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Development of Stability Indicating RP- HPLC Method for the Drugs Used in Type 2 Diabetes Mellitus

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Linagliptin and Empagliflozin in Tablet dosage form. The retention time of Linagliptin and Empagliflozin was 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.

INTRODUCTION:

Pharmaceutical product quality [1] is of vital importance for patient safety. The presence of impurities may influence the efficacy and safety of pharmaceuticals. Impurities and potential degradation of products can cause changing of chemical, pharmacological and toxicological properties of drugs having significant impact on product quality and safety. Drug stability is considered to be the secure way to ensure the delivery of therapeutic values to the patients.

Stability is defined as the capacity of a drug substance to remain within the established specifications to maintain its identity, strength, quality and purity throughout the retest or expiration dating period.

Stability of a pharmaceutical/medicinal product is defined as the capability of a particular formulation, in a specific container/closure system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications. Pharmaceutical products are expected to meet their specifications for identity, purity, quality and strength throughout their defined storage period at specific storage conditions.

Stability [2]-5 is an essential factor of the quality, safety and efficacy of a drug substance. A drug substance, which is not of sufficient stability, can result in changes in physical (like appearance, melting point, clarity and colour of solution, water, crystal modification(polymorphism), or particle size) as well as chemical characteristics (increase in impurities and decrease in assay) and microbiological attributes (Total bacterial count, fungal count and for pathogenic microbes).

The purpose of stability testing is to provide evidence on how the quality of a drug substance varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a retest period for the drug substance and recommended storage conditions. The degree of variability of individual batches affects the confidence that a future production batch will remain within specification throughout the assigned re-test period.

Stability [6-9] plays an important role in the drug development process. It explains several factors that affect the expiration dating of drug products, including the chemical and physical stability during the pre-clinical formulation stages, process development, packaging development, and post-marketing life. The evaluation of the physicochemical stability of a

given product requires an understanding of the physical and chemical properties of the drug substance. The two main aspects of drug products that play an important role in shelf life determination are assay of active drug and degredents generated during the stability studies[10-12].

The container closure system must be evaluated for compatibility with the drug substance and drug product to ensure that the container and closure system does not contribute to degradation or contamination.

MATERIALS AND METHODS

Materials:

Linagliptin and Empagliflozin, Combination Linagliptin and Empagliflozin tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, 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 Empagliflozin solutions.

Methods:

Preparation of buffer:

Buffer: (0.1 % OPA)

1 ml of con. OPA is dissolved in a 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 Empagliflozine 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 in to 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 Empagliflozine working Standards into a 25ml and 25ml clean dry volumetric flask respectively, add 20ml and 20ml of diluent, sonicated for 30 minutes and make up 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 was 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 was 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 was injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock solution LINAGLIPTIN and EMPAGLIFLOZIN, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c.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 1 ml of stock solution LINAGLIPTIN and EMPAGLIFLOZIN, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. 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.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105 °C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 50µg/ml&100µg/ml solution and10µl 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 200 Watt hours/m^2 in photo stability 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 60º. For the HPLC study, the resultant solution was diluted to 50µg/ml & 100µg/ml solution and 10 µl 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.

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 made up with Water and Acetonitrile (50:50).

Wavelength : 285nm

Temperature : 30°C

Injection Volume : 10µl

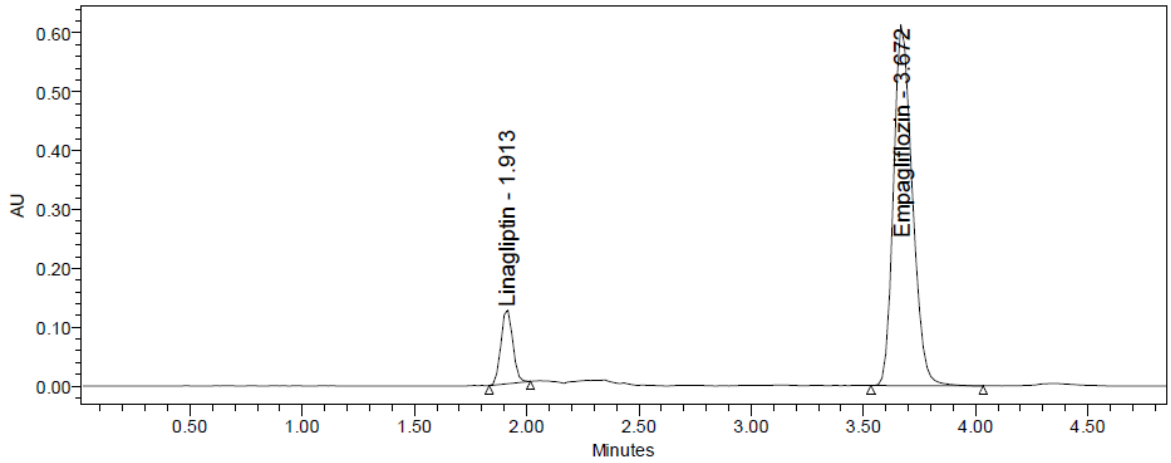

Fig 1 Optimized chromatogram of Linagliptin and Empagliflozin

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: 1 System suitability studies of Linagliptin and Empagliflozine 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

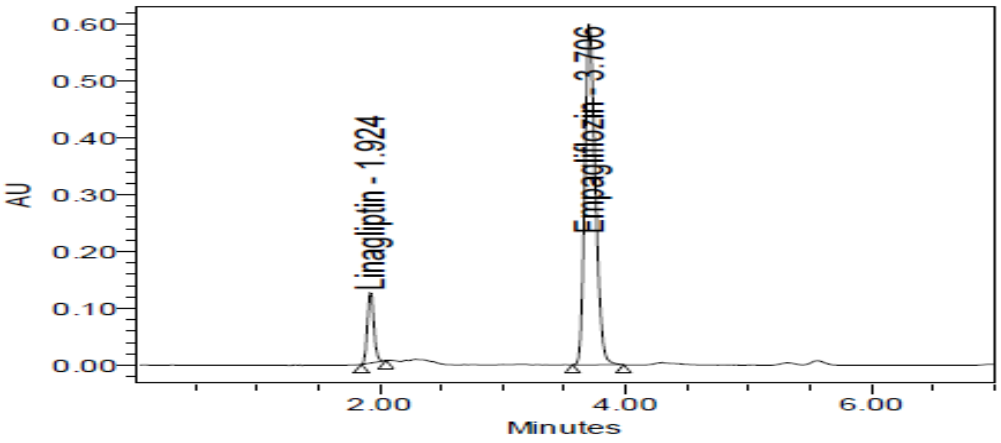

Fig: 2 Typical chromatograms of Linagliptin and Empagliflozin.

