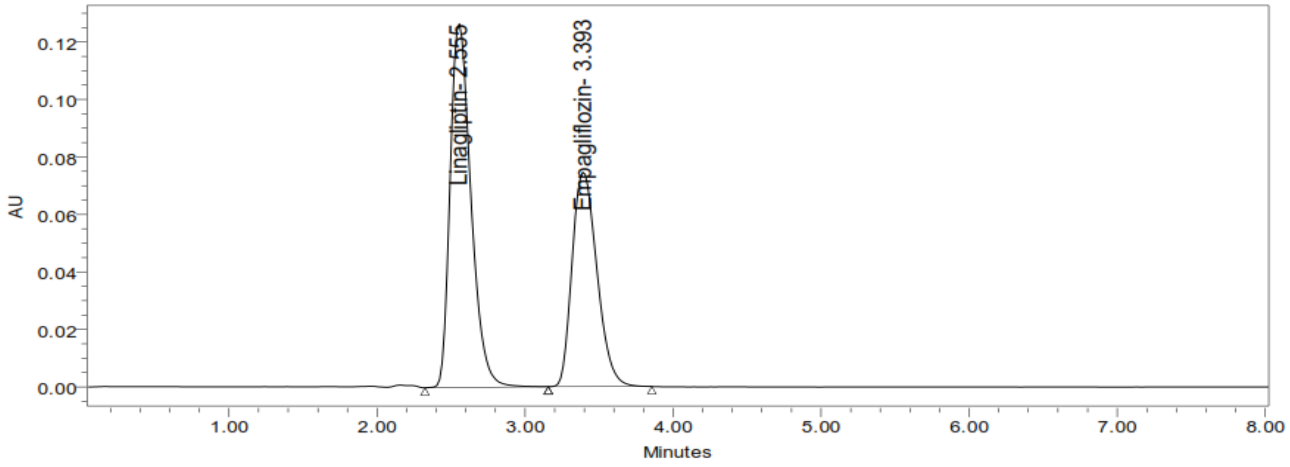


Figure-3 Chromatogram showing sample injection

The specificity test was performed for Empagliflozin and Linagliptin. It was found that there was no interference of impurities in retention time of analytical peak.

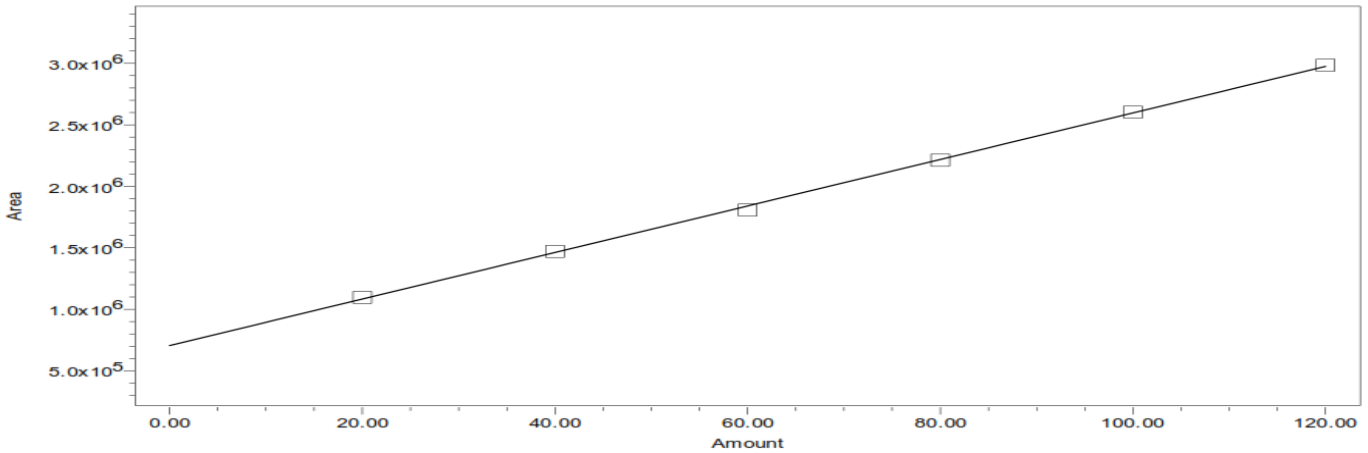
The linearity study was performed for the concentration of 10 ppm to 50 ppm Empagliflozin and 20 ppm to 100 ppm Linagliptin level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. The results are tabulated in Table-1 and 2. Calibration graph for EMPA and LINA are shown in Fig-4 and 5.

