IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
September 2019 Vol.:16, Issue:2

Analytical Method Development and Validation for Simultaneous Estimation of Linagliptin and Empagliflozin in Bulk and Pharmaceutical Formulation by RP-HPLC

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ISSN 2349-7203
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Submission: 29 August 2019
Accepted: 5 September 2019
Published: 30 September 2019

www.ijppr.humanjournals.com

Keywords: Simultaneous estimation, Linagliptin, Empagliflozin, In Bulk, Pharmaceutical Formulation

ABSTRACT

A simple, specific and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for simultaneous determination of Linagliptin and Empagliflozin in tablet dosage forms. Chromatography was performed through kromosil (250 x 4.6 mm, 5□) and isocratic elution. Mobile phase containing buffer and acetonitrile in the ratio of 70:30 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA buffer with pH 4.8 adjusted by triethylamine maintained with a temperature of 30°C. Optimized wavelength for Linagliptin and Empagliflozin was 286 nm. Retention time of Linagliptin and Empagliflozin were found to be 1.920 min and 3.699 min respectively. % RSD of the Linagliptin and Empagliflozin were 1.0 and 0.94 respectively. All the validation parameters were in the acceptable range. Simultaneously this method applied to determine the degradation products of Linagliptin and Empagliflozin. The detection wavelength of 286 nm was chosen in order to achieve high sensitivity for quantitative determination of these drugs in solid dosage form. Method can be successfully employed for simultaneous estimation of these drugs in commercial products.

INTRODUCTION

Pharmaceutical Analysis is that core branch of pharmacy education and research, which is advancing very fast. It can be categorized as synthesis of new drugs molecules and pharmaceutical analysis. Analytical chemistry is the science of making quantitative and qualitative evaluation. In practice, quantifying an analyte in a complex sample becomes an exercise in problem resolving. To be efficient and effective, analytical chemist must know the tools that are available to tackle a wide variety of problems [1,2]. Analytical chemistry is divided into two branches qualitative and quantitative. In this qualitative method provides information about the identity of atomic or molecular species or functional groups in the sample. A quantitative method provides numerical information as to the relative amount of one or more of the components. Varieties of analytical methods are used for the analysis of drugs in bulk, formulations and bioanalytical samples. In pharma industry, spectrophotometric and chromatographic methods have gained the significance in recent studies. Spectrophotometric methods [2-6] are defined as a method of analysis that embraces the measurement of absorption by chemical species of radiant energy at definite and narrow wavelength approximating monochromatic radiation. There electromagnetic spectrum extends from 100-780 nm. Traditionally, analytical chemistry has been split into two main types,

Qualitative and Quantitative: Qualitative Inorganic Analysis seeks to establish the presence of an inorganic compound in a sample or given element. Quantitative analysis seeks to establish the amount of a compound in a sample or given element. Qualitative Organic Analysis seeks to establish the presence of a given functional group or organic compound in a sample. There are various techniques used for analysis of mixture of compounds. Spectroscopy used to measure the interaction of the molecules with electromagnetic radiation. Then chromatography is the collective term for a family of laboratory techniques for the separation of mixtures and comprises passing a mixture of samples dissolved in a "mobile phase" along a stationary phase. The analyte which is separated, to be measured from other molecules in the mixture and allows it is to be isolated. Analytical Chromatography is used to determine the existence and possibly also the concentration of analyte in a compound. Analytical chemistry has played critical roles in the understanding of basic science to a variety of practical applications in industrial productions, biomedical engineering, environmental monitoring, forensic sciences and so on.

MATERIALS AND METHODS

Materials:

Linagliptin and Empagliflozin, Combination Linagliptin and Empagliflozin tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetrahydrofuran, triethylamine, ortho-phosphoric acid *etc.*

Instrument:

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Linagliptin and Empagliflozine solutions.

Methods:

Preparation of buffer:

Buffer: (0.1 % OPA)

1 ml of conc. OPA is dissolved in 1000 ml volumetric flask diluted with distilled water up to the mark. pH adjusted to 4.8 by using Triethylamine.

