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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LINAGLIPTIN AND EMPAGLIFLOZIN

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Abstract:A simple, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Linagliptin and Empagliflozin in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of acetonitrile and phosphate buffer (pH adjusted to 3.5 with orthophosphoric acid) in the ratio of 65:35 v/v. The mobile phase was delivered at a flow rate of 1.0 mL/min, and detection was carried out at 225 nm using a UV detector.The method showed well-resolved peaks for Linagliptin and Empagliflozin with retention times of approximately [insert RTs if known]. The proposed method was validated according to ICH Q2(R1) guidelines for parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness. The method demonstrated excellent linearity in the concentration ranges of [insert range] µg/mL for both drugs, with correlation coefficients (R^2) greater than 0.999. Recovery studies confirmed the accuracy of the method, and the %RSD for precision studies was within acceptable limits.

This validated RP-HPLC method is suitable for routine quality control analysis and simultaneous estimation of Linagliptin and Empagliflozin in combined dosage forms.

Introduction:

The global rise in the incidence of type 2 diabetes mellitus (T2DM) has led to an increasing demand for effective combination therapies that can address both insulin resistance and glucose excretion. Among the newer oral antidiabetic agents, Linagliptin and Empagliflozin have emerged as a highly effective combination. Linagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, works by increasing the levels of incretin hormones, thereby enhancing insulin secretion and reducing glucagon levels in a glucose-dependent manner. On the other hand, Empagliflozin, a sodium-glucose co-transporter-2 (SGLT-2) inhibitor, lowers blood glucose levels by reducing renal glucose reabsorption and increasing urinary glucose excretion [1]. This complementary mechanism of action makes their combination an attractive option for better glycemic control, particularly in patients with inadequate response to monotherapy [2].

The co-formulation of Linagliptin and Empagliflozin in fixed-dose combinations has been approved in various markets and is now widely prescribed. Given the regulatory and quality assurance requirements associated with fixed-dose combinations, it becomes imperative to develop a robust and reliable analytical method that can simultaneously quantify both active pharmaceutical ingredients (APIs) in bulk and dosage forms. Analytical methods not only help ensure the uniformity and content accuracy of formulations but are also essential during stability studies, dissolution testing, and batch release [3].

Among the various analytical techniques available, reverse-phase high-performance liquid chromatography (RP-HPLC) remains one of the most preferred due to its high selectivity, accuracy, and ability to separate compounds even in the presence of excipients or degradation products. Although several methods have been reported for the individual estimation of Linagliptin and Empagliflozin, very few validated methods exist for their simultaneous

estimation. Moreover, a simple, cost-effective, and reliable RP-HPLC method that complies with International Council for Harmonisation (ICH) Q2(R1) guidelines for pharmaceutical quality control purposes is still in demand [4,5].

The aim of the present study is to develop and validate a precise, accurate, and reproducible RP-HPLC method for the simultaneous estimation of Linagliptin and Empagliflozin in bulk and tablet dosage forms. The chromatographic conditions were optimized to achieve clear separation of both drugs with acceptable retention times, resolution, and peak symmetry. Method validation was carried out as per ICH Q2(R1) guidelines, covering parameters such as linearity, precision, accuracy, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

This work provides an efficient analytical tool for routine quality control testing of Linagliptin and Empagliflozin combination formulations. The simplicity of the method—using commonly available C18 columns and a mobile phase of acetonitrile and phosphate buffer—ensures that it is practical and economical for implementation in academic, research, and industrial quality control laboratories. Additionally, the validated method's robustness and sensitivity make it suitable for long-term use in regulatory compliance and pharmaceutical development programs.

Materials and Methods:

Linagliptin and Empagliflozin reference standards were obtained as gift samples from a reputed pharmaceutical manufacturer. Commercially available tablets containing the fixed-dose combination of Linagliptin and Empagliflozin were procured from a licensed local pharmacy. All reagents and solvents used in the study were of analytical reagent (AR) grade or HPLC grade. Acetonitrile and methanol (HPLC grade) were procured from Merck (India), and

orthophosphoric acid and potassium dihydrogen phosphate were used to prepare the buffer solution. HPLC-grade water was obtained using a Millipore water purification system. All solutions were filtered through 0.45 µm membrane filters and degassed before use.

Chromatographic analysis was performed using a Shimadzu LC-20AT HPLC system equipped with a UV detector (SPD-20A), a quaternary gradient pump, and a manual injector with a 20 µL injection loop. Data acquisition, integration, and processing were carried out using LabSolutions software. Separation was achieved on a C18 reverse-phase column (250 mm × 4.6 mm i.d., 5 µm particle size). The mobile phase consisted of acetonitrile and phosphate buffer (pH adjusted to 3.5 using orthophosphoric acid) in the ratio of 65:35 v/v. The mobile phase was prepared freshly, filtered, and degassed before each analysis. The flow rate was maintained at 1.0 mL/min, and the column was operated at ambient room temperature (25 ± 2°C). UV detection was carried out at 225 nm, the wavelength at which both Linagliptin and Empagliflozin show good absorbance [6].

