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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF LINAGLIPTIN AND EMPAGLIFLOZIN IN BULK AND IT'S DOSAGE FORM

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Keywords:

Empagliflozin; Linagliptin; RP-HPLC; Method Development; Method Validation; ICH Q2(R2); Simultaneous Estimation; Pharmaceutical Analysis

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ABSTRACT

Fixed-dose combinations of Empagliflozin and Linagliptin are widely prescribed for the management of type 2 diabetes mellitus. Reliable analytical methods are essential for routine quality control and regulatory compliance; however, simultaneous estimation of both drugs requires a simple, rapid, and validated chromatographic method.The present study aimed to develop and validate a simple, accurate, precise, and robust reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous estimation of Empagliflozin and Linagliptin in bulk drug and pharmaceutical tablet dosage forms.Chromatographic separation was achieved on a C18 column using methanol and 5 mM phosphate buffer (60:40, v/v; pH 3.5) as the mobile phase at a flow rate of 1.0 mL/min with UV detection at 228 nm. The developed method was validated according to ICH Q2(R2) guidelines for system suitability, specificity, linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD), and limit of quantification (LOQ).The developed method produced well-resolved, symmetrical peaks with retention times of approximately 4.52 min for Empagliflozin and 6.36 min for Linagliptin. Excellent linearity was observed over the concentration range of 2–20 µg/mL for both drugs ($R^2 > 0.999$). The method demonstrated satisfactory accuracy with recoveries close to 100%, while precision studies showed %RSD values within acceptable limits. Robustness and ruggedness studies confirmed the reliability of the method under small deliberate variations in chromatographic conditions. The validated method was successfully applied for quantitative estimation of both drugs in marketed tablet formulations. The proposed RP-HPLC method is simple, rapid, precise, accurate, sensitive, and economical for the simultaneous determination of Empagliflozin and Linagliptin in bulk and tablet dosage forms. The method complies with ICH validation requirements and is suitable for routine quality control and pharmaceutical analysis.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. The increasing global prevalence of T2DM has led to the widespread use of combination therapy to achieve effective glycemic control while reducing the risk of diabetes-related complications [1]. Fixed-dose combinations of antidiabetic agents with complementary mechanisms of action have become an important therapeutic strategy for improving patient compliance and treatment outcomes [2].

Empagliflozin is a selective sodium-glucose co-transporter-2 (SGLT2) inhibitor that lowers blood glucose by reducing renal glucose reabsorption, thereby promoting urinary glucose excretion. In contrast, Linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances endogenous incretin activity, leading to increased insulin secretion and reduced glucagon release in a glucose-dependent manner. The combination of these two drugs provides effective glycemic control with a lower risk of hypoglycemia and is widely marketed for the treatment of T2DM [3-5].

Quality control of fixed-dose combination formulations requires reliable analytical methods capable of simultaneously estimating both active pharmaceutical ingredients with high accuracy and precision. Reverse-phase high-performance liquid chromatography (RP-HPLC) remains the analytical technique of choice because of its excellent selectivity, sensitivity, reproducibility, and suitability for routine pharmaceutical analysis. Although several chromatographic methods have been reported for individual drugs and their combinations, there remains a need for a simple, rapid, economical, and validated method suitable for routine quality control laboratories [6].

Therefore, the present study was undertaken to develop and validate a rapid RP-HPLC method for the simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and tablet dosage forms. The developed method was optimized and validated according to the International Council for Harmonisation (ICH Q2(R2)) guidelines by evaluating system suitability, specificity, linearity, accuracy, precision, ruggedness, robustness, limit of detection, and limit of quantification to ensure its suitability for routine pharmaceutical quality control.

Methods

Identification and Characterization of Drug

The identity and physicochemical characteristics of Empagliflozin (EMP) and Linagliptin (LIN) were evaluated before method development to confirm their purity and suitability for analysis. Preliminary characterization included assessment of physical appearance, melting point, solubility, FTIR spectroscopy, and UV spectroscopy.

Selection and Procurement of Drug

Empagliflozin and Linagliptin were selected as the model drugs for RP-HPLC method development and validation. Both reference standards were obtained as gift samples from MG Lab India, Hyderabad, and used without further purification.

Physicochemical Characterization

The physicochemical properties of EMP and LIN, including organoleptic characteristics, melting point, solubility, UV absorption, and FTIR spectra, were evaluated to facilitate the selection of suitable chromatographic conditions and solvents.

Solubility Studies

The solubility of EMP and LIN was investigated in different solvents to identify a common solvent suitable for preparation of standard and sample solutions during chromatographic analysis.

