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DEVELOPMENT AND VALIDATION OF A NEW ANALYTICAL RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF ANTI-DIABETIC AGENTS EMPAGLIFLOZIN AND LINAGLIPTIN IN BULK AND MARKETED FIXED-DOSE COMBINATION

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Abstract:

Objective: *To develop a simple, selective and rapid reverse phase high performance liquid chromatography (RP-HPLC) method and validate as per ICH guidelines for simultaneous determination of Empagliflozin and Linagliptin in a combined dosage form.*

Methods: *The chromatographic separation of the two anti-Diabetic drugs were achieved using Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size, maintained at 38 °C throughout the analysis. The drugs were separated in isocratic elution mode with a mobile phase of Methanol: Tri Ethyl Amine Buffer (35:65% v/v) at a flow rate of 1.0 mL/min and a detection wavelength of 261 nm using a PDA detector.*

Results: *The linearity and range for Empagliflozin and Linagliptin were 60 to 140 mg/mL (R2> 0.9997) and 100 to 500 mg/mL (R2> 0.9997), respectively. Mean recoveries observed for Empagliflozin and Linagliptin were 100.36% and 100.15%, respectively. The precision of the method obtained was for Empagliflozin and for Linagliptin with a relative standard deviation less than 2%. The lower degree of % RSD that was obtained for intermediate precision has proved that the method is robust and rugged.*

Conclusion: *A simple and rapid RP-HPLC method was developed and validated for simultaneous determination of Empagliflozin and Linagliptin in a combined dosage form and hence, it can be used in the quality control analysis of an active pharmaceutical ingredient and pharmaceutical dosage form.*

Key Words: *Empagliflozin and Linagliptin, RP-HPLC, ICH Guidelines.*

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INTRODUCTION:

Empagliflozin is an inhibitor of sodium-glucose co-transporter-2 (SGLT2), the transporters primarily responsible for the reabsorption of glucose in the kidney. It is used clinically as an adjunct to diet and exercise, often in combination with other drug therapies, for the management of type 2 diabetes mellitus. Empagliflozin is an orally available competitive inhibitor of sodium-glucose co-transporter 2 (SGLT2; SLC5A2) with antihyperglycemic activity[1]. Upon oral administration, Empagliflozin selectively and potently inhibits SGLT2 in the kidneys, thereby suppressing the reabsorption of glucose in the proximal tubule. Inhibition of SGLT2 increases urinary glucose excretion by the kidneys, resulting in a reduction of plasma glucose levels in an insulin-independent manner[2]. SGLT2, a transport protein exclusively expressed in the proximal renal tubules, mediates approximately 90% of renal glucose reabsorption from tubular fluid. Empagliflozin is used with a proper diet and exercise program to control high blood sugar in people with type 2 diabetes[3]. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems. The IUPAC name of Empagliflozin is (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl] oxy phenyl] methyl] phenyl]-6-(hydroxy methyl) oxane-3, 4, 5-triol. The Chemical Structure of Empagliflozin is shown in following figure-1.

