METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF EMPAGLIFLOZIN AND LINAGLIPTIN IN BULK FORM AND MARKETED TABLET DOSAGE FORMS BY RP-HPLC

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ABSTRACT: A Novel Analytical simple, reproducible and efficient RP-HPLC method was developed for simultaneous estimation of Empagliflozin and Linagliptin in pure form and marketed in combined pharmaceutical dosage forms. A column having Develosil ODS HG-5 RP C18, 15cmx4.6mm, i.d. Column in isocratic mode with mobile phase containing Methanol: Acetonitrile in the ratio of 85:15% v/v was used. The flow rate was 1.0 ml/min and effluent was monitored at 258nm. The retention times and linearity range for Empagliflozin and Linagliptin was found to be (2.217, 5861min) and (0-14, 0-28), respectively. The method has been validated for linearity, accuracy and precision, robustness and limit of detection and limit of quantitation. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.08µg/ml and 0.24µg/ml for Empagliflozin and 0.1µg/ml 0.3µg/ml for Linagliptin respectively. The proposed method was found to be accurate, precise and selective for simultaneous estimation of Empagliflozin and Linagliptin in pure form and marketed combined pharmaceutical dosage forms.
Keywords: Empagliflozin and Linagliptin, RP-HPLC, Validation, Accuracy, Precision.

I. 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[1] is an orally available competitive inhibitor of sodium-glucose co-transporter 2 (SGLT2; SLC5A2) with antihyperglycemic activity. 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. SGLT2, a transport protein exclusively expressed in the proximal renal tubules, mediates approximately 90% of renal glucose reabsorption from tubular fluid. Empagliflozin[2] is indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with type 2 diabetes. It is also indicated to reduce the risk of cardiovascular death in adult patients with both type 2 diabetes mellitus and established cardiovascular disease. Empagliflozin lowers blood glucose levels by preventing glucose reabsorption in the kidneys, thereby increasing the amount of glucose excreted in the urine. It has a relatively long duration of action requiring only once-daily dosing. Patients should be monitored closely for signs and symptoms of ketoacidosis regardless of blood glucose level as Empagliflozin[3] may precipitate diabetic ketoacidosis in the absence of hyperglycemia. As its mechanism of action is contingent on the renal excretion of glucose, Empagliflozin may be held in cases of acute kidney injury and/or discontinued in patients who develop chronic renal disease. 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 as following

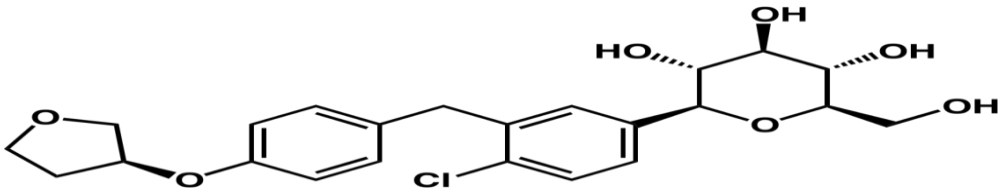

Fig-1: Chemical Structure of Empagliflozin

Linagliptin is a DPP-4 inhibitor developed by Boehringer Ingelheim for the treatment of type II diabetes. Linagliptin[4] 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. 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[5] 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 exercise5. 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. Linagliptin[6] is a competitive, reversible DPP-4 inhibitor. 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 cells. These effects together reduce the breakdown of glycogen in the liver and increase insulin release in response to glucose. 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 follows