Melting Point Determination

The melting points of EMP and LIN were determined using the capillary tube method. The observed melting point ranges were used to confirm the identity and purity of the drug substances.

FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed over the range of 4000–400 cm^{-1} to identify the characteristic functional groups and confirm the chemical identity of both drugs.

UV Spectroscopic Analysis

The UV absorption spectra of EMP and LIN were recorded in the wavelength range of 200–400 nm using a UV–Visible spectrophotometer. The maximum absorption wavelength (λmax)

was determined and selected for HPLC detection.

Selection of Mobile Phase

Various mobile phase compositions consisting of methanol, acetonitrile, water, and phosphate buffer at different pH values were investigated to obtain optimum chromatographic separation. The optimized mobile phase was selected based on peak symmetry, resolution, retention time, and system suitability parameters [7-10].

Preparation of Standard Solutions

EMP standard solution:

Accurately weighed quantity 5 mg of EMP was dissolved in methanol and volume was made up to 25 ml mark (200 µg /ml). The stock standard solution was diluted further with methanol to get final concentration of about 100 µg /ml of EMP.

LIN standard solution:

Accurately weighed quantity 5 mg of LIN was dissolved in methanol and volume was made up to 25 ml mark (200µg /ml). The stock standard solution was diluted further with methanol to get final concentration of about 100 µg /ml of LIN. As like above procedure, the standard solutions are also prepares in mobile phase.

The Methanol was allowed to equilibrate with stationary phase until steady baseline was obtained. The standard solution containingmixtureof EMP and LIN was run and different individual solvents as well as combinations of solvents have been tried to get a good separation and stable peak. Each mobile phase was filtered through Whatman filter paper No. 42. Peak, well resolved peaks with symmetry within limits and significant Based on samplesolubilityandstability,variousmobilephas ecompositionswereevaluatedtoachieve acceptable separation using selected chromatographic conditions. The mobile phases tried are as follows [11]:

1) Methanol:Water (90:10)
2) Methanol:Water (80:20)
3) Acetonitrile:Water(90:10)
4) Acetonitrile:Water(80:20)
5) Methanol:Buffer (70:30)
6) Methanol:Buffer(80:20) pH5
7) Methanol:Buffer(70:30) pH4.5
8) Methanol:Buffer(60:40)pH3.5

From various mobile phases tried, mobile phase containing Methanol: 5 mm Phosphate buffer (60:40) pH 3.5 was selected, since it gives sharp reproducible retention time for EMP and LIN.

Chromatographic Conditions

Chromatographic separation was carried out on an Inertsil ODS C18 analytical column (Grace4.6(id)x250 mm) using the optimized mobile phase under isocratic conditions. The flow rate, detection wavelength, injection volume, and other chromatographic parameters were optimized to achieve satisfactory separation of EMP and LIN [12].

Preparation of Calibration Curve

Preparationofstandard solutions

ACEstandardstocksolution:

Accurately weighed quantity 10 mg of EMP was dissolved in methanol and volume was madeup to 100 ml mark (100 µg/ml).Thestockstandardsolution was dilutedfurtherwith mobile phase to get various concentrations.

LINstandardstock solution:

Accurately weighed quantity 10 mg of LIN was dissolved in methanol and volume was madeupto100mlmark(100 µg/ml).Thestockstandardsolutionwasdilutedfur therwith mobile phase to get various concentrations.

Procedure

Themobilephasewasallowedtoequilibratewithth estationaryphaseuntilsteadybaseline wasobtained.Theseriesofconcentrationfrom2-20µg/mlforEMPand2-20µg/mlofLIN solutionswereinjectedandpeak areawas recorded.

System Suitability Test

System suitability was evaluated before analysis by performing replicate injections of the standard solution. Parameters including retention time, theoretical plates, tailing factor, and resolution were assessed to verify the performance of the chromatographic system.

Preparationofstandarddrugsolution. EMP standard solution:

Accuratelyweighedquantity10mgofEMPwasdiss olvedinmobilephaseandvolumewas madeupto100mlmark.Thestockstandardsolution wasdilutedfurtherwithmobilephase to get final concentration of about 10 µg/ml of EMP.

LINstandard solution

Accuratelyweighedquantity10mgof LINwasdissolvedinmobilephaseandvolumewas madeupto100mlmark.Thestockstandardsolution wasdilutedfurtherwithmobilephase to get final concentration of about 05 µg/ml of LIN.

Procedure

Filteredmobilephasewasallowedto equilibratewithstationaryphaseuntilsteadybaseli ne Was obtained. A 20 µL std. drug solution was injected which was made in five replicates and the system suitability parameters wererecorded [13-14].