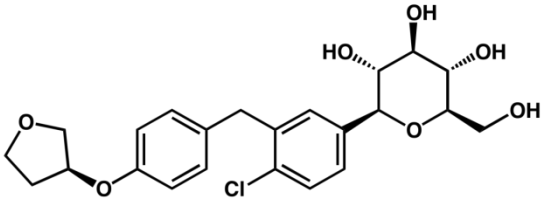

Fig-1: Chemical Structure of Empagliflozin

Linagliptin is a DPP-4 inhibitor developed by Boehringer Ingelheim for the treatment of type II diabetes. Linagliptin differs from other DPP-4 inhibitors in that it has a non-linear pharmacokinetic profile, is not primarily eliminated by the renal system, and obeys concentration dependant protein binding[4]. Linagliptin is a potent, orally bioavailable dihydropurinedione-based inhibitor of dipeptidyl peptidase 4 (DPP-4), with hypoglycemic activity. The inhibition of DPP-4 by Linagliptin appears to be longer lasting than that by some other DPP-4 inhibitors tested. Linagliptin is indicated for the treatment of type II diabetes in addition to diet and exercise[5]. It should not be used to treat type I diabetes or in diabetic ketoacidosis. An extended-release combination product containing Empagliflozin, Linagliptin, and metformin was approved by the FDA in January 2020 for the improvement of glycemic control in adults with type 2 diabetes mellitus when used adjunctively with diet and exercise[6]. The IUPAC name of Linagliptin is 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methyl quinazolin-2-yl) methyl] purine-2, 6-dione. The Chemical Structure of Linagliptin is shown in follows

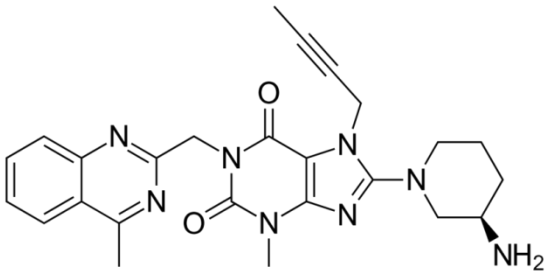

Fig-2: Chemical Structure of Linagliptin

MATERIALS AND METHODS

Instruments Used:

Table-1: Instruments Used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module. 996 PDA detector, software: Empower 2
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Digital Ultra Sonicator	Labman

Chemicals Used:

Table-2: Chemicals Used

S.No.	Chemical	Brand Names
1	Empagliflozin	SAG Health Science Pvt Ltd
2	Linagliptin	SAG Health Science Pvt Ltd
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Empagliflozin and Linagliptin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 1ml of the above Empagliflozin and 3ml of Linagliptin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Empagliflozin and Linagliptin sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent[7].

Further pipette 1ml of Empagliflozin and 3ml Linagliptin above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[18,19,20,21].

Preparation of Mobile Phase:

Accurately measured 350ml (35%) of Methanol, 650ml of Tri Ethyl Amine Buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent[8].

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Empagliflozin and 10mg of Linagliptin working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Empagliflozin and 3ml of Linagliptin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[9].

Specificity Study of Drug:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Empagliflozin and 10mg of Linagliptin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1ml of Empagliflozin and 3ml of Linagliptin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Empagliflozin and Linagliptin sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 1ml of Empagliflozin and 3ml Linagliptin above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent[10].

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Preparation of Drug Solutions for Linearity:
Accurately weigh and transfer 10 mg of Empagliflozin and 10mg of Linagliptin working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (60ppm of Empagliflozin & 100ppm of Linagliptin):
Pipette out 0.6ml of Empagliflozin and 1ml of Linagliptin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (80ppm of Empagliflozin & 200ppm of Linagliptin):
Pipette out 0.8ml of Empagliflozin and 2ml of Linagliptin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (100ppm of Empagliflozin & 300ppm of Linagliptin):
Pipette out 1ml of Empagliflozin and 3ml of Linagliptin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluen[t12].

Preparation of Level – IV (120ppm of Empagliflozin & 400ppm of Linagliptin):
Pipette out 1.2ml of Empagliflozin and 4ml of Linagliptin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (140ppm of Empagliflozin & 500ppm of Linagliptin):
Pipette out 1.4ml of Empagliflozin and 5ml of Linagliptin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:
Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient[13].

Precision
Repeatability
Preparation of Empagliflozin and Linagliptin Solution for Precision:
Accurately weigh and transfer 10 mg of Empagliflozin and 10mg of Linagliptin working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Empagliflozin and 3ml of Linagliptin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.
The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified [limits14.]

Intermediate Precision:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions[15].

Procedure:
Day 1:
The sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
Day 2:
The sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:
For preparation of 50% Sample Stock solution:
Accurately weigh and transfer 10 mg of Empagliflozin and 10mg of Linagliptin working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 0.5ml of Empagliflozin and 1.5ml of Linagliptin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent[s16].

