Method Development and Validation for the Simultaneous Estimation of Empagliflozin and Linagliptin in Bulk and Tablet Dosage Form by Using HPLC

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Abstract:
It was discovered that empagliflozin and ligandipitin may be measured simultaneously using the RP-HPLC technique.The chromatographic separations were performed using a mobile phase consisting of 0.1% and an Agilent Eclipse column (4.6 x 150mm, 5m). A 280 nm PDA detector was used in conjunction with a waters HPLC auto sampler and a separation module 2695 HPLC system to detect TEA:Methanol (30:70) at a flow rate of 1 ml/min. The duration is ten minutes. Empagliflozin had a retention duration of 1.053 minutes and ligandiliptin 2.677 minutes. Empagliflozin had a linearity correlation coefficient value of 0.999 and a concentration range of 20-100 µg/ml, while Linagliptin had a value of 10-50 µg/ml. The overall recovery for Empagliflozin was determined to be 100% and for Linagliptin it was also determined to be 100%. empagliflozin limit of detection 3.07 and 10.09. Levels of detection for ligandipitin were 2.95 and 9.93, respectively. This study's findings suggest that the suggested RP-HPLC technique might be beneficial for routinely estimating Empagliflozin and Linagliptin in bulk and tablet dose forms, as it is simple, accurate, precise, rugged, robust, rapid, and repeatable.

Keywords: Empagliflozin, Linagliptin, RP-HPLC, Simultaneous estimation.

INTRODUCTION:

Empagliflozin is an SGLT2 inhibitor used to manage type 2 diabetes mellitus. Empagliflozin is an inhibitor of sodium-glucose co-transporter-2 (SGLT2), the transporters primarily responsible for the reabsorption of glucose in the kidney.[1] 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. SGLT2 on the apical membrane of these cells then utilize this gradient to facilitate secondary active co-transport of both Na+ and glucose out of the filtrate, thereby reabsorbing glucose back into the blood – inhibiting this co-transport, then, allows for a marked increase in glucosuria and decrease in blood glucose levels. Empagliflozin is a potent inhibitor of renal SGLT2 transporters located in the proximal tubules of the kidneys and works to lower blood glucose levels via an increase in glucosuria.[2] IUPAC name of Empagliflozin is 2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl] oxyphenyl] methyl] phenyl]-6-(hydroxymethyl) oxane-3,4,5-triol. Molecular Formula is $C_{23}H_{27}ClO_7$. Molecular Weight is 450.9. Empagliflozin is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, which should be purged with an inert gas. The solubility of empagliflozin in these solvents is approximately 30 mg/ml. Empagliflozin is sparingly soluble in aqueous buffers.

Linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor used to manage hyperglycemia in patients with type 2 diabetes mellitus.Linagliptin is a competitive, reversible DPP-4 inhibitor.[3] Inhibition of this enzyme slows the breakdown of GLP-1 and glucose-dependant insulinotropic polypeptide (GIP). GLP-1 and GIP stimulate the release of insulin from beta cells in the pancreas while inhibiting release of glucagon from pancreatic beta cells5. These effects together reduce the breakdown of glycogen in the liver and increase insulin release in response to glucose.[4] IUPAC name of Linagliptin is 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl) methyl] purine-2,6-dione. Molecular formula is $C_{25}H_{28}N_8O_2$. Molecular Weight is 472.5. Linagliptin is soluble in methanol (ca. 60 mg/mL), sparingly soluble in ethanol

(ca. 10 mg/mL), very slightly soluble in isopropanol (<1 mg/mL), and very slightly soluble in acetone (ca. 1 mg/mL).