Application of the Proposed Method for Estimation of EMP and LIN in Laboratory Mixture

Preparationoflaboratorymixture(standard):

Accurately weighed quantity of EMP 10 mg was transferred to 100 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with mobile phase. Andaccuratelyweighedquantityof LIN10mgwastransferredto100mlvolumetricflask , shaken vigorously for five minutes and volume was made up to mark with mobile phase. The standard solution of EMP and LIN were mixed and diluted with mobile phase properly toobtainlaboratorymixturescontainingaconcentr ation10µg/mland05µg/mlofbothdrug respectively

Preparationoflaboratorymixture(sample)

Three different laboratory mixtures of EMP and LIN were prepared by appropriately weighingthequantities ofdrugsamples so as to get the concentration of 10 µg/ml of EMP and 05 µg/ml of LIN. Thepeakareaofstandardlaboratorymixtureandsa mplelaboratorymixturewascompared to obtain the concentration. The amount of each drug estimated in laboratory mixture was calculated using following formula –

$$\%\text{Estimation} = \frac{At}{As} \times \frac{Ds}{Dt} \times \frac{Ws}{Wt} \times 100$$

Where

At=Areacount for samplesolution, As=Areacountforstandardsolution, Ds =Dilution factor for standard, Dt= Dilutionfactorforsample, Ws = Weight of standard (mg) Wt = Weight of sample (mg)

Application of the Proposed Method for Estimation of EMP and LIN in Marketed Formulation

Standard stock solution

Accurately weighed quantity of EMP 10 mg was transferred to 100 ml volumetric flask, shaken vigorously for five minutes and volume was made up to mark with mobile phase. Andaccuratelyweighedquantityof LIN10mgwastransferredto100mlvolumetricflask , shaken vigorously for five minutes and volume was made up to mark with mobile phase. The standard solution of EMP and LIN were mixed and diluted with mobile phase properly toobtainlaboratorymixturescontainingaconcentr ation10µg/mlofEMPand05µg/mlof LIN.

Samplesolution preparation

The twenty tablets were weighed, and then average weight was determined and finely grounded. The weight of the powdered tablet equivalent to 100 mg of EMP and 50 mg of LINweretransferredintoa100mLstandardvolume tricflask.Added50mLofsolventsonicatedfor10mi nanddilutedto100mLwiththesamesolventandthe nfilteredthrough Whatmann filter paper No: 41.

Procedure

Equal volume (20µl) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content of EMP and LIN was calculated by comparing a sample peak with that of standard. Amountof drugin tablet wascalculated usingfollowingformula

$$\text{Assay}(mg/ml) = \frac{At}{As} \times \frac{Ds}{Dt} \times \frac{Ws}{Wt} \times \frac{p}{100} \times \text{wt } mg/ml \text{ oftestsample}$$

$$\%\text{Labelclaim} = \frac{\text{Assay } (mg/ml) \times 100}{label\ \ claim\ \ in\ mg/ml}$$

Where-

At=Area count for sample solution, As = Areacountforstandardsolution, Ds = Dilution factor for standard Dt= Dilutionfactorforsample,P=Potency of drug.

Validation of the Developed RP-HPLC Method

The developed analytical method was validated according to ICH Q2(R2) guidelines with respect to specificity, system suitability, linearity,

accuracy, precision, ruggedness, robustness, limit of detection (LOD), and limit of quantification (LOQ).

Accuracy

Accuracy was evaluated by the standard addition (recovery) method at different concentration levels, and the percentage recovery of each drug was calculated.

Precision

Preparation of standard solution

An accurately weighed quantityof tablet formulation was taken in 10 ml volumetric flask; to it standard solution of EMP and LIN was added in different proportions. Then volume was adjusted up to the mark with the mobile phase. The solution was diluted with mobile phasetogetfinalconcentrationandfilteredthrough whatmanfilterpaperNo.41.Theamount of drug contributed by the formulation was deduced from the total amount of respective drugs estimated and the resultant quantities were assumed to be recovered from the added pure drugs. The content of drug was calculated using same formula as in the marketed formulation [15-17].

RESULT ANDDISCUSSION

Empagliflozin (EMP) and Linagliptin (LIN) are widely used in fixed-dose combination therapy for the management of type 2 diabetes mellitus, necessitating a reliable analytical method for their simultaneous estimation. The supplied reference standards had reported purities of 99.8% (EMP) and 99.3% (LIN), as provided by the manufacturer, and these values were considered for comparison during the study. Preliminary physicochemical characterization, including solubility studies, melting point determination, and UV spectroscopic analysis, was performed to confirm the identity and suitability of both drugs and to facilitate the development of a robust RP-HPLC method.