Standard Preparation:

Accurately Weighed and transferred 12.5mg & 25mg of Linagliptin and Empagliflozin working Standards into a 25ml and 25ml clean dry volumetric flask respectively, add 20ml and 20ml of diluent, sonicated for 30 minutes and makeup to the final volume with diluents. From the above stock solutions, 1ml was pipette out into a 10ml volumetric flask and then make up to the final volume with diluent.

Sample Preparation:

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 7ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution, 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Linearity: Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions Linagliptin and Empagliflozin are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 12.5ppm, 25ppm, 37.5ppm, 50ppm, 62.5ppm, 75ppm of Linagliptin and 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm of Empagliflozin.

Accuracy:

Standard Preparation:

Accurately weighed and transferred 12.5mg & 25mg of Linagliptin and Empagliflozin working standards into a 25ml and 25ml clean dry volumetric flask respectively, add 20ml and 20ml of diluent, sonicated for 30 minutes and makeup to the final volume with diluents.

Preparation of 50% Spiked Solution: Weight equivalent to 125mg of tablet powder was transferred into a 10 ml volumetric flask, 7ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Preparation of 100% Spiked Solution: Weight equivalent to 250mg of tablet powder was transferred into a 10 ml volumetric flask, 7ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Preparation of 150% Spiked Solution: Weight equivalent to 375mg of tablet powder was transferred into a 10 ml volumetric flask, 7ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. 1ml from each standard stock solution was pipette out and taken into a 10ml volumetric flask to that 1ml of filtered Accuracy 100% Sample stock solution was spiked and made up with diluents.

Degradation:

Oxidation:

To 1 ml of stock solution of Linagliptin and Empagliflozin, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 50µg/ml & 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1ml of stock solution LINAGLIPTIN and EMPAGLIFLOZIN, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60ºC. The resultant solution was diluted to obtain 50µg/ml &100µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1ml of stock solution LINAGLIPTIN and EMPAGLIFLOZIN, 1ml of 2N sodium hydroxide was added and refluxed for 30mins at 60ºC. The resultant solution was diluted to obtain 50µg/ml & 100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105°C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 50µg/ml & 100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 500µg/ml & 1000µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200Watt hours/m^2 in photostability chamber. For HPLC study, the resultant solution was diluted to obtain 50µg/ml & 100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 50µg/ml & 100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Method Development

Method Development: Many trials were done by changing columns and Mobile phases and were reported below.

Trial: 1

Column Used : ODS 250 x 4.6 mm, 5µ.

Mobile phase : Water: Acetonitrile: methanol (30:50:20)