For preparation of 100% Sample Stock solution:
Accurately weigh and transfer 10 mg of Empagliflozin and 10mg of Linagliptin working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Empagliflozin and 3ml of Linagliptin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Sample Stock solution:
Accurately weigh and transfer 10 mg of Empagliflozin and 10mg of Linagliptin working Sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5ml of Empagliflozin and 4.5ml of Linagliptin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Empagliflozin and Linagliptin and calculate the individual recovery and mean recovery values.

Robustness:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results[17].

For preparation of Standard solution:
Accurately weigh and transfer 10 mg of Empagliflozin and 10mg of Linagliptin working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
Further pipette 1ml of Empagliflozin and 3ml of Linagliptin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:
The sample was analyzed by variation of mobile phase i.e. Methanol: Tri Ethyl Amine (35:65% v/v) was taken in the ratio and 40:60, 30:70 instead (35:65% v/v) remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION:

Analytical Method Development:

Optimized Chromatographic Condition:

Mobile phase : Methanol: Tri Ethyl Amine Buffer (35:65% v/v)
Column : Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size
Flow rate : 1 ml/min
Wavelength : 261 nm
Column temp : 38ºC
Injection Volume : 10 µl
Run time : 10 minutes

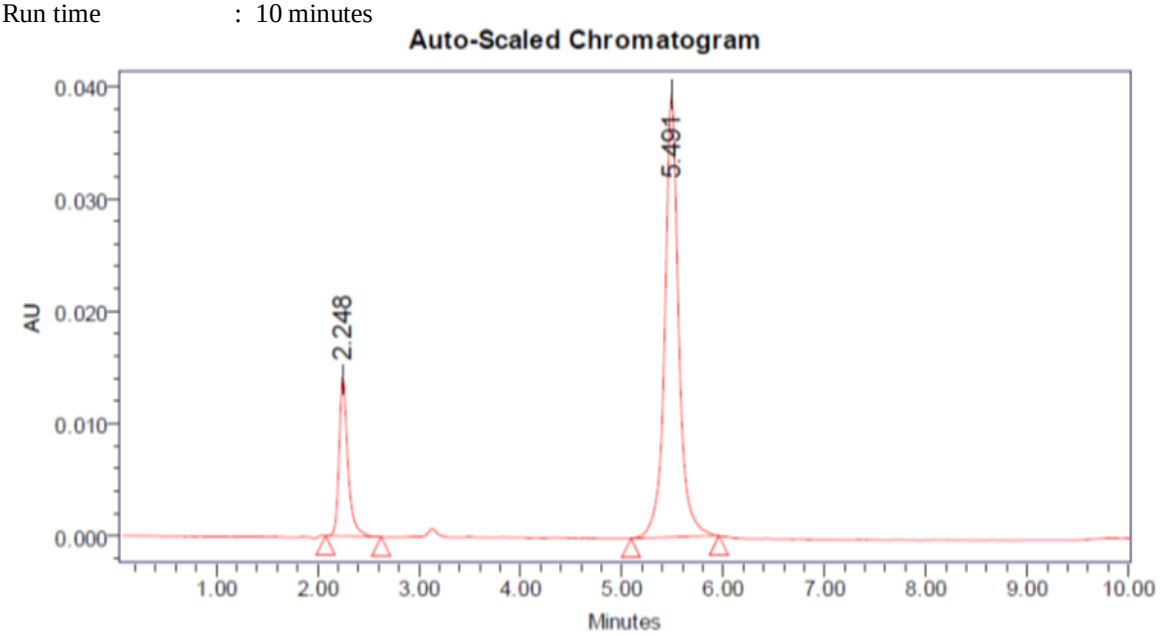

Fig-3: Optimized Chromatogram

Validation of Analytical Method:

Method was validated as per ICH guidelines with respect to linearity, accuracy, precision, specificity, and robustness, limit of detection and limit of quantification[29,30].

System Suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated[22].

Table-3: Results of System Suitability for Empagliflozin

S.No.	Name	Rt	Peak Area	Height	USP plate Count	USP Tailing
1	Empagliflozin	2.247	105698	18652	7592	1.08
2	Empagliflozin	2.246	105874	18754	7584	1.09
3	Empagliflozin	2.248	105698	18698	7562	1.08
4	Empagliflozin	2.252	105465	18689	7549	1.08
5	Empagliflozin	2.248	105236	18695	7591	1.09
Mean			**105594.2**			
Std. Dev			**247.4049**			
% RSD			**0.234298**			

Table-4: Results of System Suitability for Linagliptin

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Linagliptin	5.452	1856985	63659	6359	1.05	5.86
2	Linagliptin	5.484	1856754	63598	6384	1.04	5.85
3	Linagliptin	5.491	1856985	63845	6395	1.05	5.86
4	Linagliptin	5.482	1856574	63989	6345	1.04	5.86
5	Linagliptin	5.491	1854735	63895	6395	1.05	5.85
Mean			**1856407**				
Std. Dev			**950.2696**				
% RSD			**0.051189**				

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitates Empagliflozin and Linagliptin in marketed formulation[23].

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Observation: The % purity of Empagliflozin and Linagliptin in pharmaceutical dosage form (marketed formulation) was found to be 99.72%.

Linearity:

Linearity can be evaluated by introducing a series of standard stock solutions/diluted stock solution into the mobile phase/solvent with a minimum of five different concentrations ranging from 60–140 µg/ml and 100-500µg/ml of the expected working ranges for Empagliflozin and Linagliptin respectively[24-26].

Table-5: Chromatographic Data for Linearity Study for Empagliflozin:

Concentration µg/ml	Average Peak Area
60	648743
80	856982
100	1068542
120	1268984
140	1469853

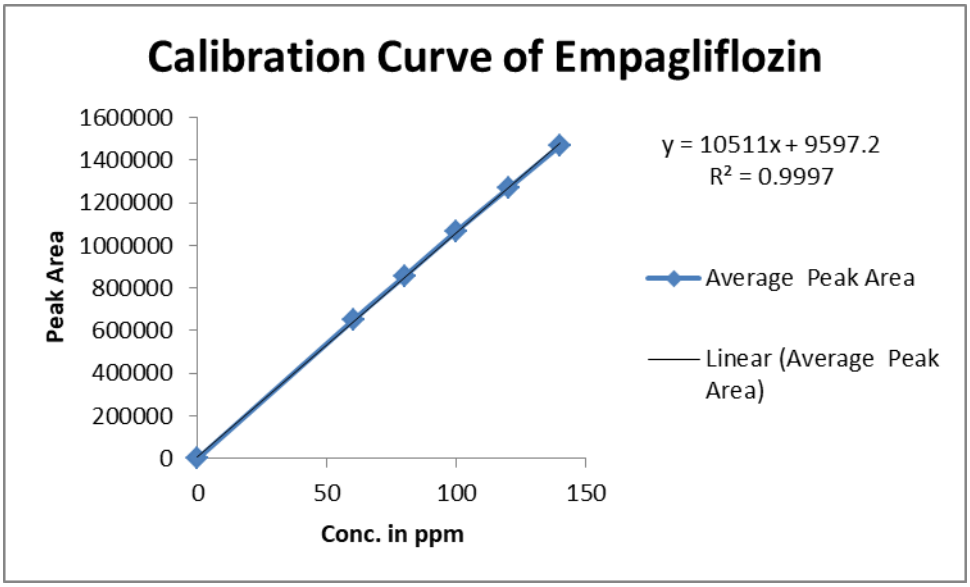

Fig-4: Calibration Graph for Empagliflozin

LINEARITY PLOT: The plot of Concentration (x) versus the Average Peak Area (y) data of Empagliflozin is a straight line.

