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A Review on Validated RP-HPLC Method Development and Validation for the Simultaneous Estimation of Empagliflozin and Linagliptin in Bulk Drug and Pharmaceutical Dosage Form

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ABSTRACT

A novel, simple, perfect and express back up point distinguished piece Liquid Chromatography (RP-HPLC) procedure has been residential and validated for the determination of Empagliflozin and Linagliptin in bulkiness and dosage amount form. Incline elution chromatography has been urban on C18 stake (5µm, 4.6mm* 250mm) with a mobile point consist of pH 4 Phosphate memory and Methanol (37:63) with a flood quotient of 1ml/min at detection of 229 nm wavelength. The custody time for Empagliflozin and Linagliptin was set up to be 3.07 and 4.37 min, respectively. Chromatography parameters were validated as apiece ICH guidelines and the approach tin be applicable for repetitive quantitative chemical analysis of drugs in pooled measure form.

INTRODUCTION:

For the treatment of Type 2 diabetes mellitus, Empagliflozin and Linagliptin are used in combined dosage form. Empagliflozin is a drug belongs to Gliflozin class. Empagliflozin is an inhibitor of sodium glucose co-transporter-2 (SGLT-2) and causes sugar in blood to be excreted by kidneys and eliminated in urine. While, Linagliptin is dipeptidyl peptidase-4 (DPP-4) inhibitor, and it increases the amount of insulin released by the body and decreases the amount of sugar made by the body. The Recommended dose of Empagliflozin and Linagliptin is 25mg and 5mg respectively.

The chemical name of Empagliflozin is (2S,3R,4R,5S,6R)-2-[4-Chloro-3-[(4-(3S)-Oxolan-3-yl] Oxyphenyl] Methyl] Phenyl-1] (-6-Hydroxymethyl)Oxane-3,4,5-Triol. It is an orally administered sodium glucose co-transporter 2 receptor (SGLT-2) inhibitor. Sodium glucose co-transporter (2) is situated in the proximal tubule of the nephron in kidneys which lowers the blood glucose level by blocking the glucose reabsorption in kidneys and excretes excess glucose through urine. (3)

The IUPAC nomenclature for Linagliptin (LINA) is 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methyl quinazolin-2-yl)methyl] -2,3,6,7-tetrahydro-1H-purine-2,6-dione. 4 LINA is an antidiabetic drug as it inhibits DPP-4 enzyme. This enzyme is responsible for the degradation of incretin hormones glucagon like peptide (GLP-1) and glucose dependent insulin tropic polypeptide (GLP-11-13). Both GLP-1 and GIP-1 increases insulin biosynthesis and pancreatic beta cells secretions, it causes elevation in blood glucose levels. GLP-1 reduces blood glucagon level which is secreted from pancreatic alpha cells. (5,6)

The reason behind the combination of EMPA and LINA is that, because EMPA is a SGLT2 inhibitor, and LINA is a DPP-4 inhibitor. SGLT2 is a protein that facilitates the reabsorption of glucose from the kidney into the blood. By inhibiting SGLT2, EMPA lowers blood glucose levels and increases glucose excretion. (7)

DPP-4 is an enzyme that cleaves GLP-1 and glucose-dependent insulinotropic polypeptide, intestinal hormones that regulate the postprandial production of insulin and glucagon by the pancreas. It increases the concentrations of these incretin hormones, which stimulates the release of insulin in a glucose-dependent manner and decreases glucagon levels in the blood. (8)

EMPA and LINA is a new drug combination. Therefore, there are very few reports of HPLC method development for this new combination. Therefore, this is an attempt to develop novel, simple, robust, accurate method for the determination of efficacy and safety of EMPA and LINA combination. This method was fully validated according to International Conference on Harmonization (ICH) and ready for the application in routine analysis without interference of an excipients.

Published Papers on this drug combination and in combination with other drug by UV (9,10,11), HPLC (12,13,14,15,16,17,18).