Flow rate : 1ml/min

Wavelength : 285 nm

Temperature : 30°C

Injection Volume : 10µl

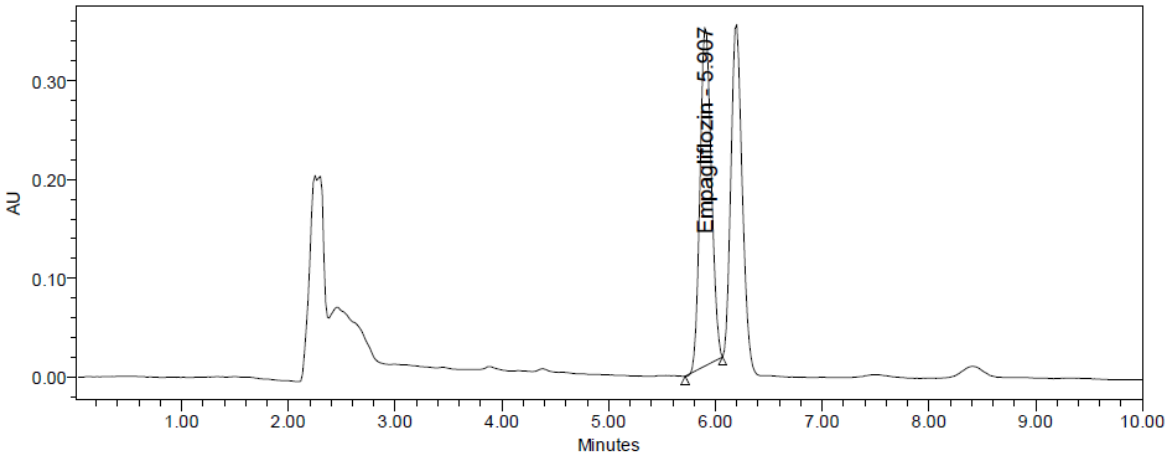

Figure No. 1: Trial chromatogram 1

Observation: Linagliptin and Empagliflozin is eluting with low resolution.

Trial: 2

Column Used : ODS 250 x 4.6 mm, 5µ.

Mobile phase : Buffer: Acetonitrile (30:70)

Buffer : 0.1% Perchloric acid

Flow rate : 1ml/min

Wavelength : 285nm

Temperature : 30°C

Injection Volume : 10µl

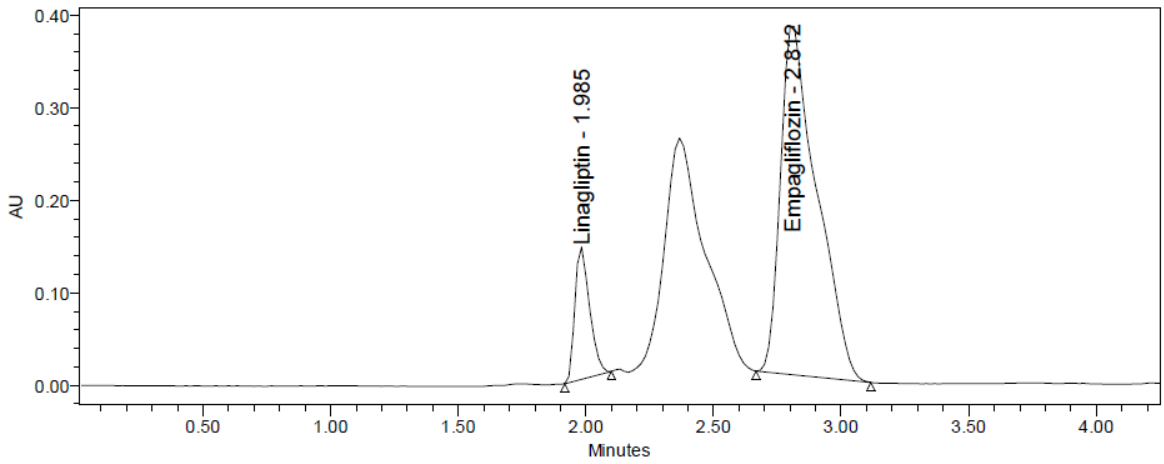

Figure No. 2: Trial chromatogram 2

Observation: Linagliptin and Empagliflozin both peaks eluted but additional peaks observed. So further trial is carried out to decrease retention time.

Trial: 3

Column Used : KROMOSIL 250 x 4.6 mm, 5µ.

Mobile phase : Buffer: Acetonitrile (42:58)

