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

ABSTRACT

A simple, accurate and precise RP-HPLC method was developed for the simultaneous estimation of the linagliptin and empagliflozin in tablet dosage form. Chromatogram was run through Kromasil 250 x 4.6 mM, 5mM column, mobile phase containing 0.1% *o*-phosphoric acid buffer and acetonitrile in the ratio of 60:40%V/V was pumped through column at a flow rate of 1 mL/min. The optimized wavelength was 230 nm. Retention times of linagliptin and empagliflozin were found to be 2.759 min and 2.139 min. %RSD of the Linagliptin and Empagliflozin were found to be 0.5 and 0.6 respectively. Percentage assay was obtained as 99.91% and 100.15% for linagliptin and empagliflozin, respectively. LOD, LOQ values obtained for linagliptin and empagliflozin were 0.23 µg/ml and 0.44 µg/mL and 0.70 µg/mL and 1.34 µg/mL, respectively. Thus, the current study showed that the developed RP-HPLC method is sensitive and selective for the estimation of linagliptin and empagliflozin in combined dosage form.

Key Words: Linagliptin, Empagliflozin, RP-HPLC, Dosage form, LOD, LOQ.

INTRODUCTION

Linagliptin is chemically 8-[(3*R*)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl) methyl]-2,3,6,7-tetrahydro-1*H*-purine-2,6-dione. It is a competitive and reversible di-peptidyl peptidase (DPP)-4 enzyme inhibitor that slows the breakdown of insulinotropic hormone glucagon-like peptide (GLP)-1 for better glycemic control in diabetic patients[1]. GLP and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones that increase the production and release of insulin from pancreatic beta cells and decrease the release of glucagon from pancreatic alpha cells. This results in a overall decrease in glucose production in the liver and increase of insulin in a glucose-dependent manner[2].

Empagliflozin is chemically (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-({4-[(3*S*)-oxolan-3-yloxy]phenyl}methyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol. It is a sodium glucose co-transporter-2 (SGLT-2) inhibitor, indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with type-2 diabetes[3-4]. SGLT-2 co-transporters are responsible for re-absorption of glucose from the glomerular filtrate in the kidney[5]. The glucuretic effect resulting from SGLT-2 inhibition reduces renal absorption and lowers the renal threshold for glucose, therefore resulting in increased glucose excretion. Additionally, it contributes to reduced hyperglycemia and also assists weight loss and blood pressure reduction[6].

The survey of literature reveals that few analytical methods[7-18] have been reported for estimation of linagliptin and empagliflozin individually[7,8] and in combined dosage form[9,10]. The reported methods suffer from drawbacks like long run times and more organic phase. Hence, the main objective of the present work was to develop and validate a specific, sensitive, accurate, rapid and precise RP-HPLC method for quantitative determination of linagliptin and empagliflozin in bulk drug and pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrument

WATERS HPLC 2695 SYSTEM with Auto Injector and PDA Detector

Chemicals and Reagents

Linagliptin and empagliflozin pure drugs (API) are procured from Spectram Labs, Hyderabad, Combination of linagliptin and empagliflozin tablets (Glyxambi) are procured from local market. Distilled water, acetonitrile, methanol, *o*-phosphoric acid were purchased from Rankem Chemicals Ltd., Mumbai, India.

Preparation of solutions

Buffer (0.1 % OPA)

1 mL of concentrated *o*-phosphoric acid was dissolved in 1000 ml volumetric flask diluted with distilled water up to the mark. pH wasadjusted to 2.5 by using triethyl amine.

Standard preparation:

Accurately weighed 12.5 mg and 25 mg of linagliptin and empagliflozin working standards were transferred