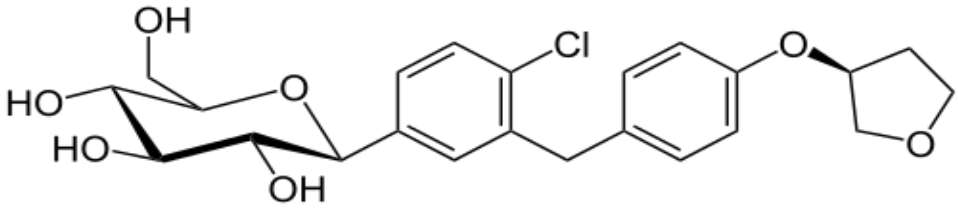

Figure No. 1: Structural formula of Empagliflozin

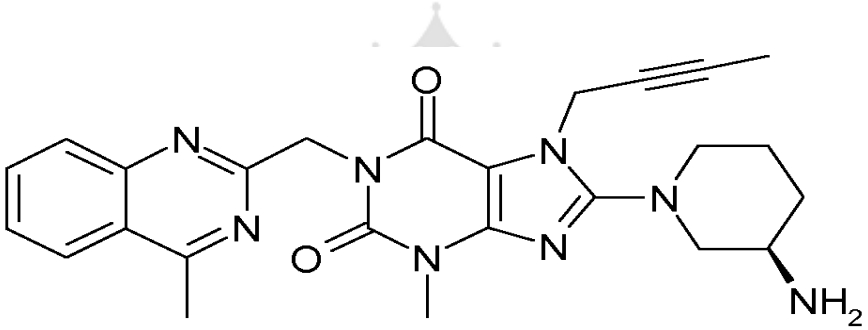

Figure No. 2: Structural formula of Linagliptin

MATERIALS AND METHODS

Instruments:

A Systronics HPLC-8600 chromatographic system is used for the quantitative analysis. It consists of a prominence solvent delivery module, a manual injector with a 20µL fixed loop, Pressure pump, UV-visible detector, operated by computer software Chemitochrom 2000. The separation was performed on Hibar® (Merck Germany) RP-Purospher Star C18 column with dimensions 5µm, 4.6mm*, 250mm at ambient temperature. A Fast Clean ultrasonicate sonicator was used for the degassing purpose. Weighing balance Sansui Vibra DJ-150S-S was used for the weighing of samples and reagents, pH meter (Equiptronics EQ 621) was

used for the checking and maintaining pH of the mobile phase and filter papers of Sartorius Stedim grade 292 was used for the filtration of mobile phase and other chemical reagents.

Chemicals and Reagents:

EMPA and LINA pure drugs were obtained as generous gift samples from Boehringer Ingelheim Pharmaceuticals India. The combined formulation Glyxambi® (25 mg/5 mg) were purchased from Vikram Pharmacy Jalgaon. HPLC grade methanol was purchased from Rankem Chemicals Mumbai Pvt. Ltd. Ortho-phosphoric Acid of AR grade was purchased from Qualigens Fine Chemicals Mumbai Pvt. Ltd. All other chemicals and reagents used were analytical grade unless otherwise indicated.

Method Development:

Various pre-trials of the mobile phases have been carried out before selecting the proper mobile phase. Finally, Phosphate buffer and Methanol with concentrations 37 ml and 63 ml respectively, has been selected as a mobile phase and pH value for the same is 4.0. Flow rates between 0.5 ml/min and 1.5 ml/min has been studied. But optimal signal can't be obtained on those flow rates. So that, flow rate of 1.0 ml/min was tested, and it gave optimal signal with noise ratio and reasonable separation using C18 column. Total analysis time is less than 12 minutes. The maximum absorption of EMPA and LINA was detected at 229 nm. At this wavelength, both Empagliflozin and Linagliptin were showed complete resolution.

Preparation of Buffer: According to Indian Pharmacopoeia 2007, the assay of Phosphate buffer is as follows. Dissolve 5.04 gm of disodium hydrogen phosphate and three.01 gm of potassium dihydrogen phosphate in sufficient water to provide 1000 ml. Adjust the pH with phosphoric acid.

Preparation of Mobile Phase: Phosphate buffer and Methanol were taken in the ratio of 37:63.

Preparation of Standard Solution: Accurately weighed and transferred 0.00848 gm of EMPA and 0.0376 gm of LINA drugs to the two separate 10 ml volumetric flasks and added a diluent like Methanol and sonicated to 30 minutes, after sonication make up the volume up to 10 ml with the same diluent.

Preparation of Sample Solution: 10 tablets of EMPA and 10 tablets of LINA were weighed accurately and calculated the average weight of each tablet, and then the weight of the 1 equivalent amount was transferred into 10 ml standard volumetric flask, and added diluent methanol and sonicated for 30 minutes. After sonication make up the volume up to 10 ml and filtered it through Nylon filter papers.