Buffer **:** 0.1% Perchloric acid

Flow rate : 1ml/min

Wavelength : 285nm

Temperature : 30◦C

Injection Volume : 10µl

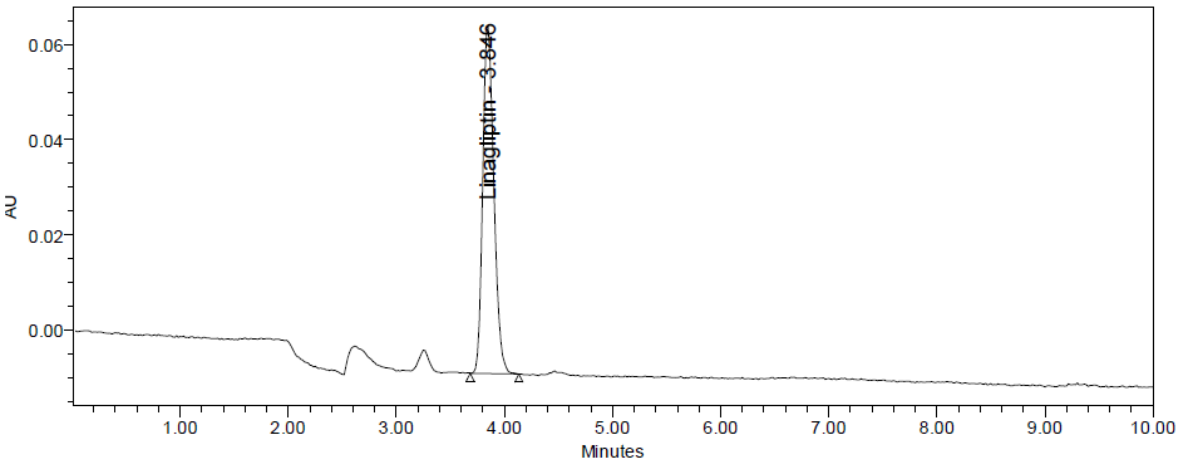

Figure No. 3: Trial chromatogram 3

Observation: Empagliflozin not yet eluted.

Trial: 4

Column Used : KROMOSIL 250 x 4.6 mm, 5µ.

Mobile phase : Buffer: Acetonitrile: Methanol (55:40:5)

Buffer **:** OPA

Flow rate : 1ml/min

Wavelength : 285nm

Temperature : 30◦C

Injection Volume : 10µl

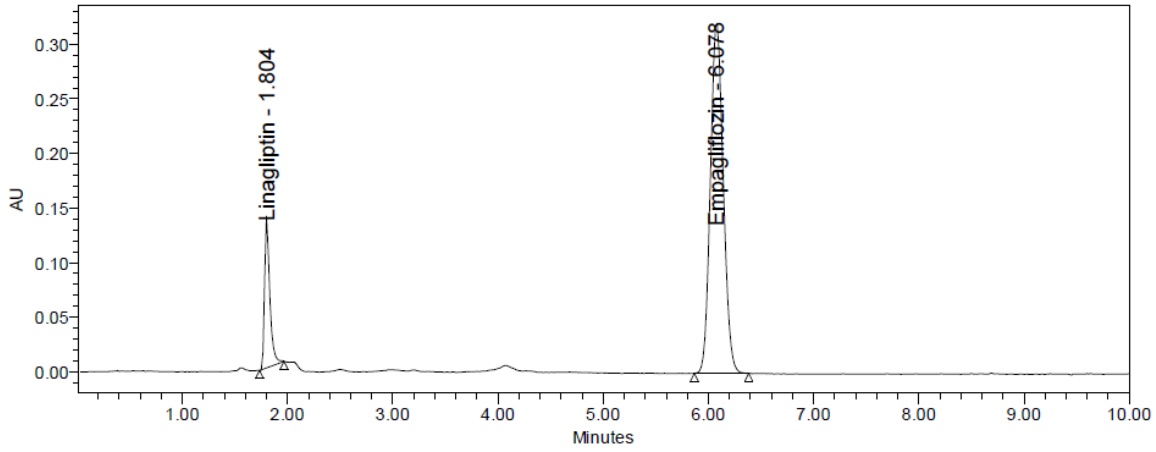

Figure No. 4: Trial chromatogram 4

Observation: Linagliptin and Empagliflozine peaks are sharp. Empagliflozin eluted at longer retention time so further tail is carried out.

Optimized Method: Drugs were eluted with good retention time, resolution; all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used : KROMOSIL 250 x 4.6 mm, 5µ.

Buffer used : 0.1% OPA

(Ortho phosphoric acid which is adjusted to 4.8 pH by using triethylamine)

Mobile phase : Buffer: Acetonitrile (70:30A)

Flow rate : 1ml/min

Diluent : Firstly dissolved in methanol and makeup with Water and Acetonitrile (50:50).