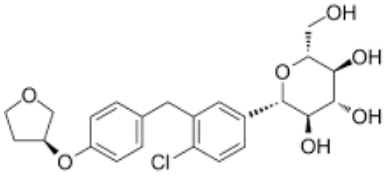

Figure 1: Structure of Empagliflozin

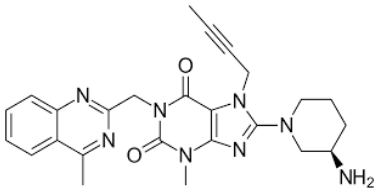

Figure 2: Structure of Linagliptin

The literature survey revealed that There are very few methods reported in the literature for analysis of Empagliflozin and Linagliptin alone or in combination with other drugs in the pure form and pharmaceuticals formulations by UV [5-8,] UPLC [9,] LCMS [10], RP-HPLC [11-25]. In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Simultaneous estimation of Empagliflozin and Linagliptin in dosage form, attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Empagliflozin and Linagliptin. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Empagliflozin and Linagliptin in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form. To apply the developed method for the simultaneous estimation of Empagliflozin and Linagliptin in bulk and tablet dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Empagliflozin and Linagliptin were Purchased from Honour labs. KH_2PO_4 was analytical grade supplied by Finerchemcal limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 280 nm with column Agilent Eclipse coloumn (4.6 x 150mm, 5µm), dimensions at 25^0C temperature. The optimized mobile phase consists of 0.1% TEA: Methanol (30: 70). Flow rate was maintained at 1 ml/min and run time for 10 min.

Preparation of solutions:

Preparation of 0.1% TEA:

Take 1ml Orthophosphoric acid in 1000ml volumetric flask and make up mith HPLC water and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of 0.1% TEA Buffer and 700 ml (60%) of Methanol were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Standard Solution Preparation:
Accurately weigh and transfer 40 mg of Empagliflozin and 20 mg of Linagliptin working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:
Accurately weigh and transfer equivalent to 40 mg of Empagliflozin and 20 mg of Linagliptin sample into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.
Procedure:
Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Empagliflozin and Linagliptin peaks and calculate the %Assay by using the formulae.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.
System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 10 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Agilent Eclipse coloumn (4.6 x 150mm, 5µm), the mobile phase of composition 0.1% TEA: Methanol (30: 70) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.
Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Empagliflozin and Linagliptin in their tablet dosage form. The result obtained for Empagliflozin and Linagliptin was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:
Linearity and Range: Stock solution was prepared by dissolving the appropriate amount of Empagliflozin and Linagliptin in 7 ml of diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 20 µg/ml to 100 µg/ml of Empagliflozin and 10 µg/ml to 50 µg/ml of Linagliptin. 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. The results are shown in table 3,4.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Linagliptin and Empagliflozin and calculate the individual recovery and mean recovery values. The results are shown in table 5,6.
Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard 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. The results are shown in table 7.
Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard 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. The results are shown in table 8.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1 ml/min. The results are shown in table 9,10,11,12.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 13.
LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

DEGRADATION STUDIES:
The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Empagliflozin and Linagliptin using the proposed method. The results are shown in table 14

Preparation of stock: Accurately weigh and transfer 40 mg of Empagliflozin and 20 mg of Linagliptin working standard into a 100 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Hydrolytic degradation under acidic condition: Pipette 1.5 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.
Hydrolytic degradation under alkaline condition: Pipette 1.5 ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 24 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.44 microns syringe filters and place in vials.
Thermal induced degradation: Empagliflozin and Linagliptine sample was taken in petridish and kept in Hot air oven at 1100 C fo 3 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.
Oxidative degradation: Pipette 1.5 ml above stock solution into a 10ml volumetric flask and 1ml of 30% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.
Photo degradation: Pipette 1.5 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