Table 1.Resultof physicochemical properties

Solvent	Empagliflozin	Linagliptin
Water	Slightlysoluble	Slightlyto moderatelysoluble
Methanol	Freelysoluble	Freelysoluble
Ethanol	Soluble	Soluble
Acetonitrile	Sparinglysoluble	Soluble
Meltingpoint	156 ºC	252 ºC

FT-IRanalysis

IRofEmpagliflozin

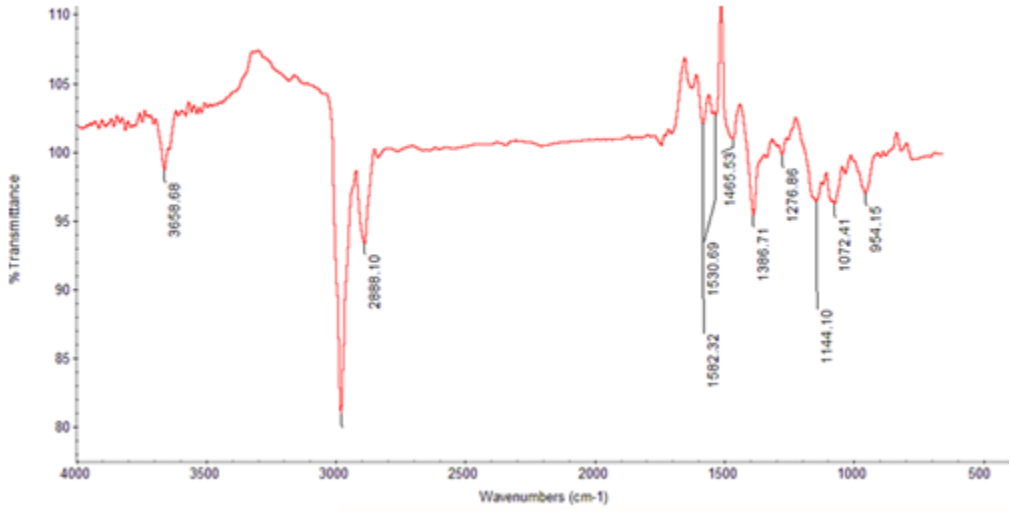

Figure 1. FTIR Spectra of EMP

The FTIR spectrum of Empagliflozin exhibited characteristic absorption peaks corresponding to its functional groups, confirming the identity and purity of the drug. A broad absorption band at 3368.86 cm^{-1} was attributed to O–H stretching vibrations, while the peak at 2888.10 cm^{-1} indicated aliphatic C–H stretching. The absorption bands at 1622.33 and 1569.05 cm^{-1}

corresponded to aromatic C=C stretching vibrations. Peaks at 1466.53 and 1388.71 cm^{-1} were assigned to C–H bending vibrations, whereas bands at 1276.86 and 1144.10 cm^{-1} represented C–O–C stretching of ether linkages. Additional peaks at 1072.41 and 954.15 cm^{-1} were attributed to C–O stretching and C–H bending vibrations, respectively. The observed FTIR spectrum was consistent with the reported functional groups of Empagliflozin, confirming its chemical identity.

FT-IRofLinagliptin (LIN)