1. Linearity: Six Linear concentrations of Linagliptin (12.5-75µg/ml) and Empagliflozin (25-150 µg/ml) are prepared and Injected. The 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: 2 Calibration data of Linagliptin and Empagliflozin method.

S.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

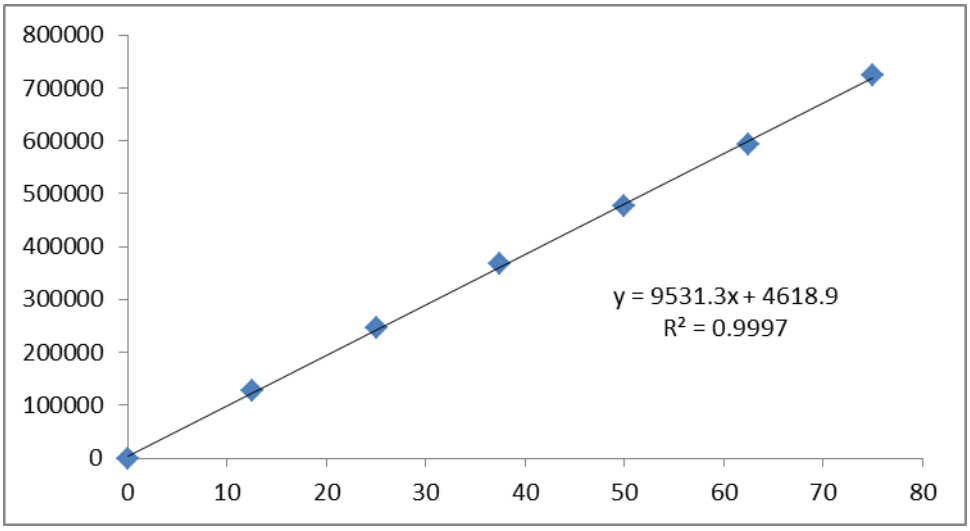

Fig: 3 Calibration curve of Linagliptin

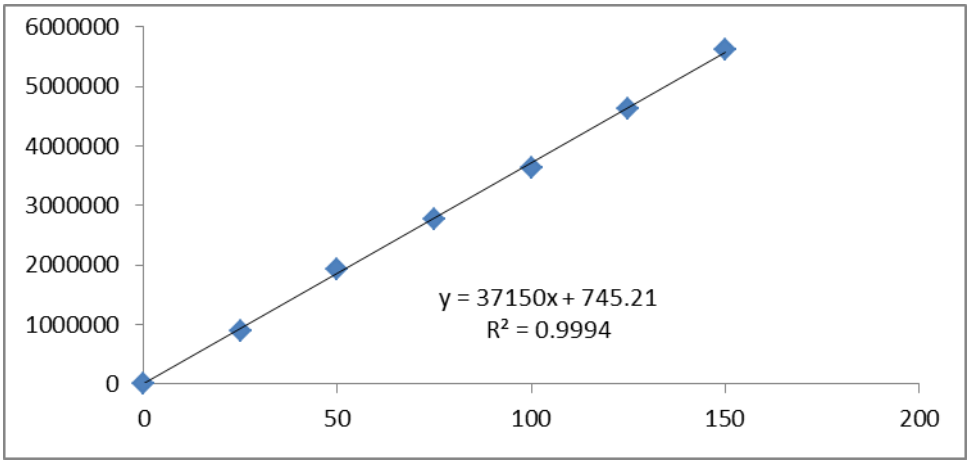

Fig: 4 Calibration curve of Empagliflozin

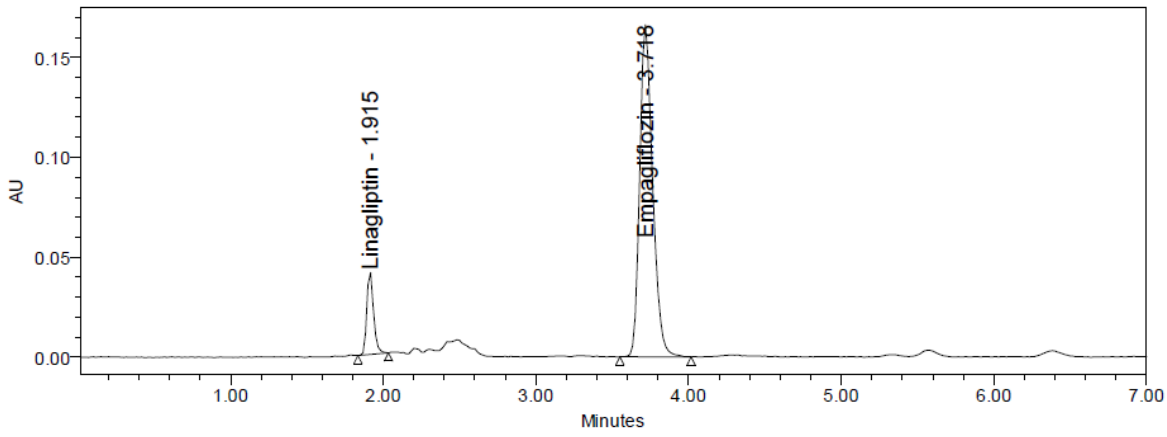

Fig: 5 Linearity 25% Chromatogram of Linagliptin and Empagliflozin

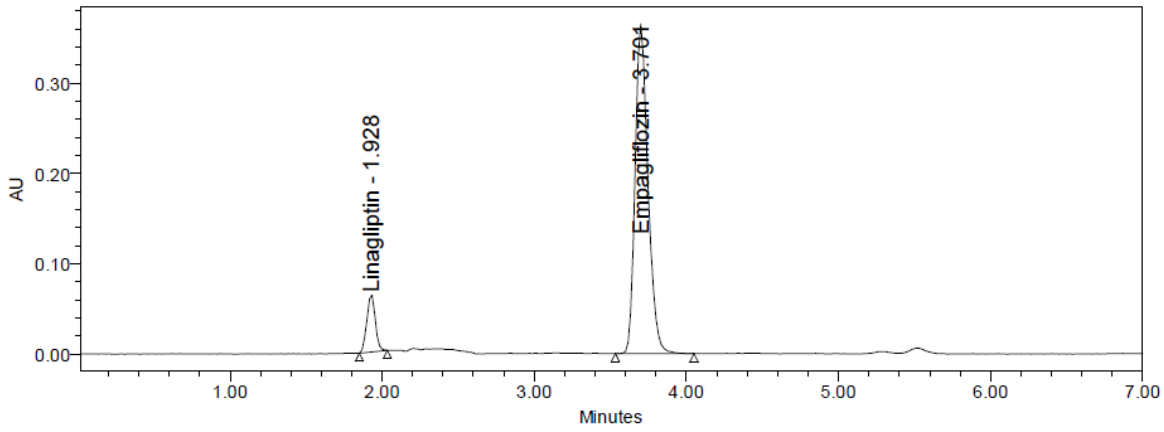

Fig: 6 Linearity 50% Chromatogram of Linagliptin and Empagliflozin

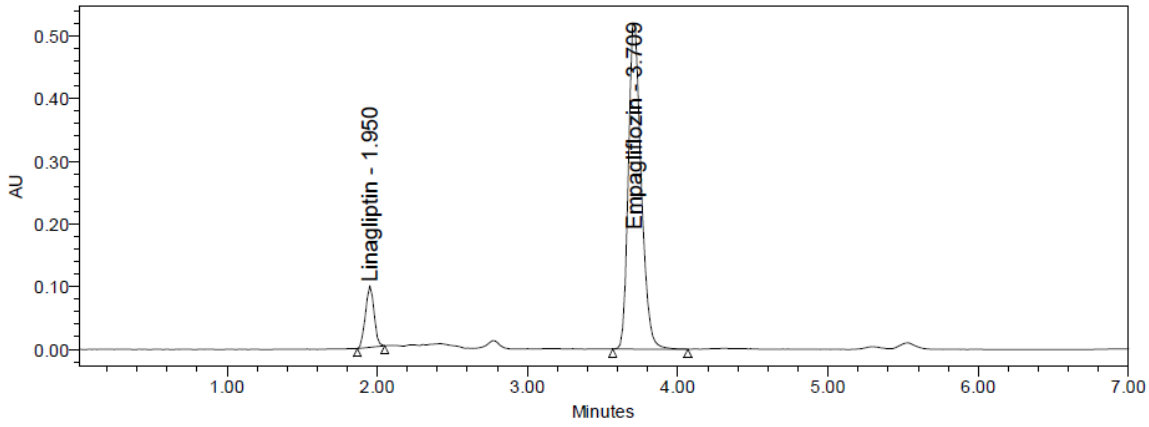

Fig: 7 Linearity 75% Chromatogram of Linagliptin and Empagliflozin

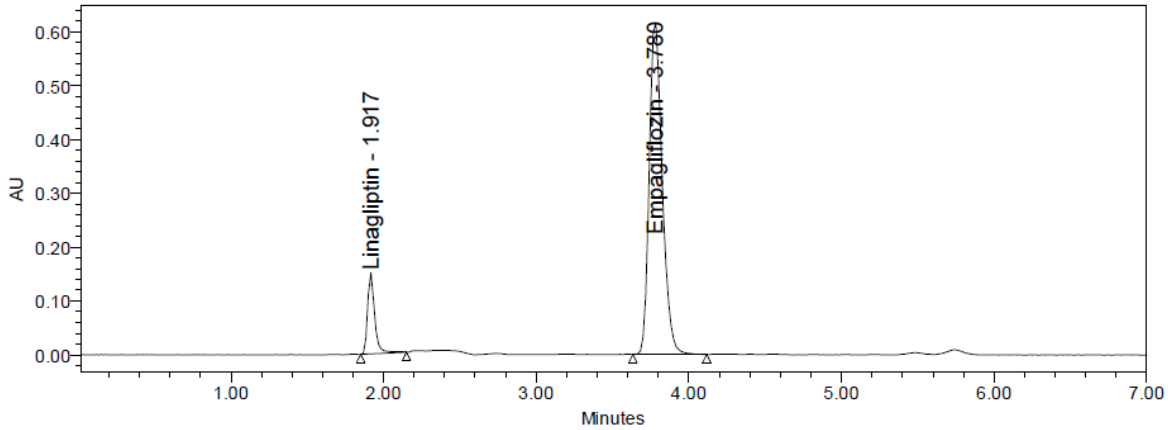