Maintained Chromatographic conditions:

Mobile Phase:	Phosphate buffer and Methanol
HPLC Column:	Octadecylsilyl C18 Column (5µm, 4.6mm* 250mm)
Detection wavelength:	229 nm
Flow rate:	0.8 ml/min
Column Temperature:	Ambient
Run Time:	12 minutes
Injection Volume:	20 µL
Diluent:	Methanol

Method Validation:

System Suitability:

The standard solution was injected into the Chromatographic system. Percentage relative standard deviations have been found satisfactory. System suitability results are tabulated in table.

Calibration curve (Linearity):

Linearity of an analytical method is its ability to elicit test results that are directly proportional to concentration of analyte in sample within given range, this was studied by analyzing five different concentrations of drug starting from 10µgm/ml to 50µgm/ml (10µgm/ml, 20µgm/ml, 30µgm/ml, 40µgm/ml, 50µgm/ml) for Empagliflozin and Linagliptin were transferred to series of 10mL volumetric flasks and therefore the contents of the flasks

were diluted up to the mark with diluent. A 20µL aliquot of every solution was injected into the chromatographic system. The conditions including the flow 0.8ml/min and detection wavelength was set to the 229 nm and also the run time program was set to the 12 minutes. A calibration curve for every drug was obtained by plotting area under the height versus concentration. Linearity results tabulated in Table 2. Linearity graphs for Empagliflozin and Linagliptin are presented in Figure 3 and 4 respectively.

Accuracy (% Recovery):

Accuracy refers to the closeness of a measured value to a customary value. Accuracy studies were dispensed by adding a known amount of pure drugs of EMPA and LINA to the pre-analyzed sample solution. the share recovery studies were dispensed by spiking 80%, 100% and 120% of respective drug, each level was injected 3 times, shown in Table 4. consistent with the results the tactic is capable to estimate both drug components accurately within the tablet dosage form at a time and therefore the results were within acceptable limits, i.e., above 99 try to below 101 %.

Precision (Repeatability):

The precision of the strategy has been evaluated by injecting the six replicate sample preparations. The share assay for both Empagliflozin and Lingaliptin were calculated and tabulated in Table 3. The % R.S.D. values of the results reminiscent of the height area and retention time were expressed for intra-day precision and on 3 days for inter-day precision. % RSD results show that the strategy is precise and might be wont to estimate the drug components within the tablet dosage form.

Intermediate Precision (Reproducibility):

The intraday and interday precisions of the proposed method were determined by estimating the corresponding responses 5 times on the identical day and on 5 different days for present method. The results are reported in terms of relative variance (RSD).

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ of the drug were calculated using the equations per International Conference on Harmonization (ICH) guidelines**.**

Robustness:

Robustness of the strategy make up my mind by making slight changes in chromatographic conditions. Effect of percentage of methanol in mobile phase on the retention time and slight changes in flow were applied as variable parameters. Rate varied at three levels (-1, 0, 1). One factor at the time was changed to estimate the effect. Thus, standard solution at varied pH (pH 2.9, 3.0 and 4.0) three pH levels were performed.

Specificity:

Specificity of an analytical method is its ability to measure accurately, and specifically, the concentration of analyte without interference from other API, diluents, mobile phase, Specificity was checked by determining EMPA, LINA in laboratory prepared binary mixture and in binary mixture containing different degradation products.

System Suitability Test:

(n=6) was prepared and injected. Then the system suitability parameters like retention time, In the system suitability test, the binary solution of fifty µg/ml of EMPA, 50 µg/ml of LINA theoretical plates, tailing factor and backbone were calculated from the chromatogram.

RESULTS AND DISCUSSION:

The absorption spectra of EMPA and LINA greatly overlap; so conventional determination of those compounds in mixture isn't possible. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for EMPA and LINA were obtained with a mobile phase consisting of methanol: phosphate buffer (63:37 v/v), pH 4.0 adjusted using 10% o-phosphoric acid. Quantification of the drugs was performed at 229 nm. Resolution of the components with clear baseline separation was obtained.

Validation of the proposed method:

Linearity:

Linear correlation was obtained between peak areas and concentrations of EMPA and LINA in range of 10µgm/ml to 50µgm/ml, for both drug compounds. The linearity of calibration curves was found to be acceptable over the concentration ranges of 10µgm/ml-50µgm/ml for

Empagliflozin and Linagliptin, with a R2 values 0.9967 and 0.9966. The results show that good correlation existed between the height area and concentration of the analysts.

Accuracy:

The recovery experiments were performed by the quality addition method. The recoveries obtained were 99.72 and 99.44% for EMPA and LINA, respectively. The high values indicate that the strategy was accurate.