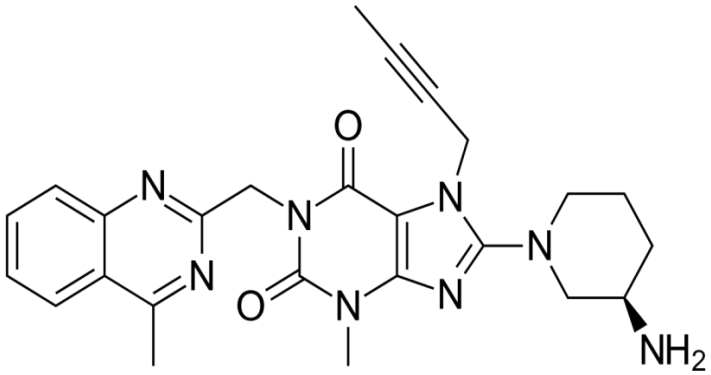

Fig-2: Chemical Structure of Linagliptin

As per the literature review[36-40], Empagliflozin and Linagliptin was estimated by few methods like spectrophotometric liquid chromatography-mass spectrometry (MS), high performance liquid chromatographic (HPLC) and gas chromatography-MS. Therefore, there is a need for a reliable, sensitive and rapid method for its analysis in bulk and pharmaceutical preparations. The objective of the work is to develop reverse phase-HPLC (RP-HPLC) method for estimation of Empagliflozin and Linagliptin in bulk form and pharmaceutical dosage form with the simple, rapid, accurate and economical method and validated for system suitability, linearity, accuracy, precision, and robustness as per ICH guidelines[35]. The method has been satisfactorily applied to the determination of Empagliflozin and Linagliptin in bulk form and pharmaceutical preparations.

II. EXPERIMENTAL

Table-1: List of Instrument used

S. No.	Instruments/Equipment/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	T60-LAB INDIA UV – Vis spectrophotometer
3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator(Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5□m, 15mm x 4.6mm i.d.
7.	P^H Analyzer (ELICO)
8.	Vacuum filtration kit (BOROSIL)

Table-2: List of Chemicals used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai

Selection of Wavelength

Selectivity of HPLC method[7] that uses UV detector depends on proper selection of Wavelength. A wavelength which gives good response for the drug to be detected is to be selected.

Empagliflozin Standard Solution Preparation

Weigh accurately 10 mg of standard Empagliflozin and it transferred into a clean & dry 100 ml of volumetric flask. Add 10ml mobile phase and further do sonication in order to dissolve. Finally make up to the volume up to mark with the mobile phase. The final resulted solution contained about 100 µg/ml of Empagliflozin.

Linagliptin Standard Solution Preparation

Weigh accurately about 10 mg of standard Linagliptin and transferred into a clean and dry 100 ml volumetric flask. Add 10ml mobile phase and further do sonication in order to dissolve. Finally make up the volume with the same mobile phase i.e. same solvent system. The volume was made up to the mark with same solvent. The final solution contained about 100µg/ml of Linagliptin.

Initialization of the HPLC instrument

First switched on the HPLC instrument. The selected column[8] was washed with the HPLC grade water for 45 minutes. Then selected column was saturated with the mobile phase for 45 minutes. Then keep the mobile phase for stabilization. The mobile phase was run to obtain the peaks. After completion of stabilization. After 20 minutes the standard drug solution was injected in HPLC.

OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS:

The different HPLC chromatographic conditions[9] were used to find out the optimum chromatographic condition for best elution of drugs.

Table- 3: Different Chromatographic used and their Optimizations

S.No.	Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
1	Symmetry C i8, 5pni, 25emx4.6mm i.d.	Water: Methanol =30:70	1.0 ml/min	258nm	Peaks didn't Separate	Method rejected
2	Waters C18, 5pm, 25cmx4.6inini.d.	Water : ACN = 55:45	1.0 ml/min	258nm	Early elution of peak	Method rejected
3	Waters C18, 5pm, 25cmx4.6inini.d.	ACN: methanol= 60: 40	1.0 ml/min	258nm	Low resolution peak	Method rejected

4	Develosil ODS HG-5 RP C18, 5pin,15emx4.6mm i.d.	ACN: methanol 90:10	1.0 ml/ min	258nm	Resolution increasesbut Peak shapes not good	Method rejected
5	Develosil ODS HG-5 RP C18, 5pni, l5emx4.6mm i.d.	Methanol : Acetonitrile = 85:15	1.0 ml/min	258nm	Nice and Good Peaks	Method Accepted

III. METHOD VALIDATION

This method was validated[10] according to ICH guidelines to establish the performance characteristics of a method to meet the requirements for the intended application of the method. They were tested using the optimized chromatographic conditions and instruments.