Wavelength : 285nm

Temperature : 30°C

Injection Volume : 10µl

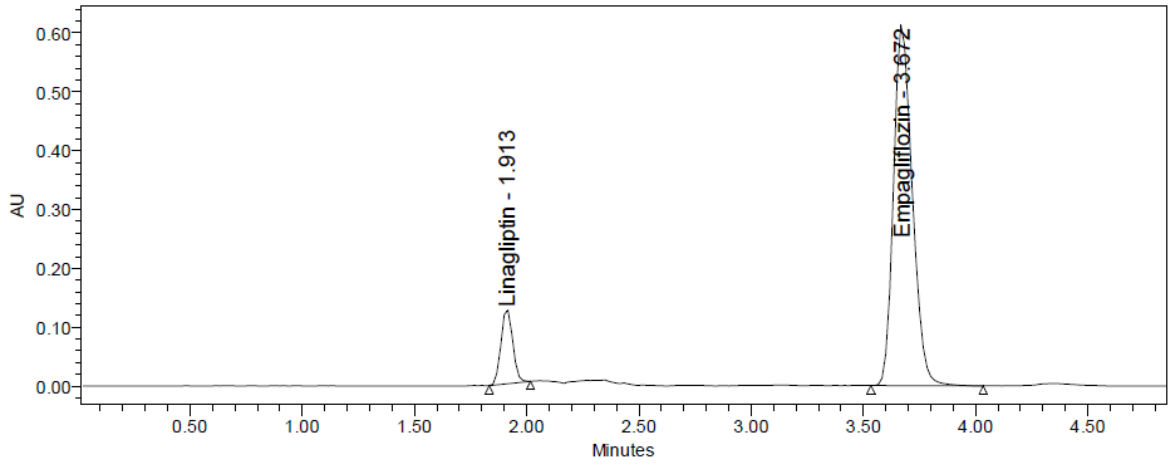

Figure No. 5: Optimized chromatogram of Linagliptin and Empagliflozin

Observation: peak shape and retention time is good.

RESULTS AND DISCUSSION

System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines.

Table No. : 1 System suitability studies of Linagliptin and Empagliflozin method

Property	Linagliptin	Empagliflozin
Retention time (t_R)	1.920min	3.690min
Theoretical plates (N)	7217 ± 63.48	8554 ± 63.48
Tailing factor (T)	1.08 ± 0.117	1.22± 0.117

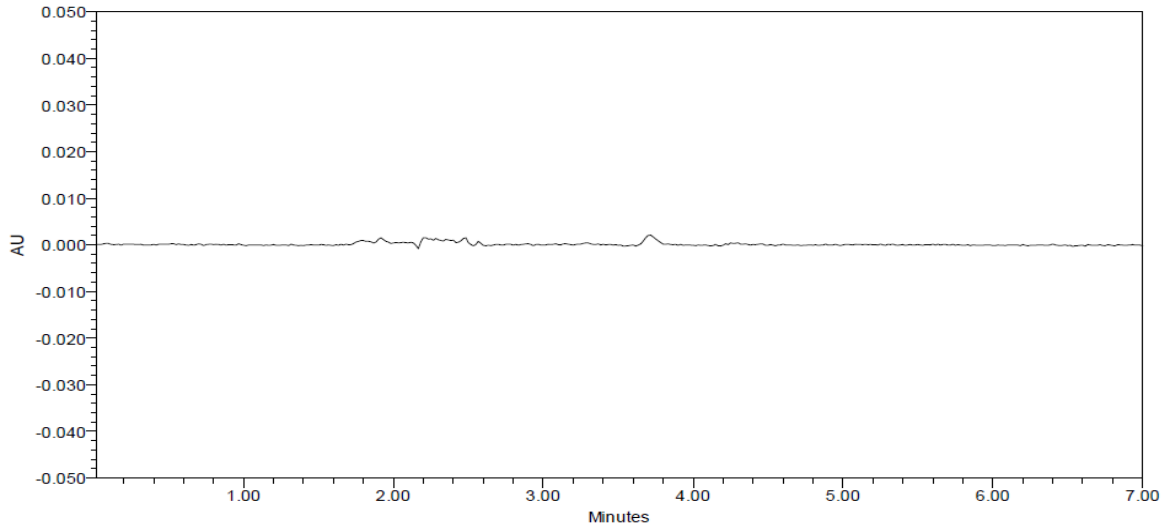

Fig 6: Chromatogram of blank.