Fig: 8 Linearity 100% Chromatogram of Linagliptin and Empagliflozin

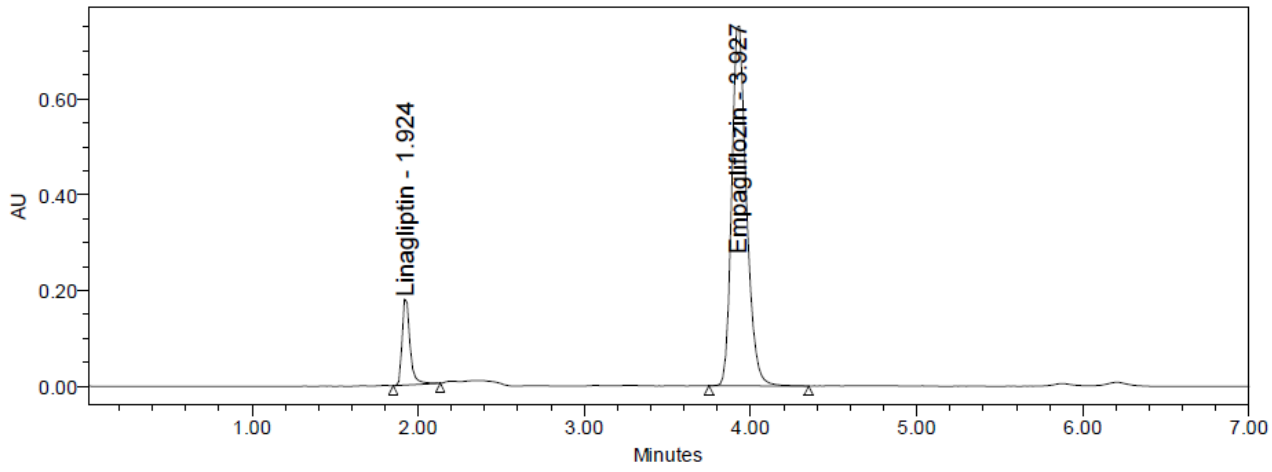

Fig: 9 Linearity 125% Chromatogram of Linagliptin and Empagliflozin

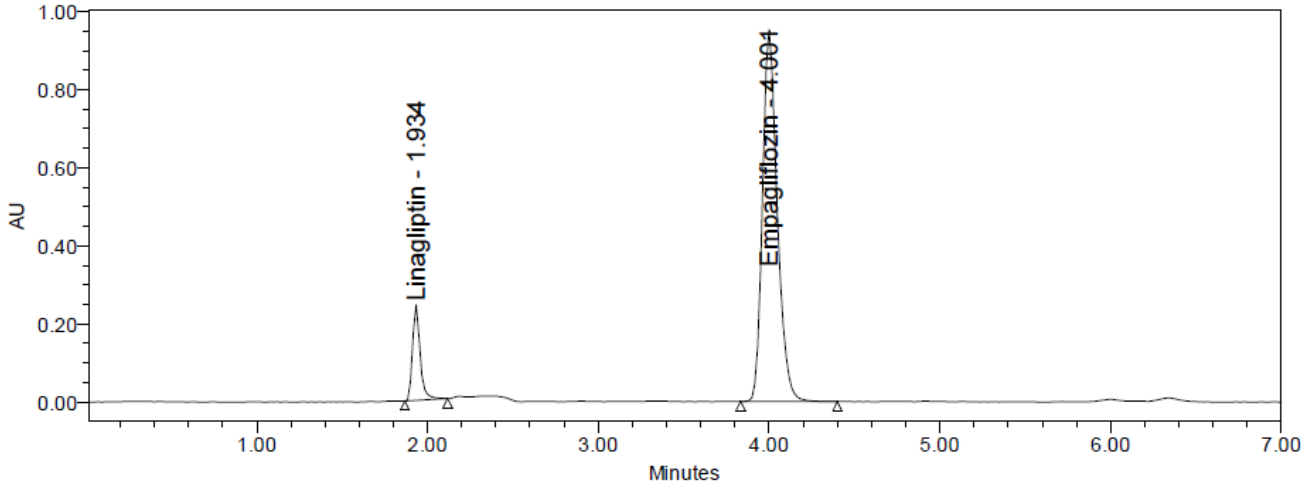

Fig: 10 Linearity 150% Chromatogram of Linagliptin and Empagliflozin

2. Precision:

Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Linagliptin and Empagliflozin were found to be 1.0% and 0.94% respectively.

Table: 3 Repeatability results for Linagliptin and Empagliflozin.

Sr. No.	Linagliptin	Empagliflozin
1	462762	3598346
2	463892	3598711
3	467252	3587845
4	469414	3565861
5	473880	3656485
6	472737	3563766
Mean	468323	3595169
Std. Dev.	4544.4	33708
%RSD	1.0	0.94

*Average of six determinations

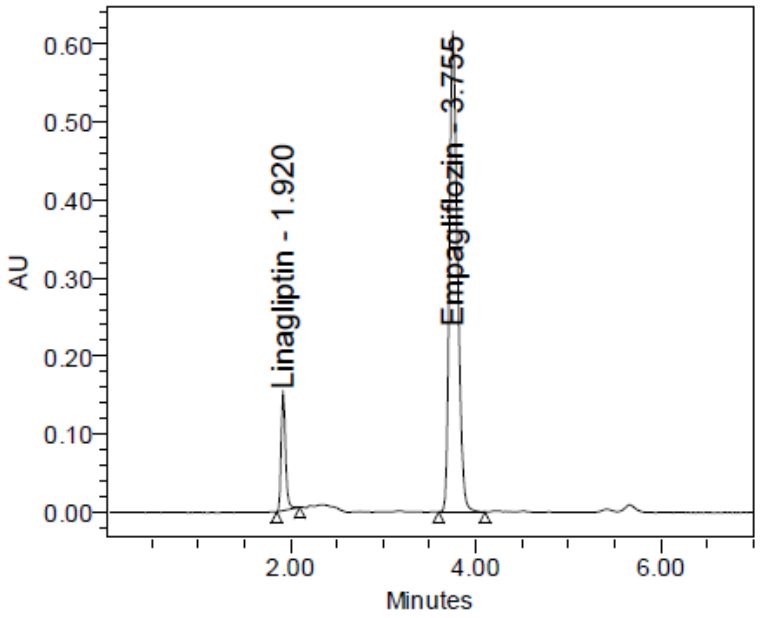

Fig: 11 Repeatability Chromatogram of Linagliptin and Empagliflozin