Specificity

It is the ability to asses unequivocally the analyte in the presence of components that may be expected to be present. Excipients that are commonly used were spiked into a pre-weighed quantity of drugs. Appropriate dilutions[11] were injected into chromatographic system, and the quantities of the drugs were determined. The chromatogram did not show any other peaks, which confirmed the specificity[12] of the method.

System Suitability

System performance parameters of HPLC method were determined by injecting standard solutions. Parameters such as a number of theoretical plates (N), tailing factor, and retention time[13] were determined. From system suitability studies it is observed that % relative standard deviation (RSD) values are within the limit, i.e., not more than two which indicates good performance of the system. Results are tabulated in Table 14.

Linearity

A series of solutions were prepared using Empagliflozin and Linagliptin working standard solution at a concentration levels from 30 to 70 mg/ml and the peak area response of all solutions are measured. A graph[14] was plotted against the concentration (mg/ml) on X-axis versus area/response on Y-axis. The detector response was found to be linear with a correlation coefficient of 0.999. Linearity[15] results are tabulated in Table 5 & 6 and Fig. 5 & 6.

Precision

The precision of the method expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. Precision[16] studies were performed, and the results are reported in term of RSD. The repeatability studies were conducted by estimating response of five different concentrations of Empagliflozin and Linagliptin and reported in terms of % RSD[17]. The results are tabulated in Table 9.

Accuracy (% recovery)

Accuracy[18] of the method was determined by calculating the recovery of Empagliflozin and Linagliptin by the spiked method. A known quantity of Empagliflozin and Linagliptin was added to a pre-determined sample solution, and the amount of Empagliflozin and Linagliptin was estimated by measuring peak areas. Mean % recovery[19] values are within the limit (limit is 98-102%). Accuracy data were presented in Table 7 & 8.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberately variations in method parameters and provides an indication of its reliability during normal usage. The results of robustness[20] are tabulated in table no.12 & 13.

RESULTS AND DISCUSSION

Method Development

Selection of Wavelength

Selectivity[21] of HPLC method that uses UV detector depends on proper selection of Wavelength. A wavelength which gives good response for the drug to be detected is to be selected. From the UV spectra 258 nm was selected as the wavelength for study. The λ max[22] of this method can be determined as 258 nm.

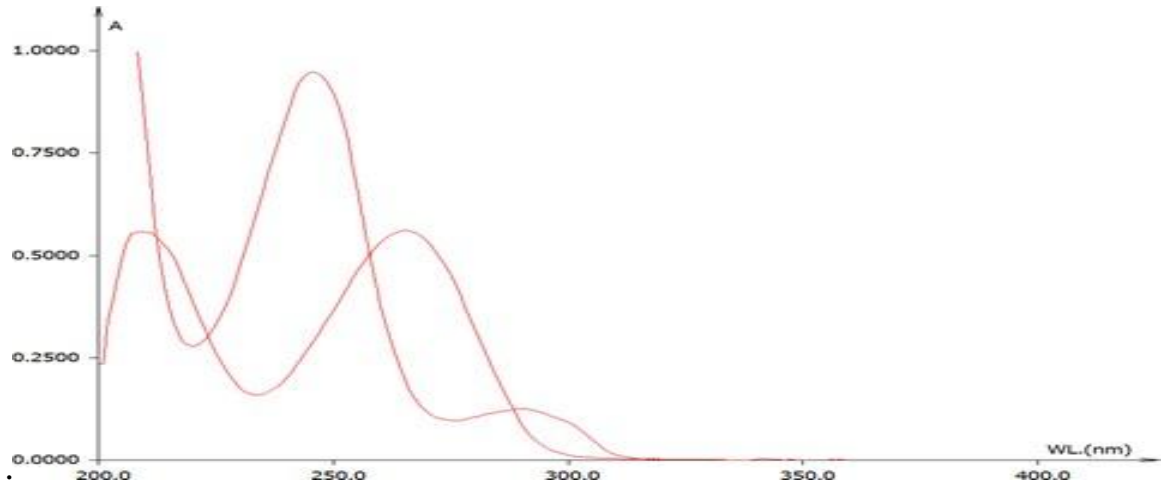

Fig-3: Isobestic Point Empagliflozin and Linagliptin (258nm)