Y = mx + c
Slope (m) = 10511
Intercept (c) = 9597
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 9597. These values meet the validation criteria[27].

Table-6: Linearity Data of Linagliptin

Concentration µg/ml	Average Peak Area
100	667564
200	1268547
300	1868598
400	2465487
500	3085864

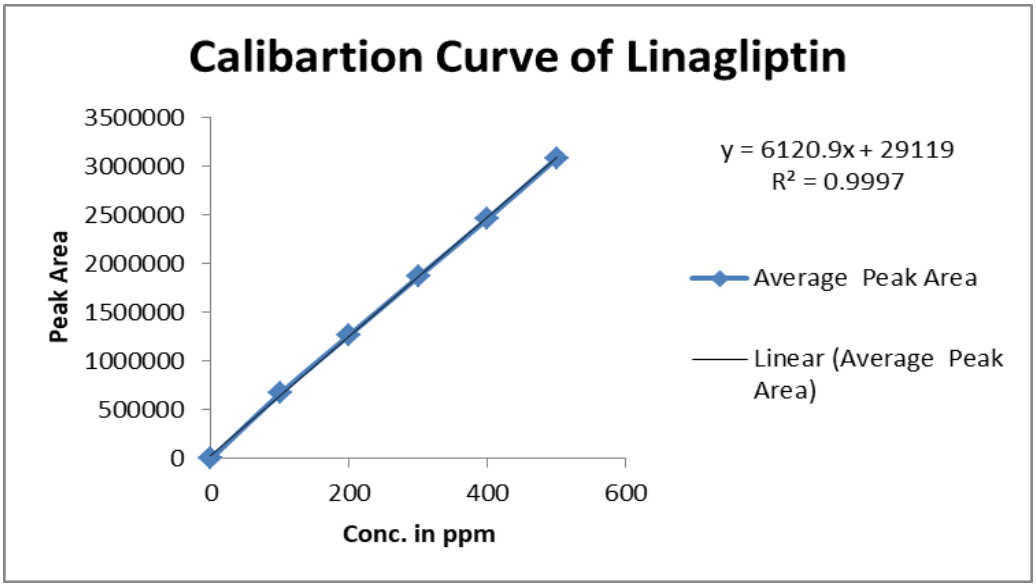

Fig-5: Calibration Graph for Linagliptin

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Linagliptin is a straight line.

Y = mx + c
Slope (m) = 6120
Intercept (c) = 29119
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater[28].

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 29119. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability: Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-7: Results of Repeatability for Empagliflozin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Empagliflozin	2.269	105698	18569	7598	1.08
2	Empagliflozin	2.255	105684	18547	7546	1.09
3	Empagliflozin	2.252	105421	18594	7549	1.09
4	Empagliflozin	2.267	105879	18574	7538	1.08
5	Empagliflozin	2.260	105326	18563	7582	1.08
Mean			105601.6			
Std. Dev			224.5023			
% RSD			0.212594			

Table-8: Results of Method Precision for Linagliptin

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Linagliptin	5.274	1856985	63598	6359	1.05	5.86
2	Linagliptin	5.266	1857458	63579	6357	1.04	5.85
3	Linagliptin	5.265	1854795	63547	6358	1.04	5.86
4	Linagliptin	5.278	1857469	63592	6357	1.05	5.86
5	Linagliptin	5.305	1857685	63569	6345	1.04	5.85
Avg			1856878				
Std. Dev			1192.4				
% RSD			0.064215				

Intermediate Precision:

Day 1:

Table-9: Results of Intermediate Precision for Empagliflozin

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Empagliflozin	2.248	115246	19685	7698	1.09
2	Empagliflozin	2.245	116985	19654	7685	1.09
3	Empagliflozin	2.242	115847	19675	7645	1.09
4	Empagliflozin	2.239	116985	19682	7682	1.09
5	Empagliflozin	2.243	115848	19654	7691	1.09
6	Empagliflozin	2.246	116582	19647	7642	1.10
Mean			**116248.8**			
Std. Dev			**710.3091**			
% RSD			**0.611025**			