Inter day precision: Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Linagliptin and Empagliflozin were 0.60% and 0.43%.

Table 4 Inter-day precision results for Linagliptin and Empagliflozin .

Sr. No.		Linagliptin	Empagliflozin
1		457175	3611613
2		457484	3572397
3		459261	3587699
4		464175	3598528
5		459196	3574390
Mean		459155	3590575
Std. Dev.		2616	15326
%RSD		0.6	0.43

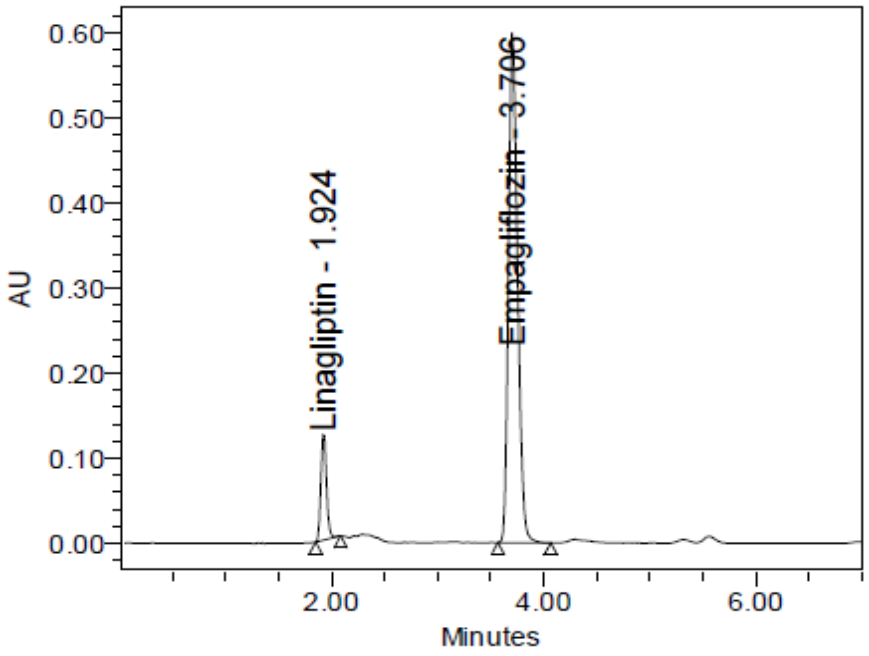

Fig: 12 Inter-precision Chromatogram of Linagliptin and Empagliflozin

3. Accuracy: Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 6.5.

Table: 5 Table of Accuracy

Sample	Concentration (%) (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Linagliptin	25	25.28	101.11	0.94
	50	49.89	99.79	0.54
	75	75.15	100.20	0.25
Empagliflozin	50	50.56	101.12	0.57
	100	100.58	100.58	0.85
	150	151.06	100.71	0.34