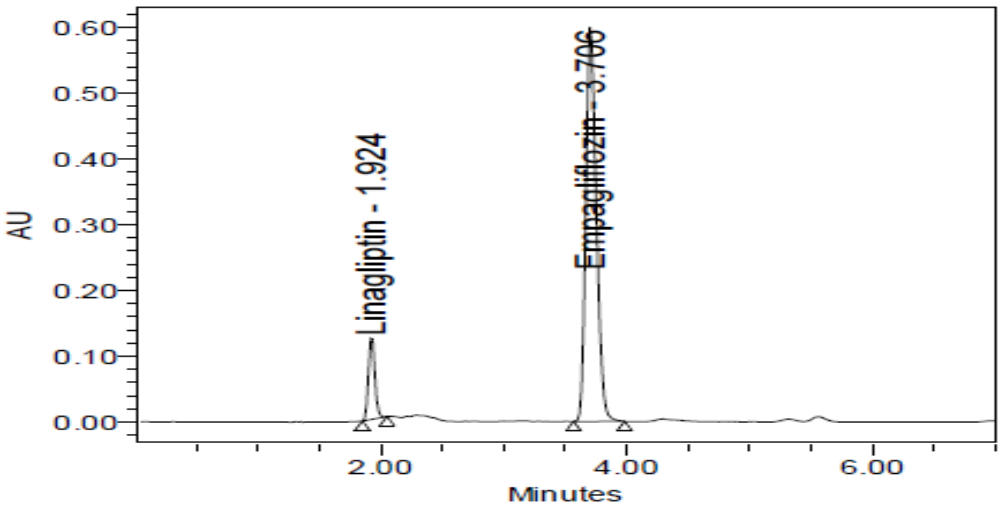

Figure No. 7: Typical chromatogram of Linagliptin and Empagliflozin.

Linearity: Six Linear concentrations of Linagliptin (12.5-75µg/ml) and Empagliflozin

(25-150 µg/ml) are prepared and Injected. Regression equation of the Linagliptin and Empagliflozin are found to be, y = 9531.x + 4618, and y = 37150x + 745.2. And regression co-efficient was 0.999.

Table No. : 2 Calibration data of Linagliptin and Empagliflozin method.

Sr. No.	Concentration Linagliptin	Response	Concentration Empagliflozin	Response
1	0	0	0	0
2	25%	126420	25%	905911
3	50%	245671	50%	1934386
4	75%	367825	75%	2778580
5	100%	477424	100%	3645445
6	125%	593849	125%	4628418
7	150%	723119	150%	5616375