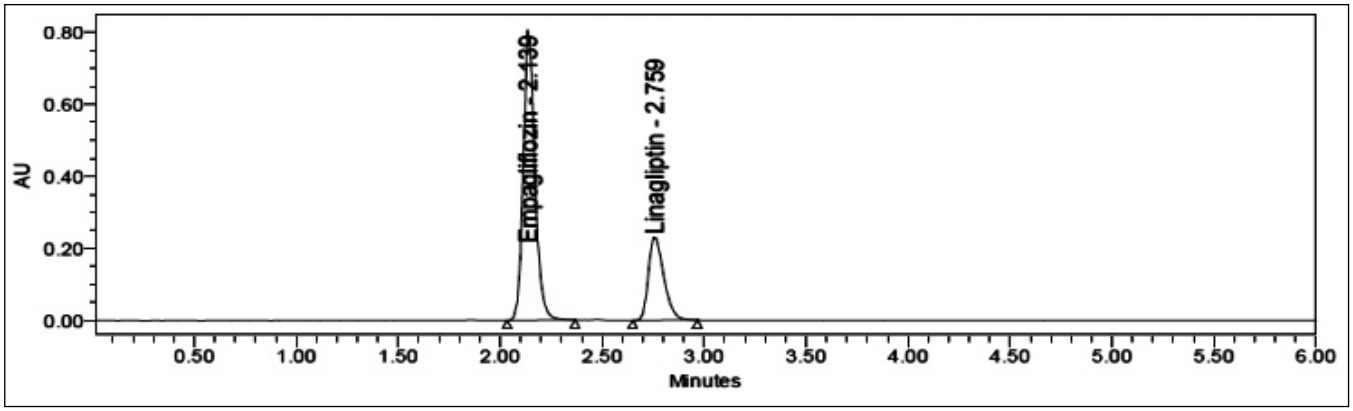

Fig.1: Standard chromatogram of linagliptin and empagliflozin

Table I: Validation Parameters

Parameters	Linagliptin	Empagliflozin
Linearity range (µg/ mL)	12.5-75	25-150
Optimized wavelength	260 nm	260 nm
Retention time (min.)	2.759	2.139
Regression equation (y)	y = 23585x + 20233	y = 32242x + 54991
Correlation coefficient (r2)	0.999	0.999
Precision (%RSD)	0.5	0.6
% Recovery	100.34	100.47
Limit of Detection (µg/mL)	0.23	0.7
Limit of Quantitation (µg/mL)	0.44	1.34

into a 25 mL clean dry volumetric flask, add 20 mL of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1 mL was pipetted out into a 10 mL volumetric flask and then made up to the final volume with diluent.

Sample preparation

20 tablets were weighed and calculate the average weight and transfer the weight equivalent to 10 mg into a 10 mL volumetric flask, 7 mL of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1 mL was pipette out into a 10 mL volumetric flask and made up to 10 mL with diluent.

RESULTS

Method development

Based on the solubility and polarity of drugs various mobile phase mixtures in different proportions were used for separation. Depending on peak shape, theoretical plates, tailing factor and retention time the mixture of 0.1% OPA (*o*- phosphoric acid which is adjusted to 2.5 pH by using triethyl amine) and acetonitrile in proportion of ratio 60:40% V/V proved to be the most suitable of all the combinations. The spectra of the both linagliptin and empagliflozin showed that a balanced wavelength was found to be 260 nm. Flow rate of the mobile phase was maintained at 1.0 mL/min for optimum separation. Water and acetonitrile in the ratio of 50:50V/V was used as diluent.

METHOD VALIDATION

System suitability

These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. All the system suitability parameters were found to be within range and satisfactory as per ICH guidelines.

Linearity

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. Here six linear concentrations of linagliptin (12.5-75 µg/mL) and empagliflozin (25-150 µg/mL) were prepared and injected.

Specificity

The term specific refers to a method that produces a response for single analyte only. The chromatograms of blank, placebo, standard and sample revealed that there is no interference by the excipients.

Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Intra-day precision (Repeatability): Intra-day precision was performed and % RSD for linagliptin and empagliflozin were found to be 0.5 % and 0.6 %, respectively.

Inter-day precision: Inter-day precision was performed with 24 hrs time lag and the %RSD obtained for linagliptin and empagliflozin were 1.5% and 1.7%, respectively.

ACCURACY

Accuracy is the measure of how close the experimental value is to the true value. Three concentration 50%, 100%, 150%, were injected in a triplicate manner and amount recovered and % recoveries were calculated.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ values were found to be 0.23 µg/ml and 0.70 µg/mL for Linagliptin and 1.34 µg/mL and 0.44 µg/mL for Empagliflozin respectively.

Robustness

Robustness is a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters. Small deliberate changes in method like flow rate, mobile phase ratio, and temperature were made but there were no recognized changes in the results.

Assay

Drug in the formulation was estimated by taking the standard as the reference. Linagliptin/Empagliflozin: 5 mg/10 mg. The average % assay was calculated and found to be 99.91% and 100.15% for linagliptin and empagliflozin, respectively.

Forced degradation studies

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

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 in bulk samples and their tablet dosage forms. The optimized wavelength used for the analysis was found to be 260 nm. The linearity was found satisfactory in the concentration range of 12.5-75 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 peak area were found to y = 25385x + 20233 and y = 32242x + 54991 (Table I). The correlation coefficient (R^2) was found to be 0.999 for both linagliptin and empagliflozin. Precision of the method was studied by repeated measurements of drug solution and results showed lower %RSD values. The %RSD for intra and inter-day precision for linagliptin were found to be 0.5 % and 1.5 % respectively (limit %RSD<2.0%) and for Empagliflozin they were found to be 0.6% and 1.7% respectively (limit %RSD<2.0%). This reveals that the method is quite precise. 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/mLand 0.70 µg/mL and 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. 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 are 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.

CONCLUSION

A stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of linagliptin and empagliflozin in their combined dosage form. The linearity range for described method was 12.5-75 µg/mL for linagliptin and 25-150 µg/mL for empagliflozin. The specificity of the method was established by performing degradation studies and there are no interfering peaks were observed due to excipients. %RSD values were less than 2 showed that the method was precise. Percentage mean recoveries for accuracy were found to be satisfactory proved that the method was accurate. As the method was stable to small changes the method was said to be robust. Hence the proposed method was simple, sensitive,

specific, reproducible, accurate and robust which can be used for simultaneous estimation of linagliptin and empagliflozin in bulk drug and combined dosage form for routine analysis.

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