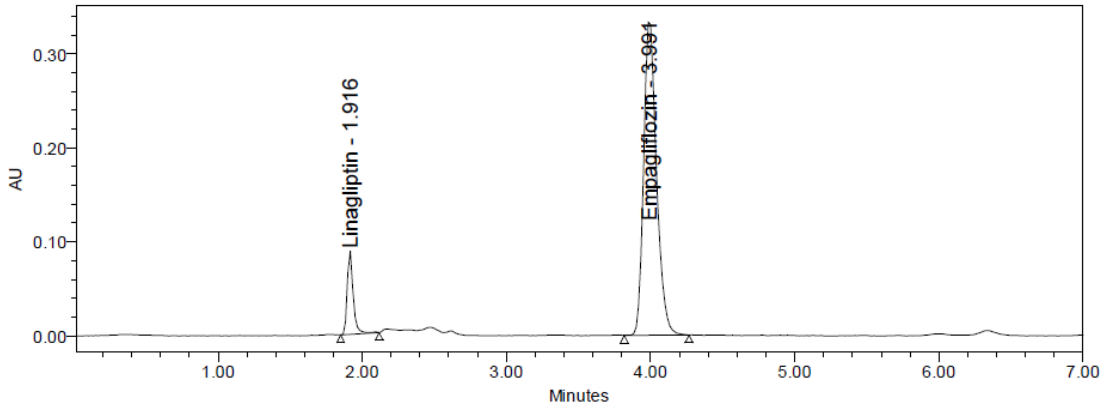

Fig: 13 Accuracy 50% Chromatogram of Linagliptin and Empagliflozin

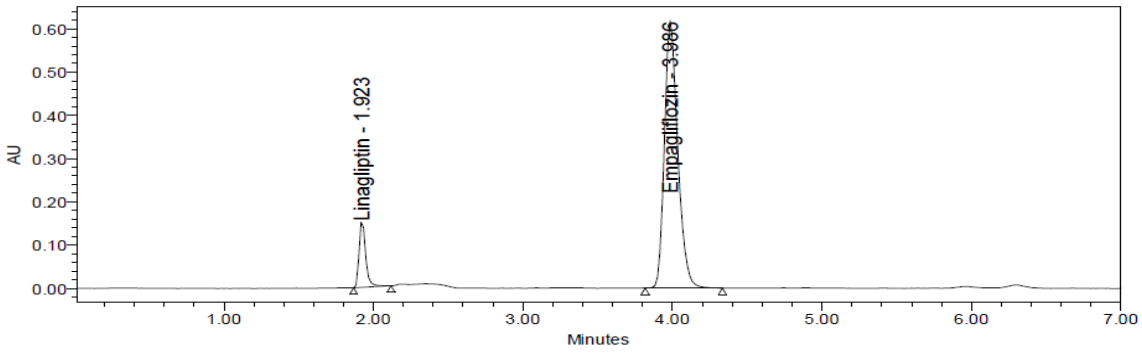

Fig: 14 Accuracy 100% Chromatogram of Linagliptin and Empagliflozin

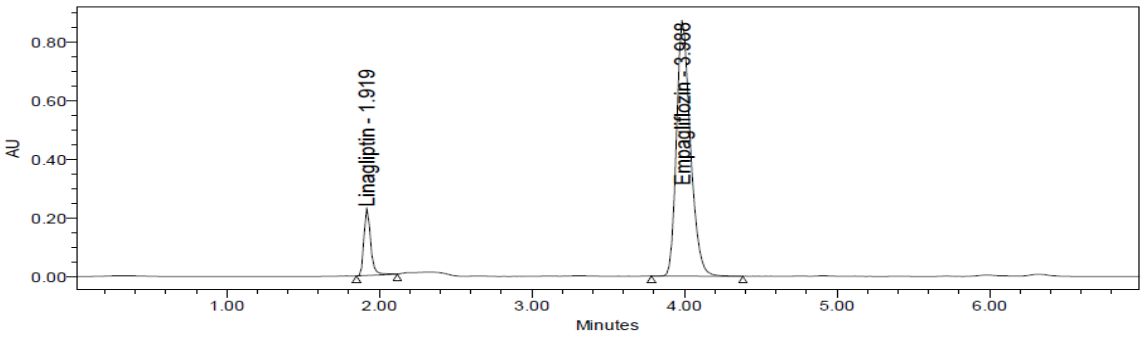

Fig: 15 Accuracy 150% Chromatogram of Linagliptin and Empagliflozin

4. LOD: Limit of detection was calculated by std deviation method Linagliptin and Empagliflozin and LOD for Linagliptin and Empagliflozin were found to be 0.24and 0.17respectively.

5. LOQ: Limit of Quantification was calculated by std deviation method Linagliptin and Empagliflozin and LOQ for Linagliptin and Empagliflozin were found to be 0.72 and 0.51 respectively.