Precision:

Precision study was applied using parameter like method repeatability study which showed that results were within acceptable limit 0.094 and 1.073 *i.e.* % RSD below 2.0 indicating that the tactic is reproducible. The results are shown in (Table No.2).

Intermediate Precision:

The intraday RSD values for EMPA and LINA were 0.3371, 1.5665 and respectively. The interday RSD values for EMPA, and LINA were 0.1290, 0.8525 respectively. The kids RSD (< 2%) values indicate that the tactic was sufficiently precise (Table 2).

LOD and LOQ

LOD values for EMPA and LINA were found to be 0.127036 µg/mL, 0.256725 µg/mL, respectively. LOQ values for EMPA and LINA were found to be 0.42345 µg/mL, 0.85575 µg/mL, respectively (Table 2). These data showed that the method was sensitive enough for the determination of EMPA and LINA.

Robustness:

The method was found to be robust with no significant changes on test result upon change of analytical conditions like different flow, % methanol in mobile phase and pH of mobile phase with the quality deviation was found to be below 1 and zippers RSD is a smaller amount than 2 for all results. It had been found that under small deliberate changes of chromatographic factors, there was no considerable change in understudy parameters.

System Suitability Test:

A binary solution of 50µg/mL of EMPA and 50µg/mL LINA (n=5) was prepared and same was injected, then the system suitability parameters were calculated from the chromatogram. The parameters, retention times, resolution factor, tailing factor and theoretical plates were evaluated. The results (Table 4) obtained from system suitability tests are in agreement with the official requirements.

CONCLUSION:

The proposed LC method presented in this paper has advantages of simplicity, accuracy, precision and convenience for separation and quantitation of EMPA and LINA in combination and can be used for the assay of their respective dosage form. Moreover, the proposed HPLC method is stability indicating assay method that can determine EMPA and LINA in presence of their degradation products. Thus, the proposed HPLC method can be used for the quality control of EMPA and LINA in typical laboratories.

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Table No. 1: Regression Analysis of the calibration curves for Empagliflozin and Linagliptin in the proposed HPLC method.

Parameters	Empagliflozin	Linagliptin
Linearity Range (µgm/ml)	10 – 50 µgm/ml	10 – 50 µgm/ml
Detection Wavelength	229 nm	
Slope ± S.D.	40.74	18.21
Intercept ± S.D.	2.7216	2.0647
Correlation Coefficient (R^2)	0.9967	0.9966

Table No. 2: Summary of the validation parameters for the proposed HPLC Method:

Parameters	Empagliflozin	Linagliptin
LOD	0.127036	0.256725
LOQ	0.42345	0.85575
Accuracy %	99.6	99.2
Repeatability (% RSD, n=5)	0.094	1.073
Precision (RSD %)		
Interday, n=3	0.1290	0.8525
Intraday, n=3	0.3371	1.5665

LOD = Limit of Detection

LOQ = Limit of Quantification

RSD = Relative Standard Deviation

Table No. 3: Assay results for the combination dosage form using the proposed HPLC method.

Formulation	Empagliflozin	Linagliptin
Glyxambi	99.42 ± 0.4155	97.16 ± 1.222

Table No. 4: System suitability test parameters for EMPA and LINA for the proposed HPLC method.

System suitability parameters	Proposed method	
	EMPA	**LINA**
Retention Time (t_R)	3.06	4.37
Number of Theoretical plates	2318	2365
Asymmetry factor	1.094	1.052
Resolution factor (R)	2.43	4.190