RESULTS AND DISCUSSION

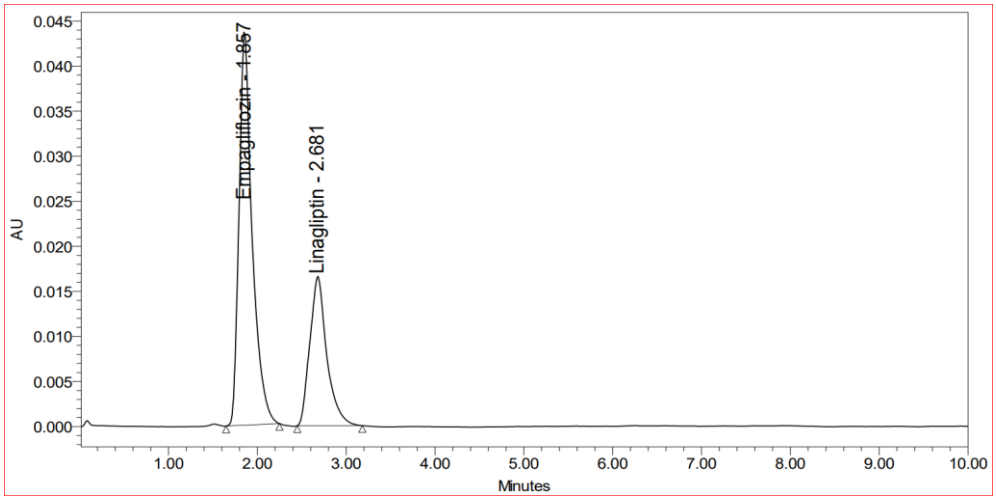

Figure 3: Standard chromatogram

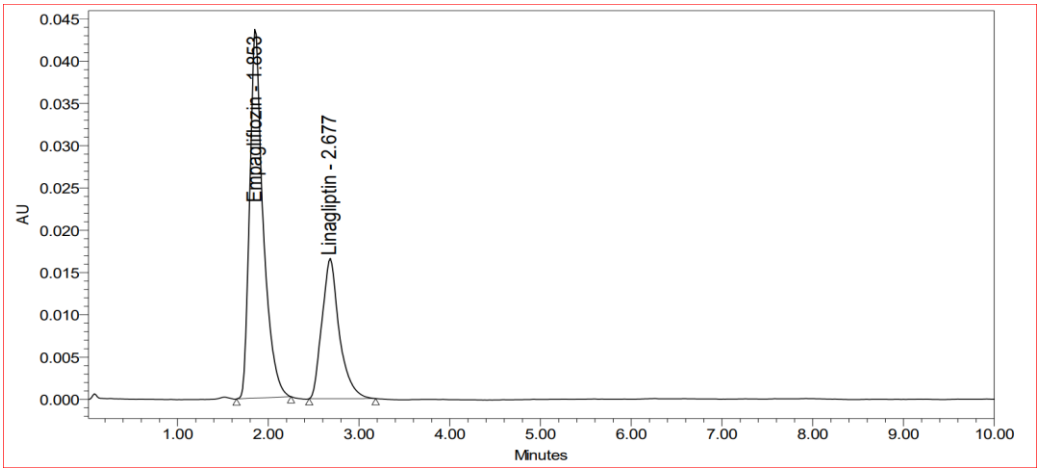

Figure 4: Sample chromatogram

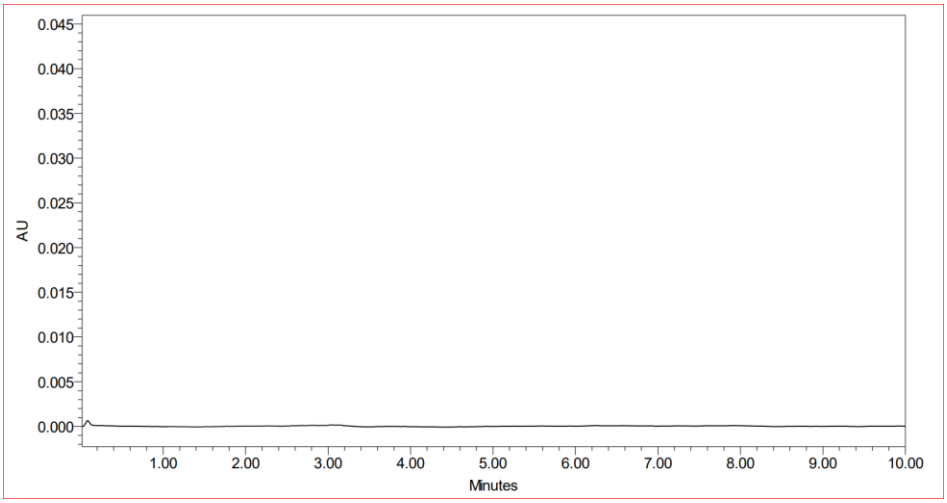

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Empagliflozin	Linagliptin
Retention time	1.053	2.677
USP Plate count	4725.92	6256.39
USP Tailing	1.46	1.29