Optimized Chromatographic Method:

Table-4: Summary of Optimized Chromatographic Conditions

Mobile phase	Methanol: Acetonitrile 85:15% v/v
Column	Develosil ODS HG-5 RP C_{18}, 15cmx4.6mm, i.d. Column.
Column Temperature	Ambient
Detection Wavelength	258 nm
Flow rate	1.0 ml/ min.
Run time	15 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic

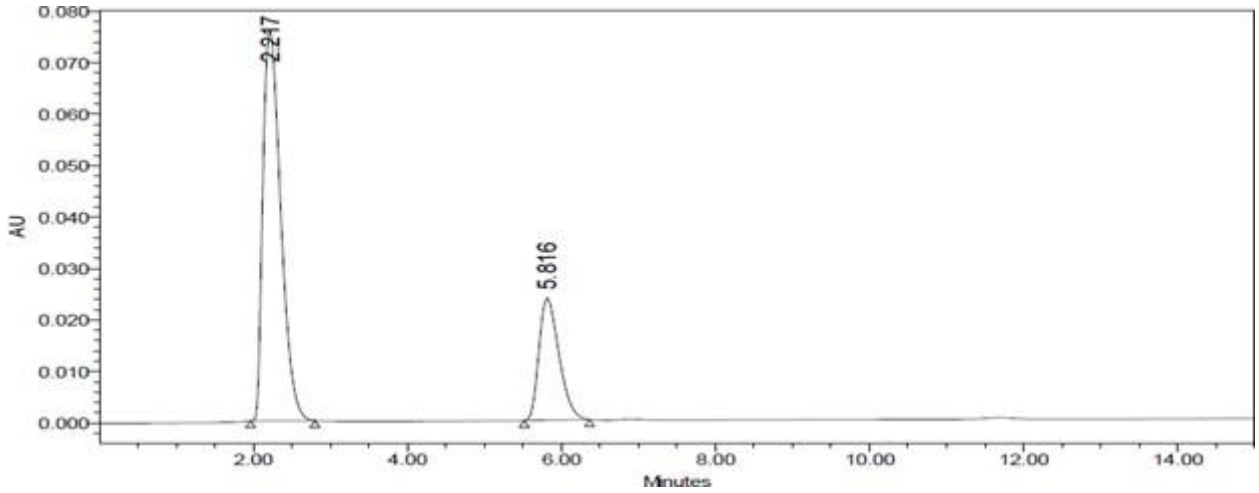

Fig-4: Chromatogram of Empagliflozin and Linagliptin in Optimized Chromatographic Condition

Method Validation

1. Linearity and Range:

Linearity range was found to be 0-14 µg/ml for Empagliflozin and 0-28 µg/ml for Linagliptin respectively. The correlation coefficients[23] were found to be 0.999 & 0.999, the slopes were found to be 21516 & 14059 and intercept were found to be 3613.2 & 3514.9 for Empagliflozin and Linagliptin respectively.