Table-10: Results of Intermediate Precision for Linagliptin

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Linagliptin	5.284	1948592	64582	6459	1.05	5.96
2	Linagliptin	5.293	1958245	64256	6475	1.06	5.95
3	Linagliptin	5.306	1947584	64598	6498	1.05	5.96
4	Linagliptin	5.319	1948675	64785	6472	1.06	5.95
5	Linagliptin	5.346	1959854	64585	6493	1.05	5.96
6	Linagliptin	5.352	1958246	64924	6438	1.06	5.96
Mean			**1953533**				
Std. Dev			**5792.661**				
% RSD			**0.296522**				

Day 2:

Table-11: Results of Intermediate precision Day 2 for Empagliflozin

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Empagliflozin	2.255	102658	62584	6259	1.03
2	Empagliflozin	2.260	102856	62359	6276	1.02
3	Empagliflozin	2.242	102658	62451	6215	1.03
4	Empagliflozin	2.245	102698	62584	6285	1.02
5	Empagliflozin	2.260	102451	62758	6235	1.03
6	Empagliflozin	2.255	102368	62154	6298	1.02
Mean			**102614.8**			
Std. Dev			**176.9592**			
% RSD			**0.17245**			

Table-12: Results of Intermediate precision for Linagliptin

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Linagliptin	5.266	1798952	62859	6265	1.03	5.42
2	Linagliptin	5.265	1789854	62985	6289	1.02	5.43
3	Linagliptin	5.306	1798659	62895	6279	1.03	5.42
4	Linagliptin	5.293	1789898	62785	6285	1.02	5.43
5	Linagliptin	5.265	1796856	62354	6249	1.03	5.42
6	Linagliptin	5.266	1798568	62589	6245	1.02	5.43
Mean			**1795465**				
Std. Dev			**4390.879**				
% RSD			**0.244554**				

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (50, 100 and 150 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery Empagliflozin and Linagliptin by standard addition method[31].

Table-13: The Accuracy Results for Empagliflozin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	539070	50	50.373	100.746%	100.36%
100%	1063578	100	100.274	100.274%	
150%	1587149	150	150.085	100.056%	

Table-14: The accuracy results for Linagliptin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	949127	150	150.328	100.218%	100.15%
100%	1867824	300	300.441	100.147%	
150%	2785321	450	450.359	100.079%	

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value[32].

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Empagliflozin:

=2.63µg/ml

Linagliptin:

=3.84µg/ml

Limit of Quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined[33].

LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Empagliflozin:

= 7.92µg/ml

Linagliptin:

= 11.54µg/ml

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Empagliflozin and Linagliptin[34].

The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The samples (marketed formulation) of Empagliflozin and Linagliptin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count[35-39].

Table-15: Robustness Data for Empagliflozin:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	105265	2.256	7589	1.08
Less Flow rate of 0.9 mL/min	109898	2.505	7256	1.05
More Flow rate of 1.1 mL/min	102365	2.046	7469	1.07
Less organic phase	101548	2.505	7358	1.06
More organic phase	104645	2.046	7659	1.02

Table-16: Robustness Data for Linagliptin:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1858475	5.427	6354	1.04
Less Flow rate of 0.9 mL/min	1925684	5.599	6253	1.05
More Flow rate of 1.1 mL/min	1863525	4.576	6248	1.03
Less organic phase	1825471	5.599	6415	1.02
More organic phase	1836594	4.576	6529	1.06

SUMMARY AND CONCLUSION:

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 261 nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Tri Ethyl Amine Buffer (35:65% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 10 min because analyze gave peak around 2.248, 5.491 ± 0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 60-140µg/ml of Empagliflozin and 100-500µg/ml of Linagliptin of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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