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# RPHPLC Method Development and Validation for Simultaneous Determination of Linagliptin and Empagliflozine in Tablet Dosage Form

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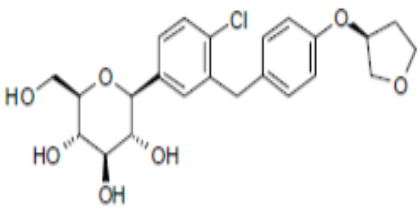
**Abstract:** A novel, simple rapid reverse phase high performance chromatography (RPHPLC) method has been developed and validated for the determination of Linagliptin and Empagliflozine in tablet dosage form. Isocratic chromatography has been developed on a ODS column (250 x 4.6mm, 5µ) with a mobile phase consists of buffer and Acetonitrile (45:50) with the flow rate of 1ml/min with PDA detector at 245 nm. The total run time was 7 minutes. The retention time for Lingaliptin and Empagliflozine were found to be 2.2 and 3.6 min respectively. Chromatography parameters were validated as per ICH guidelines and can be applied for routine quantitative analysis of drugs in combined tablet dosage form.

**Keywords:** ICH, Validation, Empagliflozine, Linagliftin.

## INTRODUCTION

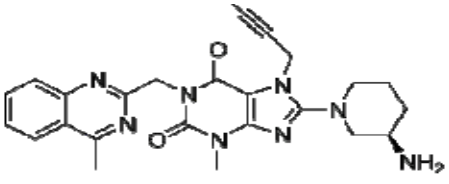
Empagliflozine and Linagliftin are available in combined tablet dosage form for oral use. This tablet dosage form acts as inhibitor of Sodium Glucose Co-transporter (SGLT-2) and Di Phenyl Peptidase-4(DPP-4) to reduce glucose levels in blood (glycemic control) in adults suffering with type-2 diabetes mellitus. Generally recommended dose with 10mg of Empagliflozine and 5mg of Linagliptin once daily in the morning.

Empagliflizone chemically known as (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




Structure of Empagliflizone

Linagliptin chemically known as (8-[ (3R)-3-Aminopiperdin-1-yl]- (But-2-yn-1-yl)-3-Methyl-1 (4 MethylQuinazoline-2yl)Methyl -2,3,6,7 Tetrahydro-1H-Purin-2,6-Dione)




Structure of Linagliptin

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## MATERIALS AND METHODS

**Instrumentation:** A waters HPLC system consisting of Alliance 2695 with 2996 PDA detector operated by Empower 2 software and with auto injector. A Octadecylsilane (ODS) column (250mm x 4.6mm, 5µ) from GL Science Inc. Weighing Balance (Make: ASCOSET, Model: ER200A), Sonicator (Make: Enertech, Model: SC60US), pH meter (Make. ADWA, Model AD102U), Nylon filter paper 0.45 microns (Millipore)

**Method Development:** The mobile phase was chosen after several trials performed with Acetonitrile, water and Buffer solutions in various proportions and at different pH values. Flow rates between 0.5ml/min and 1.5ml/min were studied. A flow rate of 1 ml/min gave an optimal signal to noise ratio with a reasonable separation time using ODS column. Total time of analysis was less than 8 minutes. The maximum absorption of Lingaliptin and Empagliflozine together detected at 245nm. The chromatogram at 245nm showed a complete resolution between Linagliptin and Empagliflozine. Figure 1 & 2 represents the Blank (diluent) and standard solution chromatograms.

**Preparation of buffer:** 1ml of Orthophosphoric acid was added to 1000ml of milli-Q water added then sonicated.

**Mobile phase:** Buffer and Acetonitrile were taken in the ratio of 45:55

**Preparation of standard solution:**
Accurately weighed and transferred 12.5 mg of Linagliptin and 25 mg of Empagliflozine into 250ml of standard volumetric flask and added about 150 ml of diluent and sonicated for 30 minutes and made upto the mark with diluent. (50 µg/mL Linagliptin & 100 µg/mL Empagliflozine).

**Sample preparation:** 5 tablets were weighed accurately and calculated the average weight of each tablet then the weight of the 1 tablet equivalent amount was transferred into 100ml standard volumetric flask, and added 70ml of diluent and sonicated for 30 minutes, further it was diluted to the mark with diluent and filtered through Nylon 0.45 micro membrane filter.