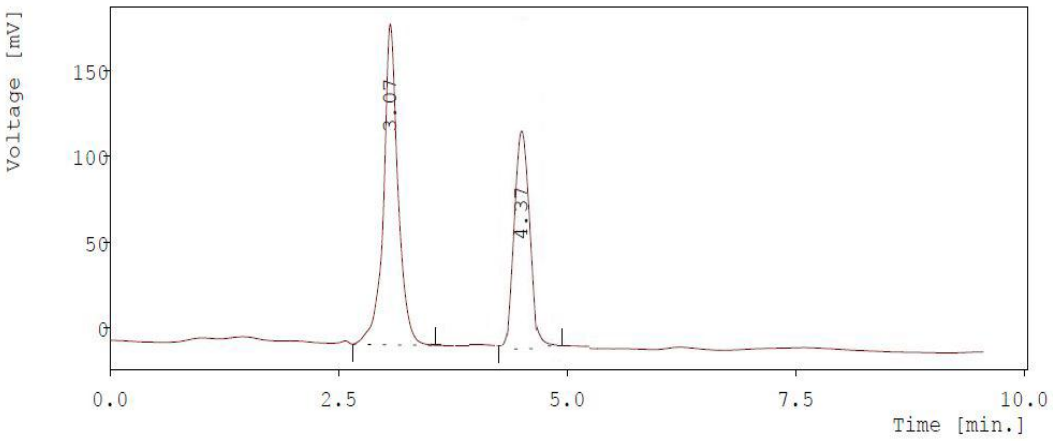

Figure No. 3: Typical liquid chromatograms obtained for a 20 µL injection of a binary mixture of EMPA and LINA

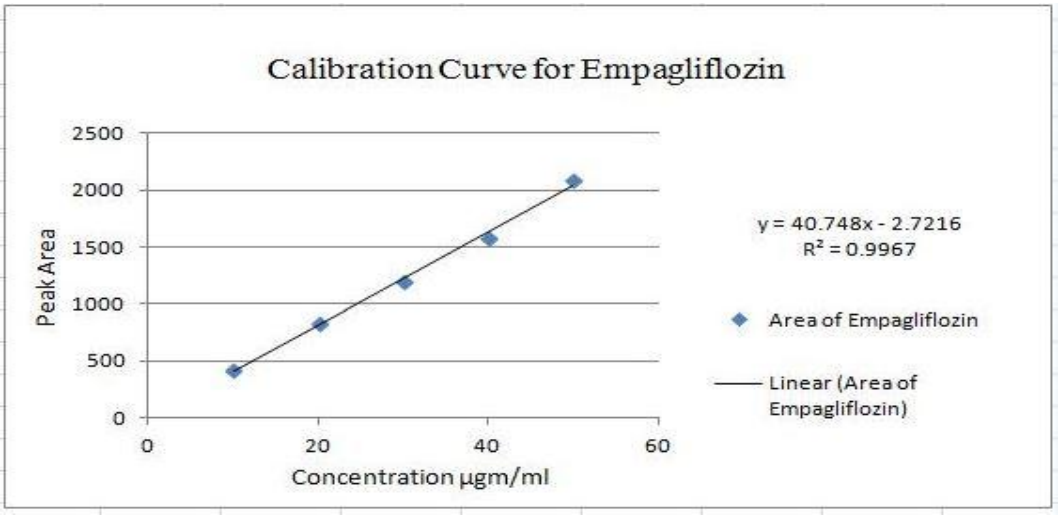

Figure No. 4: Calibration curve of Empagliflozin

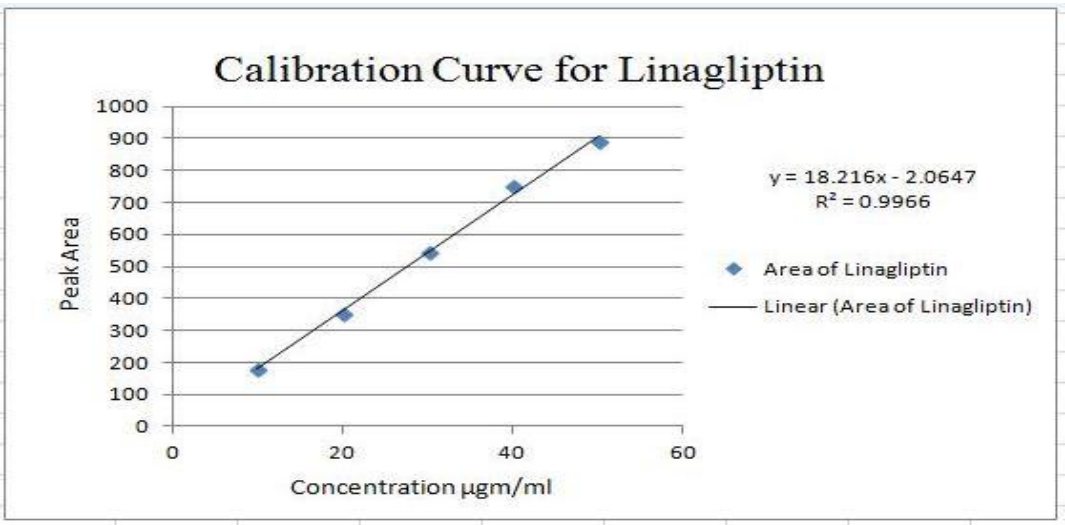

Figure No. 5: Calibration curve of Linagliptin

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