6. Robustness: Small deliberate changes in a method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guidelines.

Table 6 Robustness data of Linagliptin and Empagliflozin

S.NO	Robustness condition	Linagliptin %RSD	Empagliflozin %RSD
1	Flow minus	1.34	0.65
2	Flow Plus	0.75	0.20
3	Mobile phase minus	0.23	0.26
4	Mobile phase Plus	0.02	0.15
5	Temperature minus	0.94	0.66
6	Temperature Plus	1.36	0.06

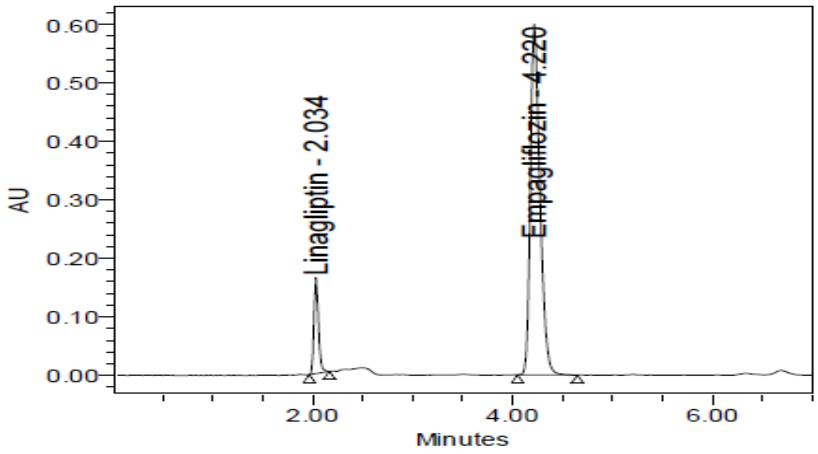

Fig: 16 Flow minus Chromatogram of Linagliptin and Empagliflozin

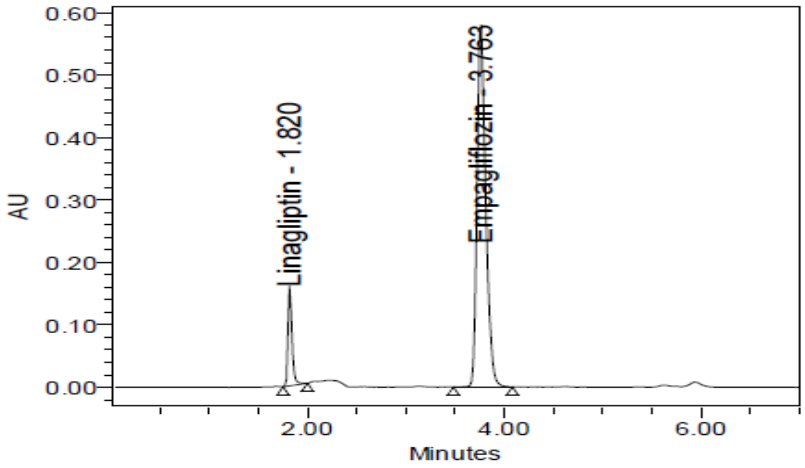

Fig: 17 Flow plus Chromatogram of Linagliptin and Empagliflozin

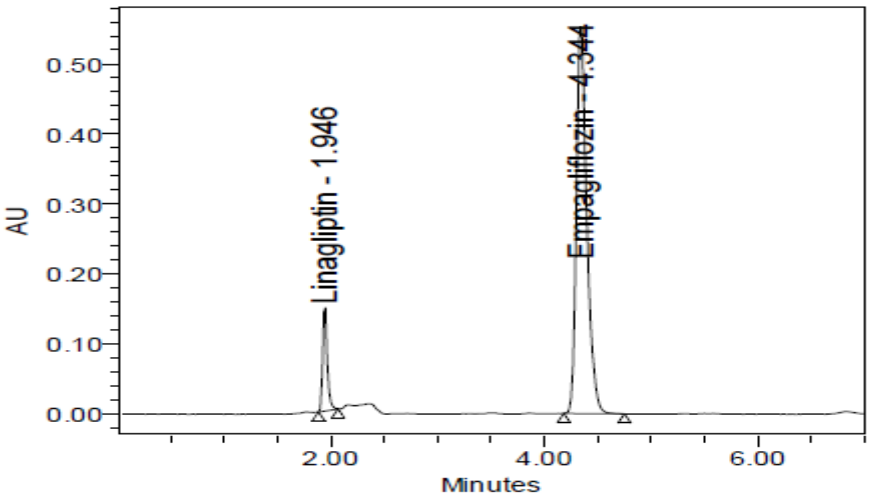

Fig: 18 Mobile phase minus Chromatogram of Linagliptin and Empagliflozin

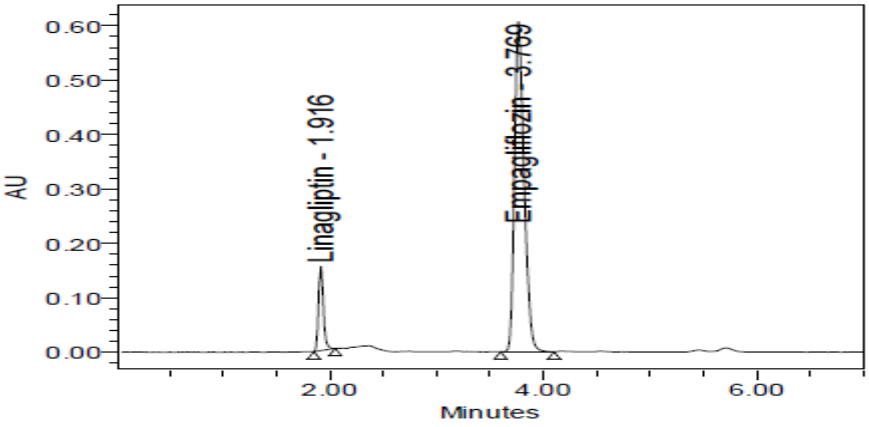

Fig: 19 Mobile phase Plus Chromatogram of Linagliptin and Empagliflozin

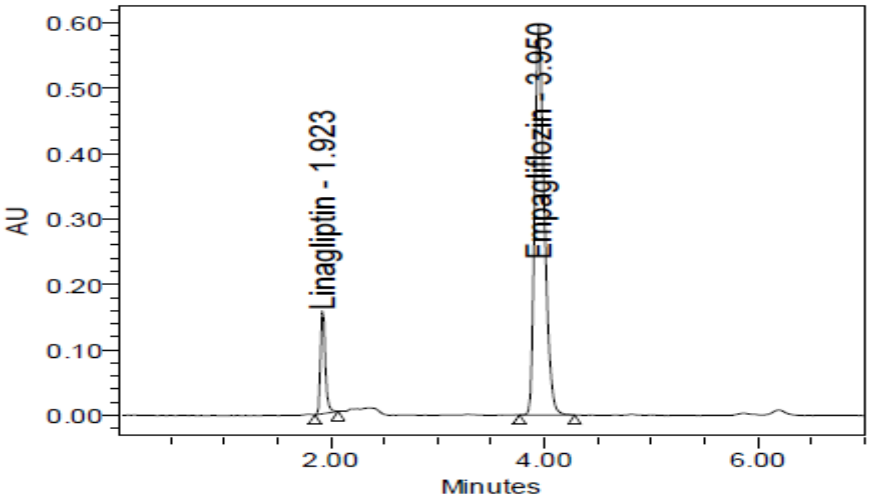

Fig: 20 Temperature minus Chromatogram of Linagliptin and Empagliflozin

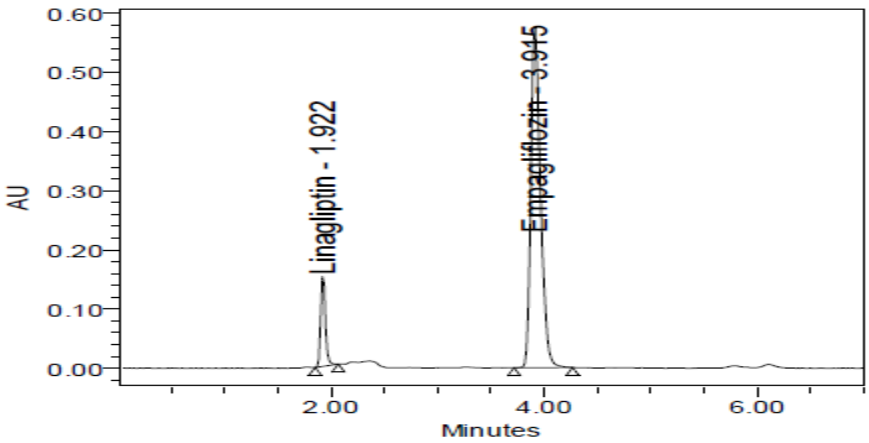

Fig: 21Temperature Plus Chromatogram of Linagliptin and Empagliflozin