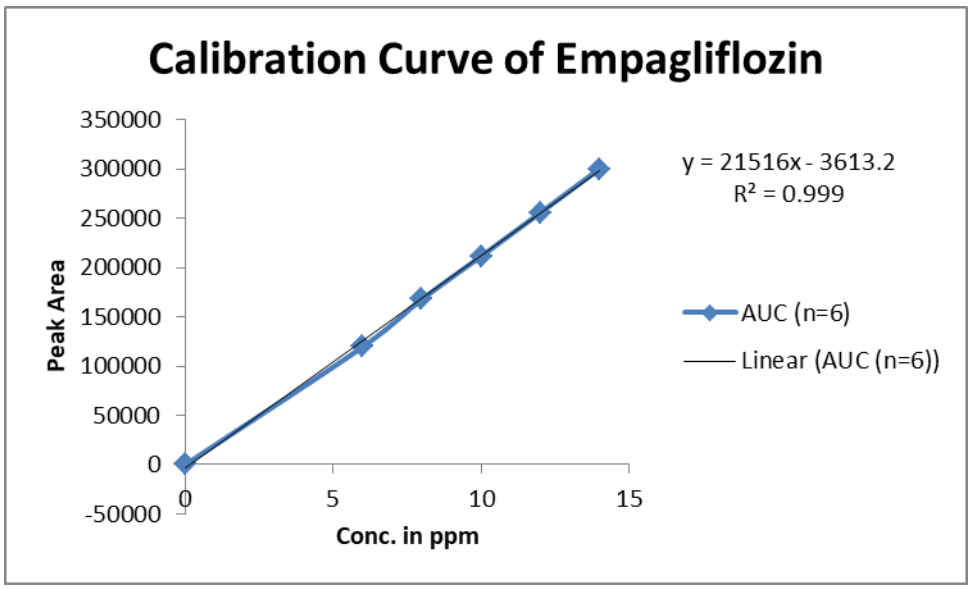

Fig-5: Standard curve for Empagliflozin

Table-5: Linearity Results for Empagliflozin

CONC. (µg/ml)	AUC (n=6)
0	0
6	119571
8	167873
10	211264
12	255428
14	299987

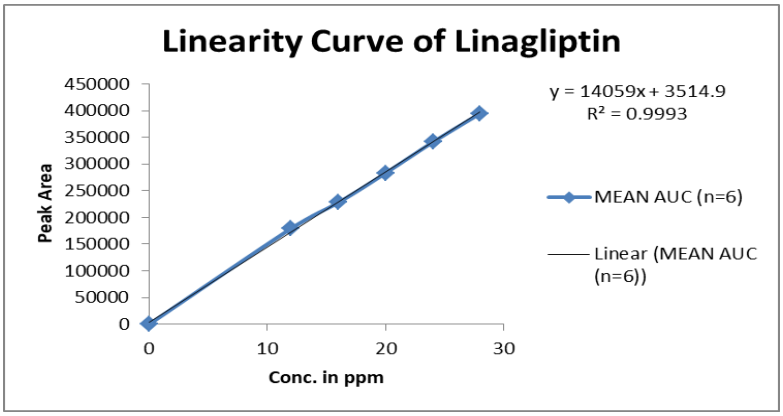

Fig-6: Standard Curve for Linagliptin

Table-6: Linearity Results for Linagliptin

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	179371
16	227893
20	283264
24	341428
28	394987

2. Accuracy:

Inject the Three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions[24]. 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.

Table-7: Accuracy Readings for Empagliflozin

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	8	7.997368	115949	99.9671	Mean= 100.7003%
S_2 : 80 %	8	8.106622	117485	101.3328	S.D. = 0.6884036
S_3 : 80 %	8	8.064087	116887	100.8011	% R.S.D.= 0.683616%
S_4 : 100 %	10	9.904901	142767	99.04901	Mean= 100.36157%
S_5 : 100 %	10	10.02966	144521	100.2966	S.D. = 1.346221
S_6 : 100 %	10	10.17391	146549	101.7391	R.S.D.= 1.3413706%
S_7 : 120 %	12	12.01807	172476	100.1506	Mean= 100.183756%
S_8 : 120 %	12	11.88079	170546	99.00657	S.D. = 1.19411
S_9 : 120 %	12	12.16729	174574	101.3941	% R.S.D. = 1.19191%