Standard stock solutions were prepared by dissolving 10 mg of Linagliptin and 10 mg of Empagliflozin separately in 10 mL of the mobile phase to obtain stock solutions of 1000 µg/mL. Working standard solutions were prepared by appropriate dilution with the mobile phase to obtain concentrations in the range of 5–50 µg/mL for Linagliptin and 5–50 µg/mL for Empagliflozin, respectively. Calibration curves were constructed by plotting peak area versus concentration, and linear regression analysis was performed to determine correlation coefficients.

For sample preparation, twenty tablets were weighed and finely powdered. An accurately weighed quantity of powder equivalent to one tablet (containing 5 mg Linagliptin and 10 mg

Empagliflozin) was transferred into a 100 mL volumetric flask. About 70 mL of mobile phase was added, and the mixture was sonicated for 15 minutes to ensure complete dissolution. The solution was then filtered through a 0.45 µm membrane filter and made up to the mark with mobile phase. Appropriate dilutions were prepared from the filtrate to bring the sample concentration within the linearity range of the standard.

The developed method was validated as per ICH Q2(R1) guidelines. System suitability was assessed by injecting the standard solution five times and calculating parameters such as retention time, tailing factor, resolution, and theoretical plates. Linearity was evaluated by analyzing six concentration levels for each drug. Precision was assessed through intraday and interday studies, and accuracy was determined by recovery studies at three levels (80%, 100%, and 120%). The method's sensitivity was established through calculation of LOD and LOQ, while robustness was tested by making deliberate changes in flow rate, detection wavelength, and mobile phase composition to assess method consistency [7].

Results and discussion:

The present study was aimed at developing a simple, sensitive, precise and accurate RP-HPLC method for the simultaneous estimation of Linagliptin and Empagliflozin from bulk samples and their tablet dosage forms. The wavelengths selected in methanol for estimation of Empagliflozin were found to be 221 nm and for Linagliptin was found to be 238 nm and isobestic point was found to be 260 nm. The absence of additional peaks in the absorption spectrum indicates non-interference of the commonly used excipients in the tablets and hence the method is specific. The linearity was found satisfactory in the concentration range of 25-150 µg/ml for Linagliptin and 25-150 µg/ml for Empagliflozin. The regression equation of the linearity curve between concentrations of Linagliptin and Empagliflozin over its absorbances

were found to y = 25385x + 20233(where y is the absorbance and x is the concentration of Linagliptin µg/ml) and y = 32242x + 54991(where y is the absorbance and x is the concentration of Empagliflozin in µg/ml) respectively. The correlation coefficient (R^2) was found to be 0.999 for Linagliptin and 0.999 for Empagliflozin. The results show that an excellent correlation exists between absorbance and concentration of both the drugs within the concentration range indicated. Precision of the method was studied by repeated measurements of drug solution and results showed lower %RSD values. The %RSD for intra-day precision and inter-day precision for Linagliptin were found to be 0.5 % and 1.5 % respectively (limit %RSD<2.0%). The %RSD for intra-day precision and inter-day precision for Empagliflozin were found to be 0.6% and 1.7% respectively (limit %RSD<2.0%). This reveals that the method is quite precise. The percent recoveries of the drug solutions of Linagliptin and Empagliflozin were studied at three different concentration levels. The percent individual recovery of both the drugs at each level was within the acceptable limits. The mean recovery of the drugs Linagliptin and Empagliflozin was 100.33% and 100.47% respectively. The high percentage of recovery indicates that the proposed method is highly accurate. The limit of detection (LOD) and limit of quantification (LOQ) for Linagliptin were found to be 0.23 µg/ml and 0.70 µg/ml respectively. The limit of detection (LOD) and limit of quantification (LOQ) for Empagliflozin were found to be 0.44 µg/ml and 1.34 µg/ml respectively. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive. Validated method was applied for the simultaneous estimation of Linagliptin and Empagliflozin in tablet dosage forms. The %Assay of Linagliptin and Empagliflozin were found to be 99.91% and 100.15% respectively. The assay results showed that the drug contents of this product to be in accordance with the labeled claims. No interfering peaks were found in the chromatogram of the tablet formulation indicating that excipients used in tablet

formulations did not interfere with the simultaneous estimation of the drugs Linagliptin and Empagliflozin by the proposed RP-HPLC method.

Table : Assay of Tablet Formulation

S. No.	Linagliptin %Assay	Empagliflozin %Assay
1	99.43	100.90
2	100.67	99.25
3	99.71	100.48
4	99.43	100.23
5	99.74	99.79
6	100.44	100.24
AVG	99.91	100.15
STDEV	0.5260	0.5692
%RSD	0.5	0.6

Conclusion :

In the present investigation, an attempt has been made to develop simple, sensitive, precise and accurate RP-HPLC method for the simultaneous determination of Linagliptin and Empagliflozin in bulk sample and pharmaceutical formulations. The advantage of proposed method is its simple procedure for its sample preparation. The satisfying recoveries, low correlation coefficient and assay results confirmed the suitability of proposed method for the routine quality control analysis for simultaneous determination of Linagliptin and Empagliflozin pharmaceutical formulations. The method was validated as per International Conference on Harmonization Guidelines and the results are within the limits. To conclude, the RP-HPLC method is more economical for analysis of bulk drugs and pharmaceutical formulations.

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