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected in six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.63% and 100.20% for Linagliptin and Empagliflozin respectively.

Table 7 Assay of Tablet

S. No.	Linagliptin %Assay	Empagliflozin %Assay
1	99.44	100.29
2	99.68	100.30
3	100.40	100.00
4	100.87	99.38
5	101.83	101.91
6	101.58	99.33
AVG	100.63	100.20
STDEV	0.9765	0.9395
%RSD	1.0	0.94

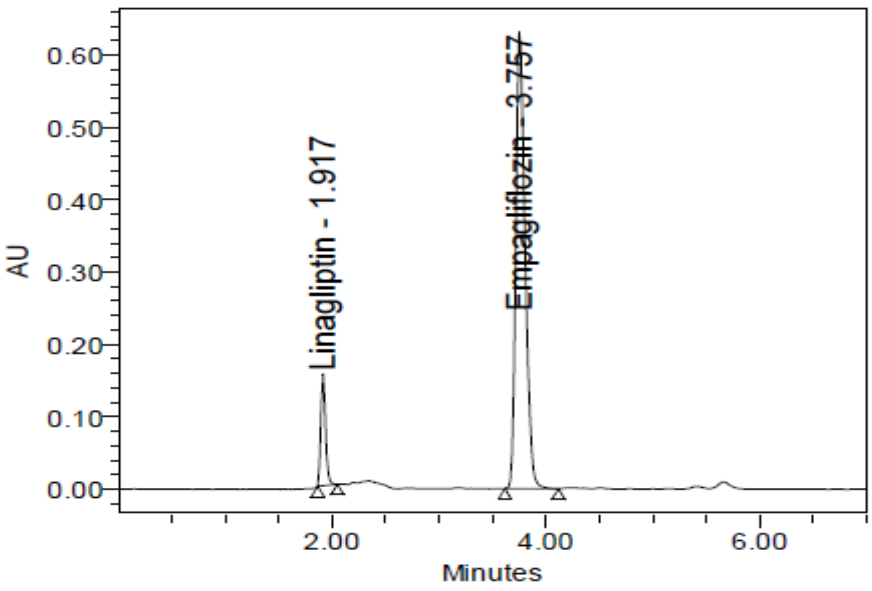

Fig: 22 Assay of Tablet

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.

Table 8 Degradation Data of LINAGLIPTIN

S.NO	Degradation Condition	AREA	% ASAAY	AMOUNT DEGRADED %
1	Acid	452252	97.18	2.82
2	Alkali	459880	98.82	1.18
3	Oxidation	449176	96.52	3.48
4	Thermal	462236	99.32	0.68
5	UV	461745	99.22	0.78
6	Water	463578	99.61	0.39

Table 9 Degradation Data of EMPAGLIFLOZIN

S.NO	Degradation Condition	AREA	%ASSAY	AMOUNT DEGRADED %
1	Acid	3503766	97.65	2.35
2	Alkali	3528711	98.35	1.65
3	Oxidation	3486053	97.16	2.84
4	Thermal	3561306	99.26	0.74
5	UV	3551594	98.99	1.01
6	Water	3564912	99.36	0.64