Table-1 Linearity Results for Empagliflozin

SI.NO	Linearity level	Concentration	Area
1	I	20 ppm	2000516
2	II	40ppm	2420416
3	III	60ppm	2905813
4	IV	80ppm	3270034
5	V	100ppm	3671138
Correlation Coefficient			0.999

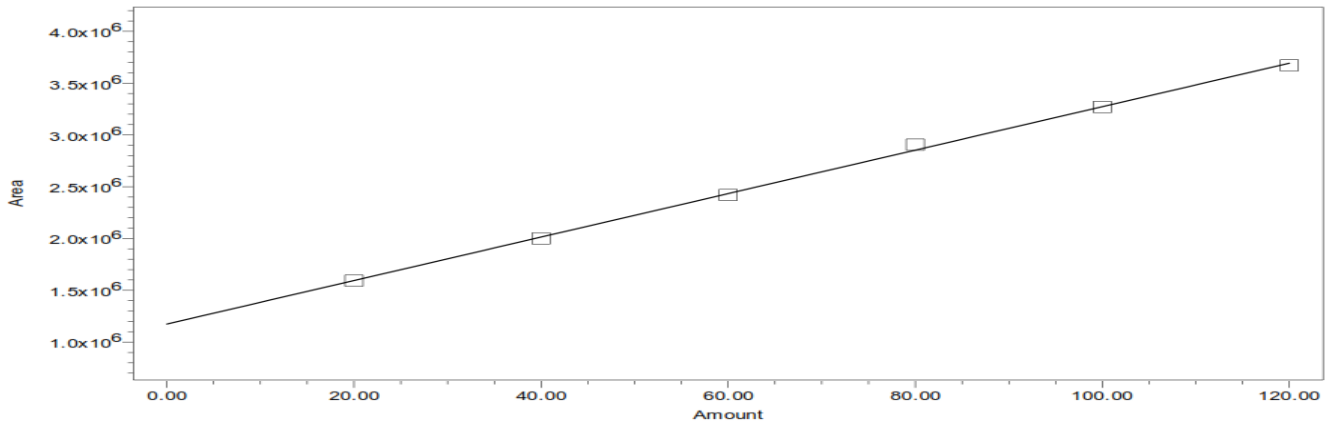
Table-2 Linearity Results for Linagliptin

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	1097254
2	II	20 ppm	1471214
3	III	30 ppm	1807502
4	IV	400 ppm	2212400
5	V	50 ppm	2603215
Correlation Coefficient			0.999



Empagliflozin $r^2 = 0.999$

Figure-4 Showing calibration graph for Empagliflozin



Linagliptinr^2 = 0.999

Figure- 5 Showing calibration graph for Linagliptin

The linearity study was performed for concentration range of 10µg Empagliflozine -50µg Linagliptin and 20µg of Empagliflozine and 100 µg Linagliptin and the correlation coefficient was found to be 0.999 and 0.999.(NLT 0.999)respectively.

The accuracy study was performed for 50%, 100% and 150 % for Empagliflozin and Linagliptin. Each level was injected in triplicate into chromatographic system. The area of each level was used for c alculation of % recovery, results are tabulated in Table-3 and 4.

Table-3 Showing accuracy results for Empagliflozin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1193706	5	4.96	99.91%	99.56%
100%	1601741	10	9.98	99.18%	
150%	2243270	15	15.02	99.60%	

Table-4 Showing accuracy results for Linagliptin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	484733	0.5	0.99	99.53%	99.47%
100%	967998	1.0	1.05	99.38%	
150%	145437	1.5	1.495	99.52%	

The accuracy study was performed for % recovery of Empagliflozin and Linagliptin. The % recovery was found to be 99.56% and 99.47% respectively (NLT 98% and NMT 102%)

Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision/Ruggedness

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

The intermediate precision was performed for %RSD of Empagliflozin and Linagliptin was found to be 1.1 and 1.1 respectively (NMT 2).

The LOD was performed for Empagliflozin and Linagliptin was found to be 2.17and 0.0372 respectively.

The LOQ was performed for Empagliflozin and Linagliptin was found to be 6.60 and 0.112 respectively.

CONCLUSION

A new method was established for simultaneous estimation of Empagliflozin and Linagliptin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Empagliflozin and Linagliptin by using Agilent C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid),detection wavelength was 254 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 3.345 mins and 2.523 mins. The % purity of Empagliflozin and Linagliptin was found to be 99.87% and 100.27% respectively. The system suitability parameters for Empagliflozin and Linagliptin such as theoretical plates and tailing factor were found to be 2885, 1.27 and 2235 and 1.3, the resolution was found to be 3.4. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Empagliflozin and Linagliptin was found in concentration range of 10µg-50µg and 20µg-100µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 1.2and 2.0, % RSD for intermediate precision was 1.1 and 1.1 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Empagliflozin and Linagliptin in API and Pharmaceutical dosage form.

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