Table 2: Assay results for Empagliflozin and Linagliptin

	Label Claim (mg)	% Assay
Empagliflozin	10	100.19
Linagliptin	05	100.45

Table 3: Linearity results for Empagliflozin

S. No	Linearity Level	Concentration	Area
1	I	20	148475
2	II	40	286753
3	III	60	445725
4	IV	80	596836
5	V	100	745622
Correlation Coefficient			0.999

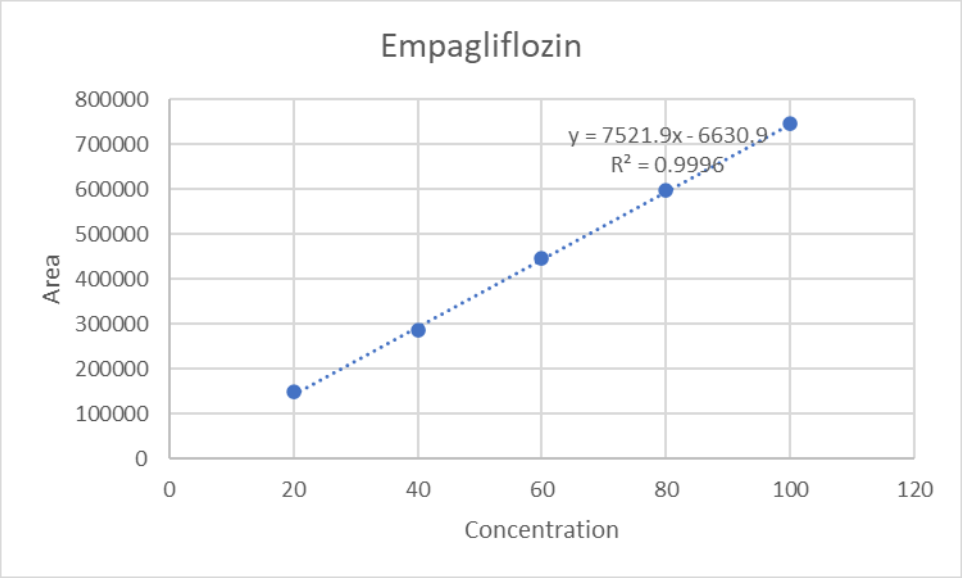

Figure 6: Linearity graph for Empagliflozin

Table 4: Linearity results for Linagliptin

S. No	Linearity Level	Concentration	Area
1	I	10	71914
2	II	20	140828
3	III	30	215732
4	IV	40	286753
5	V	50	357562
Correlation Coefficient			0.999