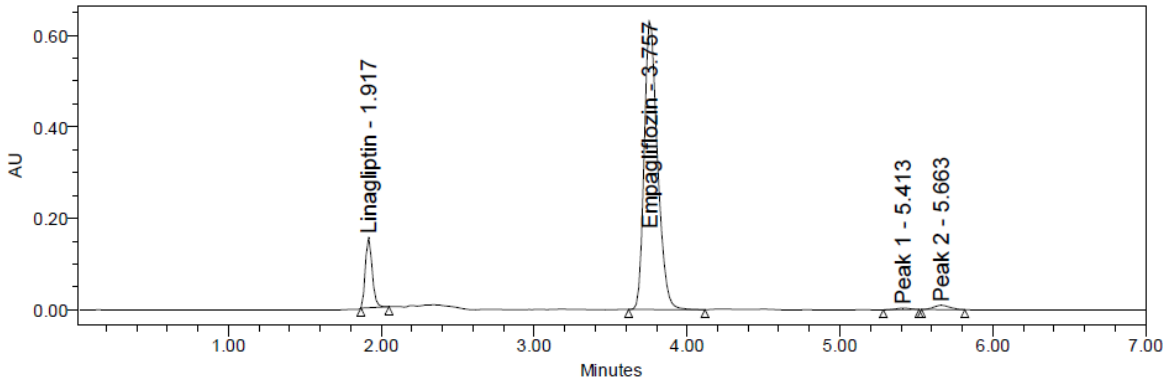

Fig 23 Acid chromatogram

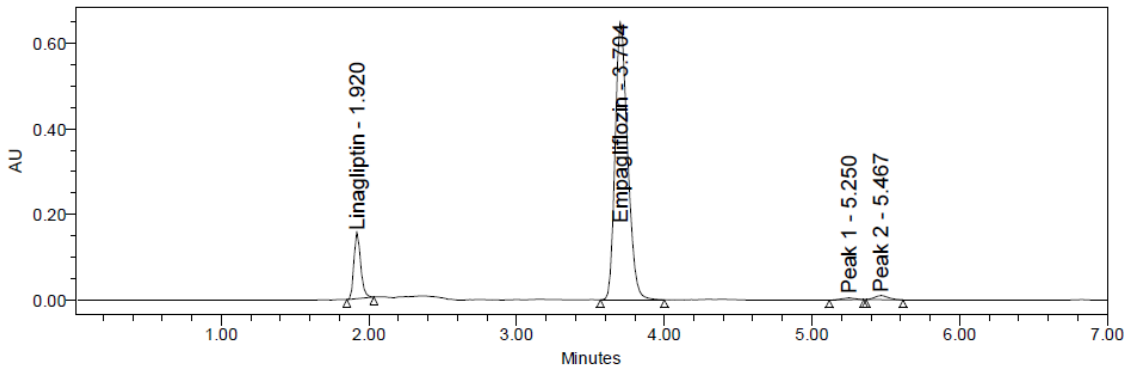

Fig 24 Base chromatogram

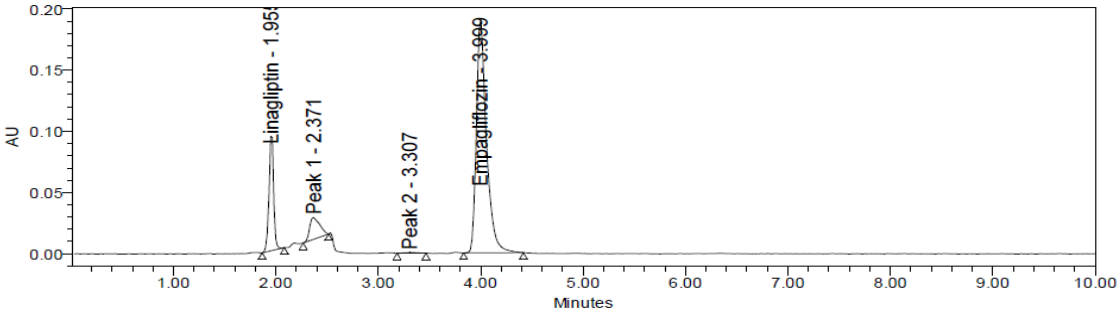

Fig 25 Peroxide chromatogram

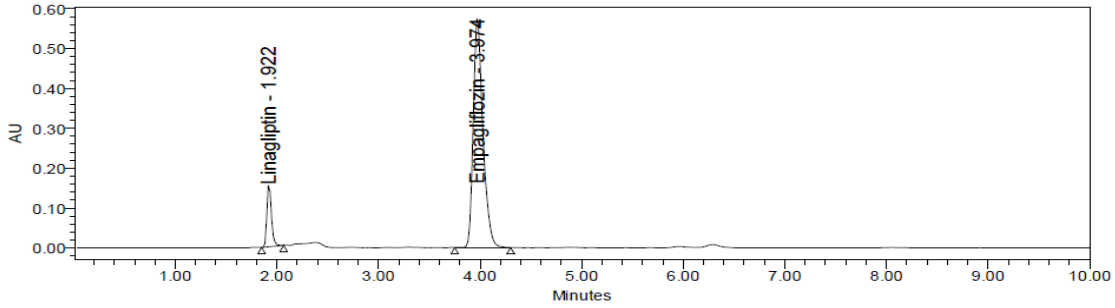

Fig 26 Thermal chromatogram

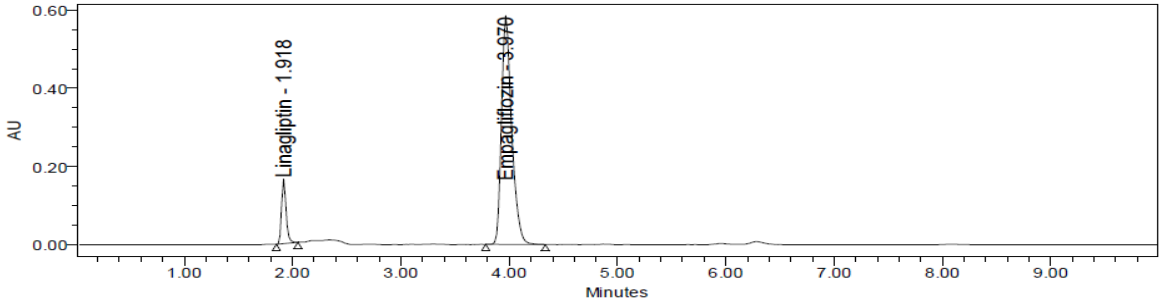

Fig 27 UV chromatogram

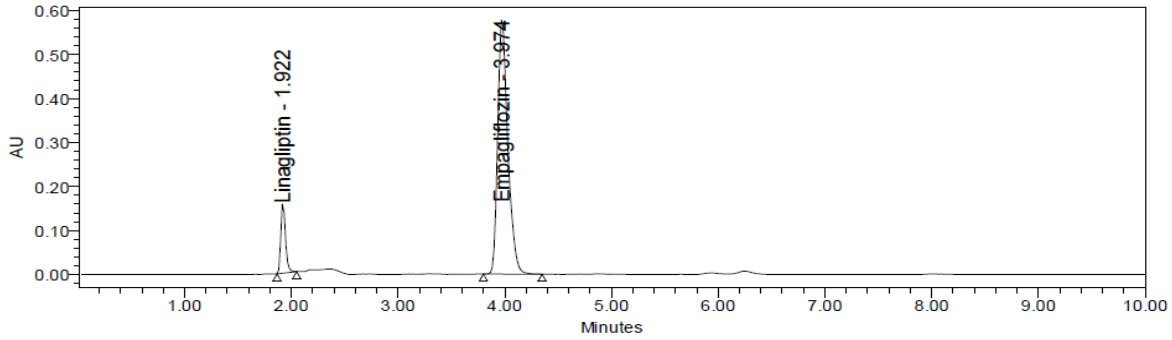

Fig 28 Water chromatogram

SUMMARY AND CONCLUSION

Summary Table

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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