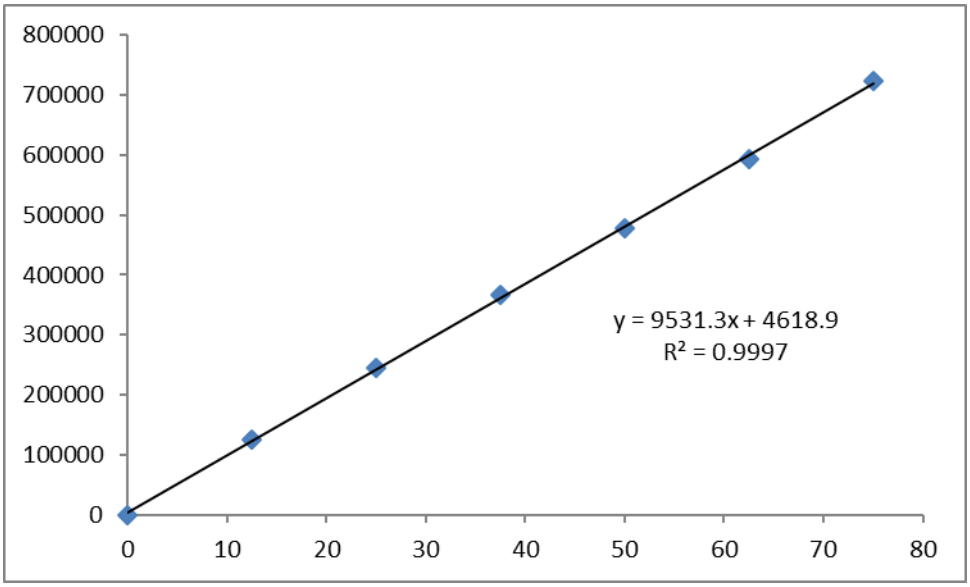

Figure No. 8: Calibration curve of Linagliptin

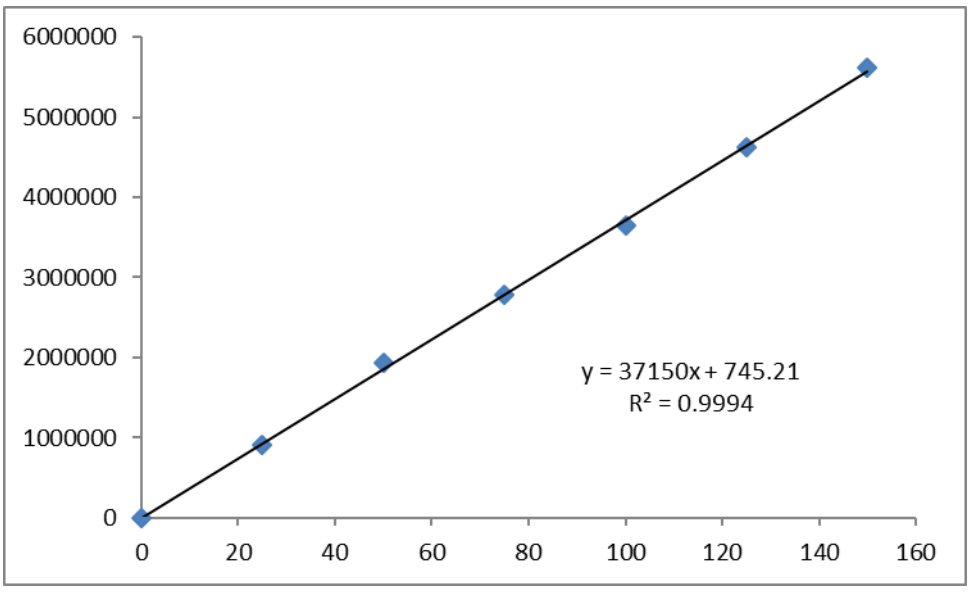

Figure No. 9: Calibration curve of Empagliflozin

SUMMARY AND CONCLUSION

Parameters	Linagliptin	Empagliflozin
Calibration range (mcg/ml)	12.5-75 ppm	25-150 ppm
Optimized wavelength	210nm	210nm
Retention time	1.920min	3.699 min
Regression equation (Y*)	y = 9531.x + 4618.	y = 37150x + 745.2
Correlation coefficient(r^2)	0.999	0.999
Precision (% RSD*)	1.0	0.94
% Assay	100.63%	100.20%
Limit of Detection (mcg / ml)	0.24ppm	0.17ppm
Limit of Quantization (mcg / ml)	0.72ppm	0.51ppm

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Linagliptin and Empagliflozin in Tablet dosage form. Retention time of Linagliptin and Empagliflozin were found to be 1.920min and 3.699 min. % RSD of the Linagliptin and Empagliflozin were and found to be 1.0 and 0.94 respectively. % assay was obtained as 100.63% and 100.20% for Linagliptin and Empagliflozin respectively. LOD, LOQ values are obtained from regression equations of Linagliptin and Empagliflozin were 0.24ppm, 0.72ppm and 0.17ppm, 0.51ppm respectively. Regression equation of Linagliptin and Empagliflozin is y = 9531.x + 4618, and y = 37150x + 745.2. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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