Recovery study: Linagliptin

Table-8: Accuracy Results for Linagliptin

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S_1 : 80 %	16	16.08685	229679	100.5428	Mean= 100.54488% S.D. = 0.97847% R.S.D.= 0.9731%
S_2 : 80 %	16	15.93079	227485	99.56745	
S_3 : 80 %	16	16.2439	231887	101.5244	
S_4 : 100 %	20	20.07632	285767	100.3816	Mean= 99.97095% S.D. = 0.395406 % R.S.D.= 0.39552%
S_5 : 100 %	20	19.98769	284521	99.93847	
S_6 : 100 %	20	19.91856	283549	99.59279	
S_7 : 120 %	24	23.75432	337476	98.97634	Mean= 100.27718%
S_8 : 120 %	24	24.11494	342546	100.4789	S.D. = 1.21262 % R.S.D. = 1.20927%
S_9 : 120 %	24	24.33032	345574	101.3763	

Precision:

The precision of the analytical developed method was studied by analysis of multiple sampling of homogeneous (same) sample. The precision expressed as standard deviation[25] (SD) or relative standard deviation (%RSD). The precision of the method can be analyzed by the intermediate precision[26]. It includes the intra-day and inter-day variation.

Repeatability

The precision of each method was achieved separately from the peak areas obtained by actual estimation of 5 injections of fixed homogenous sample concentrations of Empagliflozin and Linagliptin. The % relative standard deviation for the Empagliflozin and Linagliptin was calculated.

Table-9: Data showing repeatability analysis for Empagliflozin & Linagliptin

HPLC Injection Replicates	AUC for Empagliflozin	AUC for Linagliptin
Replicate – 1	113568	241022
Replicate – 2	113241	240137
Replicate – 3	115408	242911
Replicate – 4	117412	245245

Replicate – 5	112541	241941
Replicate – 6	112546	240444
Average	**114119.3333**	**241356.6667**
Standard Deviation	**1925.83838**	**1416.95812**
% RSD	**1.68756**	**0.58708**

Intermediate Precision:

Intra-assay & inter-assay: The intra & inter day variation[27] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Empagliflozin and Linagliptin revealed that the proposed method is precise.

Table-10: Results of intra-assay & inter-assay

Conc. of Empagliflozin (API) (µg/ml)	**Observed Conc. of Empagliflozin (µg/ml) by the proposed method**			
	Intra-Day		**Inter-Day**	
	Mean (n=6)	**% RSD**	**Mean (n=6)**	**% RSD**
8	8.09	0.97	8.03	0.96
10	10.05	0.45	10.04	0.47
12	11.98	0.37	11.90	0.12

Table-11: Data for Linagliptin intra-assay & inter-assay analysis

Conc. of Linagliptin (API) (µg/ml)	**Observed Conc. of Linagliptin (µg/ml) by the proposed method**			
	Intra-Day		**Inter-Day**	
	Mean (n=6)	**% RSD**	**Mean (n=6)**	**% RSD**
8	7.97	0.27	8.09	0.59
10	10.14	1.29	9.95	0.64
12	12.08	0.61	11.94	0.26

IV. RESULT AND DISCUSSION

The Intraday and interday related studies[28] shows that the % RSD was found to be within limit i.e. (≤2%). So it is indicated that the developed is within the limits. Hence finally we concluded that the developed method was found to be precise.

5. Limit of detection (LOD) & Limit of quantification (LOQ):

The detection limit[29] (LOD) and quantitation limit (LOQ) may be expressed as:

L.O.D. = 3.3 (SD/S).

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Result & Discussion

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified[30] (LOQ) were found to be

0.08 & 0.24 µg/ml respectively for Empagliflozin.
The LOD was found to be 0.1 µg/ml and LOQ was found to be 0.3 µg/ml for Linagliptin which represents that sensitivity of the method is high.

Method Robustness:

Influence of small changes in chromatographic conditions[31] such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness[32] of the method are also in favour of (Table-12, % RSD < 2%) the developed RP-HPLC method for the analysis of Empagliflozin (API).

Table-12: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	1.05
Flow (0.9 ml/min)	0.67
Temperature (27^{0}C)	0.58
Temperature (23^{0}C)	0.61
Wavelength of Detection (280 nm)	0.38
Wavelength of detection (270 nm)	0.17

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table-13, % RSD < 2%) the developed RP-HPLC method[33] for the analysis of Linagliptin (API).