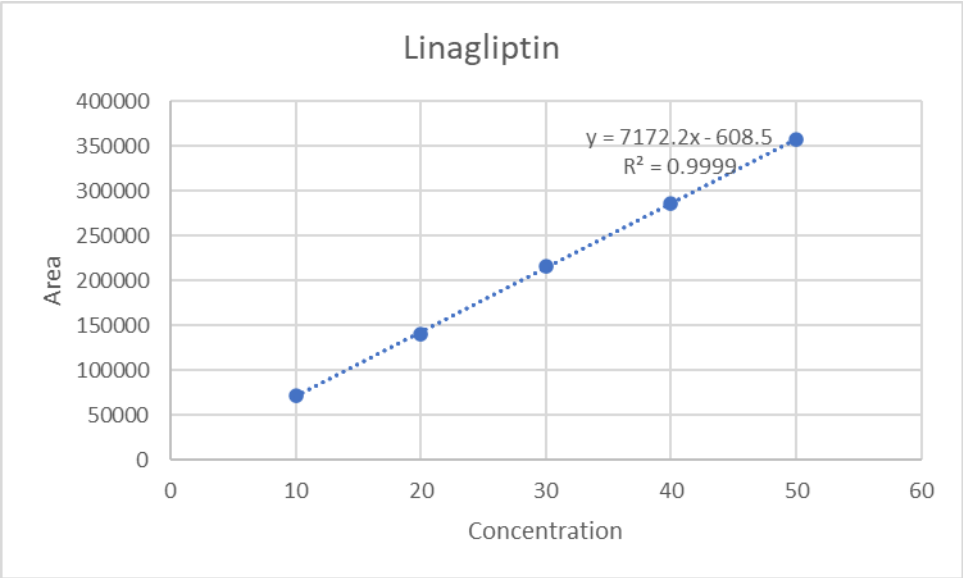

Figure 7: Linearity graph for Linagliptin

Table 5: Showing accuracy results for Empagliflozin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	225703.3	20	20.14	100.69	100.39
100%	448469.7	40	40.01	100.04	
150%	675482.7	60	60.27	100.45	

Table 6: Showing accuracy results for Linagliptin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	109553.3	10	10.04	100.44	100.39
100%	219228.7	20	20.10	100.50	
150%	327988.3	30	30.07	100.24	

Table 7: Precision results for Empagliflozin and Linagliptin

Injection	Area for Empagliflozin	Area for Linagliptin
Injection-1	448662	218753
Injection-2	446873	214829
Injection-3	446352	216426
Injection-4	447562	218452
Injection-5	447529	216468
Injection-6	446244	217567
Average	447203.7	217082.5
Standard Deviation	907.4	1468.9
%RSD	0.2	0.7

Table 8. Ruggedness results of Empagliflozin and Linagliptin

Injection	Area for Empagliflozin	Area for Linagliptin
Injection-1	448776	218573
Injection-2	445735	218562
Injection-3	447673	214652
Injection-4	448673	215354
Injection-5	445876	216454
Injection-6	448676	216457
Average	447568.2	216675.3
Standard Deviation	1424.2	1618.5
%RSD	0.3	0.7

Robustness results

Table 9: Flow variation results for Empagliflozin

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Tailing	USP Plate Count
1	0.9	1.46	4626.92
2	1.0	1.46	4725.92
3	1.1	1.46	4865.39

Table 10: Flow variation results for Linagliptin

S. No	Flow Rate (ml/min)	System Suitability Results		
		USP Resolution	USP Tailing	USP Plate Count
1	0.9	3.31	1.29	6132.29
2	1.0	3.18	1.29	6256.39
3	1.1	3.02	1.29	6352.29

Table 11: Result for effect of inconsistency in mobile phase configuration (Organic Phase) of Empagliflozin

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	1.46	4762.23
2	*Actual	1.46	4725.92
3	10% more	1.46	4767.76

Table 12: Result for effect of inconsistency in mobile phase configuration (Organic Phase) of Linagliptin

S. No	Change in	System Suitability Results

	Organic Composition in the Mobile Phase	USP Resolution	USP Tailing	USP Plate Count
1	10% less	3.37	1.29	6214.27
2	*Actual	3.18	1.29	6256.39
3	10% more	2.96	1.29	6232.23

Table 13: LOD, LOQ of Empagliflozin and Linagliptin

Drug	LOD	LOQ
Empagliflozin	3.07	10.09
Linagliptin	2.95	9.93

Table 14: Degradation results for Empagliflozin and Linagliptin

	Empagliflozin		Linagliptin	
Sample Name	**Area**	**% Degraded**	**Area**	**% Degraded**
Standard	447408.3		217707	
Acid	436522	2.43	207853	4.53
Base	428673	4.19	196762	9.62
Peroxide	439657	1.73	206752	5.03
Thermal	430876	3.70	199672	8.28
Photo	421862	5.71	195534	10.18

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Empagliflozin and Linagliptin in bulk and tablet dosage form. Hence, this method can easily and conveniently adopt for routine quality control analysis of Empagliflozin and Linagliptin in bulk and tablet dosage form.

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