**Chromatographic Conditions:**
Mobile phase: Phosphoric acid buffer and Acetonitrile
Column :ODS 250x4.6mm, 5µ Make : GL Science Inc.
Flow rate : 1ml/ min
Detection. : 245nm
Column temperature : 30°C
Injection volume : 10µL
Runtime : 7 min
Diluent : Water and Acetonitrile (50: 50)

**Method Validation:**
**System Suitability:** Standard solution was injected in five replicates into the Chromatographic System. The percentage relative standard deviations per peak area have been found to be satisfactory. System suitability results are tabulated in Table 1 **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. Representative blank chromatogram is presented in Figure 1

**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 ranging from 12.5 to 75 ppm for Linagliptin and 25 to 150 ppm for Empagliflozine. Linearity results tabulated in Table 2. Linearity graphs for Empagliflozine and Linagliptin are presented in Figure 3 and 4 respectively.

**Method Precision:** The precision of the method has been evaluated by injecting the six replicate sample preparations. The percentage assay for both Empagliflozine and Lingaliptin were calculated and tabulated in Table 3. % RSD results show that the method is precise and can be used to estimate the drug components in the tablet dosage form.

**Accuracy:** Accuracy refers to the closeness of a measured value to a standard value. The percentage recovery was studied for 50%, 100% and 150% each level was injected 3 times, shown in Table 4. Results show that the method is capable to estimate both drug components accurately in the tablet dosage form at a time.

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TABLE I: System Suitability results

| S.No. | Empa | Lina |
|---|---|---|
| 1 | 1648576 | 980204 |
| 2 | 1667929 | 972164 |
| 3 | 1664123 | 979362 |
| 4 | 1662957 | 982578 |
| 5 | 1663360 | 980901 |
| 6 | 1661215 | 973196 |
| Average | 1661360 | 978068 |
| SD | 6645 | 4317 |
| %RSD | 0.4 | 0.4 |

TABLE III: Method precision results

| S.No. | Empagliflozine | Linagliptin |
|---|---|---|
| 1 | 101.9 | 97.9 |
| 2 | 101.2 | 97.0 |
| 3 | 101.8 | 97.6 |
| 4 | 101.4 | 98.2 |
| 5 | 101.5 | 97.8 |
| 6 | 101.4 | 97.3 |
| Average | 101.5 | 97.6 |
| SD | 0.27 | 0.43 |
| %RSD | 0.26 | 0.44 |

TABLE IV: Recovery from Drug product

| S.No | Concentration (%) | Mean recovery (%) (n = 3) | |
|---|---|---|---|
| | | Empagliflozine | Linagliptin |
| **1** | 50 | 99.8 | 99.9 |
| **2** | 100 | 100.3 | 100.2 |
| **3** | 160 | 100.1 | 100.5 |

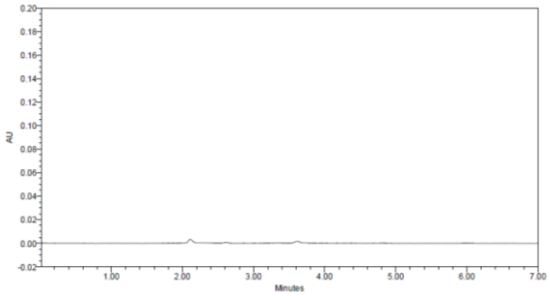


Figure 1. Blank Chromatogram

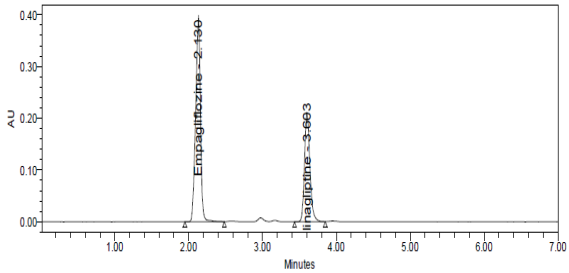


Figure 2. Standard solution Chromatogram

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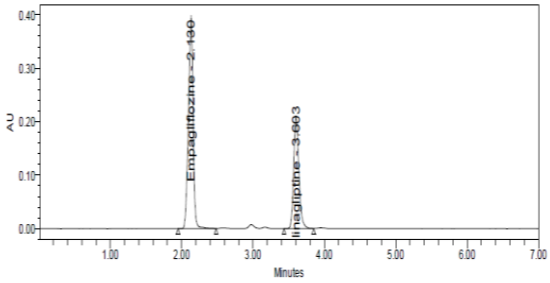