Table-13: Result of Method Robustness Test

Change in Parameter	% RSD
Flow (1.1 ml/min)	0.09
Flow (0.9 ml/min)	0.07
Temperature (27^{0}C)	0.28
Temperature (23^{0}C)	0.74
Wavelength of Detection (235 nm)	0.86
Wavelength of detection (240 nm)	0.67

System Suitability Parameter: It is an integral part of so many analytical procedures. The parameters are based on the idea that the equipment, electronics, analytical operations and the samples to be analyzed constitute as an integral system[34] which can be examined. Finally system suitability test parameters are established. The obtained data is shown in the following table-14.

Table-14: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result

1	Resolution	Rs ± 2	3.65
2	Asymmetry	T > 2	Empagliflozin = 0.35 Linagliptin = 0.23
3	Theoretical plate	N < 2000	Empagliflozin = 3771 Linagliptin = 2437

Estimation of Empagliflozin and Linagliptin in Pharmaceutical Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Finally the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make up to the mark with the mobile phase. The final solution was filtered through a selected membrane filter (0.45 µm) and in order to sonicated to degas the mobile phase (Solvent system). From this above stock solution (1 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system (Mobile phase).

The prepared solutions were injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection (Blank Solution) of the standard solution also injected into the HPLC system and the chromatograms and peak areas were recorded and calculated. The obtained data are shown in Table 15.

ASSAY:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Average weight} = \text{mg/tab}$$

Where:

AT = Test Preparation Peak Area
AS = Standard preparation Peak Area
WS = Working standard weight taken in mg
WT = Sample weight taken in mg
DS = Standard solution dilution
DT = Sample solution dilution
P = Working standard percentage purity

The assay was performed as explained in the previous chapter (Above). The results which are obtained are following:

Table-15: Recovery Data for estimation Empagliflozin and Linagliptin in Glyaxambi

Brand name of Empagliflozin and Linagliptin	Labelled amount of Drug (mg)	Mean (± SD) Amount (mg) Found By The Proposed Method (n=6)	Assay % (± SD)
Glyaxambi Tablets (Eli Lilly and Company (India) Pvt. Ltd. (Lilly India))	10mg/5mg	9.787 (±0.598)/4.898	99.875 (±0.598)/99.698 (± 0.467)

Result & Discussion: The %purity of Empagliflozin & Linagliptin for Tablets was found to be 99.875% and 99.698% respectively.

Results of Degradation Studies:

The results of the stress studies indicated the specificity of the method that has been developed. Empagliflozin and Linagliptin were stable only in photolytic stress conditions and little bit in thermal stress conditions. The results of forced degradation studies are given in the following Table-16.

Table-16: Results of Forced Degradation Studies of Empagliflozin and Linagliptin API

Stress Condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	95.62	4.38	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	97.13	2.87	100.00
Thermal Degradation (60 ^{0}C)	24Hrs.	96.24	3.76	100.00
UV (254nm)	24Hrs.	95.43	4.57	100.00
3% Hydrogen peroxide	24Hrs.	96.16	3.84	100.00

V. SUMMARY AND CONCLUSION

Isocratic elution is easy, needs only one pump & flat standard splitting up for easy and also reproducible results. So, it was preferred for the present research over gradient elution. In the case of RP-HPLC various columns are offered, however, below Develosil ODS HG-5 RP C18, 5µm, l5cmx4.6mm i.d. column was preferred since using this column top shape, resolution, as well as absorbance, were great. Mobile stage & diluent for preparation of different examples were completed after researching the solubility of API in various solvents of our disposal (methanol, Acetonitrile, water, 0.1 N NaOH, 0.1 N HCl). Discovery wavelength was picked after checking the basic remedy of the drug over 200 to 400nm. From the U.V spectrum of Empagliflozin and also Linagliptin it is evident that the majority of the HPLC work can be achieved in the wavelength variety of 200-300 nm easily. Even more, a circulation rate of 1 ml/min & an injection volume of 10µl were found to be the best evaluation.

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