Figure 2.1 Sample solution Chromatogram

TABLE II.1: Linearity of Empagliflozine

| % level | Injection No. | Conc. of Empa (µg/mL) | Area of Empa |
|---|---|---|---|
| 25 | 1 | 24.86 | 400956 |
| | 2 | 24.86 | 401105 |
| | 3 | 24.86 | 404166 |
| 50 | 1 | 49.72 | 838877 |
| | 2 | 49.72 | 839691 |
| | 3 | 49.72 | 840883 |
| 75 | 1 | 74.58 | 1267374 |
| | 2 | 74.58 | 1274370 |
| | 3 | 74.58 | 1272339 |
| 100 | 1 | 99.44 | 1676293 |
| | 2 | 99.44 | 1657219 |
| | 3 | 99.44 | 1666007 |
| 125 | 1 | 124.3 | 2106056 |
| | 2 | 124.3 | 2103135 |
| | 3 | 124.3 | 2101783 |
| 150 | 1 | 149.16 | 2481946 |
| | 2 | 149.16 | 2486042 |
| | 3 | 149.16 | 2492746 |
| Slope | | 16792 | |
| Correlation Coefficient | | 0.9998 | |

TABLE II.2: Linearity of Linagliptin

| % level | Injection No. | Conc.of Lina (µg/mL) | Area of Lina(µg/mL) |
|---|---|---|---|
| 25 | 1 | 12.76 | 243482 |
| | 2 | 12.76 | 247024 |
| | 3 | 12.76 | 240263 |
| 50 | | 25.52 | 482181 |
| | 2 | 25.52 | 482542 |
| | 3 | 25.52 | 482890 |
| 75 | 1 | 38.28 | 753554 |
| | 2 | 38.28 | 753533 |
| | 3 | 38.28 | 752276 |
| 100 | 1 | 50.72 | 970829 |
| | 2 | 50.72 | 975864 |
| | 3 | 50.72 | 960609 |
| 125 | 1 | 63.8 | 1196725 |
| | 2 | 63.8 | 1206099 |
| | 3 | 63.8 | 1209444 |
| 150 | 1 | 76.56 | 1473530 |
| | 2 | 76.56 | 1470229 |
| | 3 | 76.56 | 1459094 |
| Slope | | 19048 | |
| Correlation Coefficient | | 0.9996 | |

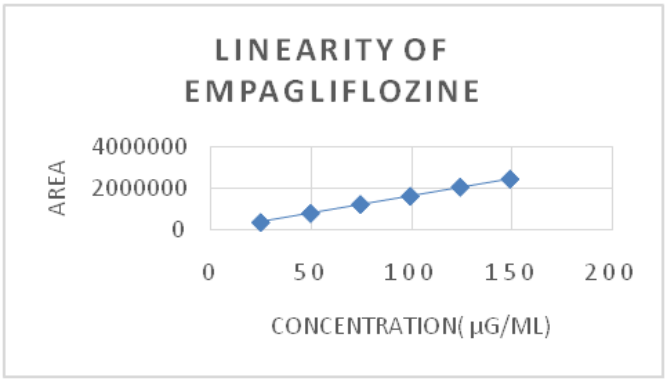


Figure 3: Linearity of Empagliflozine

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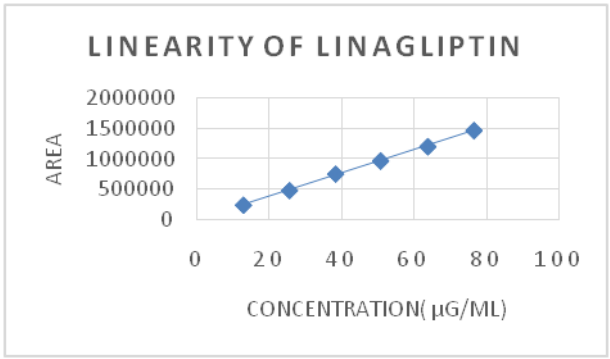


Figure 4: Linearity of Linagliptin

## CONCLUSION

An isocratic RP-HPLC method has been developed and validated for the simultaneous determination of Linagliptin and Empagliflozine in tablet dosage form. The validation results reveal that, developed method was simple, precise, accurate and economical. This developed method meets the all requirements of ICH guidelines.

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## BIOGRAPHY

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