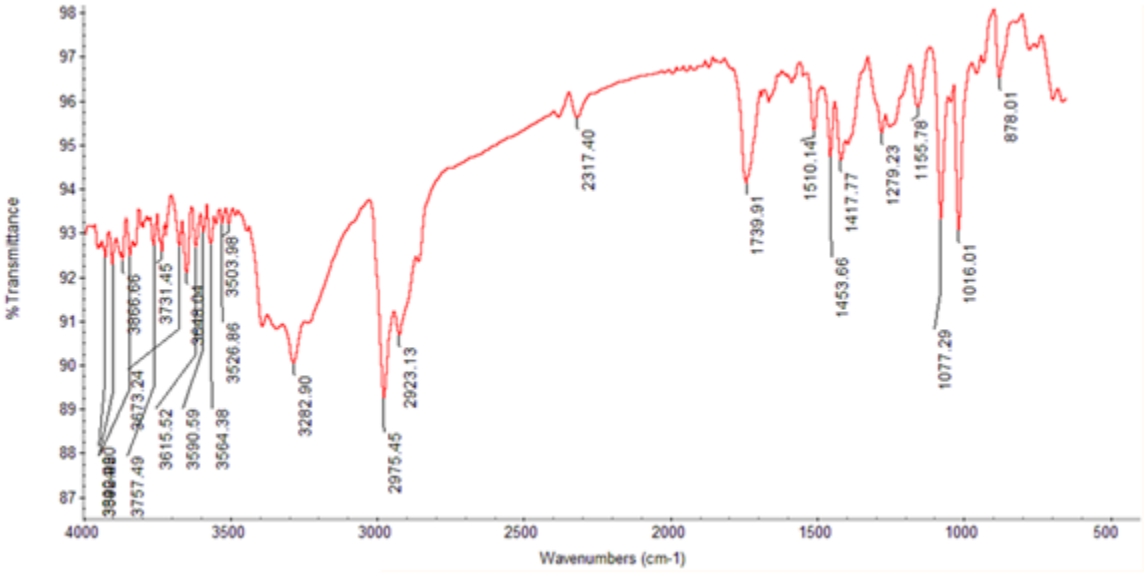

Figure 2. FT-IR Spectra of LIN

The FTIR spectrum of Linagliptin exhibited characteristic absorption peaks corresponding to its functional groups, confirming the identity and purity of the drug. A broad absorption band at 3867–3282 cm^{-1} was attributed to N–H and O–H stretching vibrations, while peaks at 2975.45 and 2923.13 cm^{-1} indicated aliphatic C–H stretching. The characteristic carbonyl (C=O) stretching peak appeared at 1739.10 cm^{-1}, whereas bands at 1593.91 and 1501.14 cm^{-1} corresponded to aromatic C=C stretching. Additional peaks at 1417.77, 1229.23, 1165.65, 1077.29, and 1016.01 cm^{-1} were assigned to C–N, C–O, and C–O–C stretching vibrations, while the peak at 818.01 cm^{-1} represented aromatic C–H bending. The observed FTIR spectrum was in good agreement with the reported functional groups of Linagliptin, confirming its chemical identity.

UVSpectroscopy Analysis

The UV absorption spectra of Empagliflozin (EMP) and Linagliptin (LIN) were recorded using a Shimadzu UV-1800 UV–Visible spectrophotometer with 1 cm quartz cells over the wavelength range of 200–400 nm. The maximum absorption wavelengths (λmax) were found to be **220 nm** for Empagliflozin and **293 nm** for Linagliptin. These characteristic absorption maxima were used for the identification of the drugs and served as a basis for selecting the appropriate detection wavelength during RP-HPLC method development.

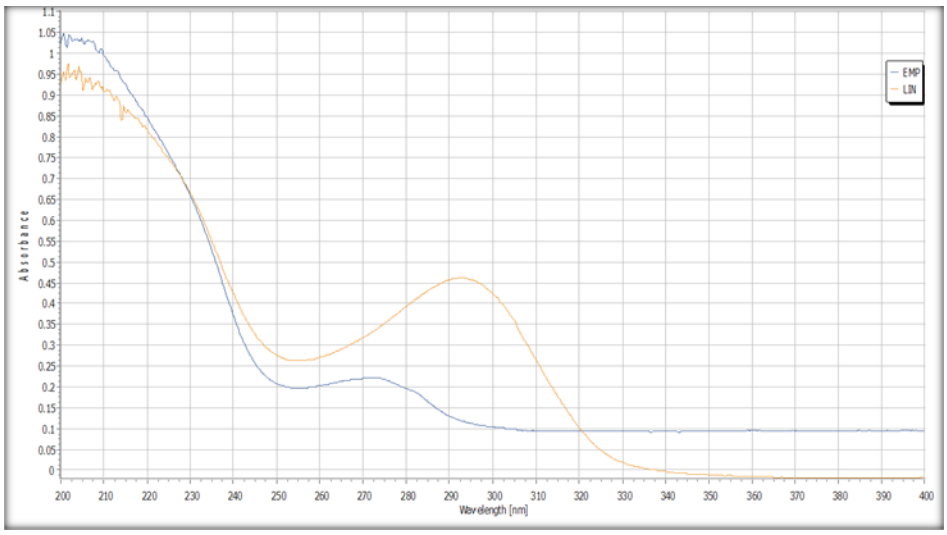

Figure 3. Overlain UV Spectra of EMP and LIN

Fromtheoverlainspectrumthewavelengthsselecte dforestimationofdrugsis228 nm.

Selectionofmobilephase:

From various mobile phases tried, mobile phase containing Methanol: 5 mm Phosphate buffer (60:40) pH 3.5 was selected, since it gives sharp reproducible retention time for EMP and LIN.

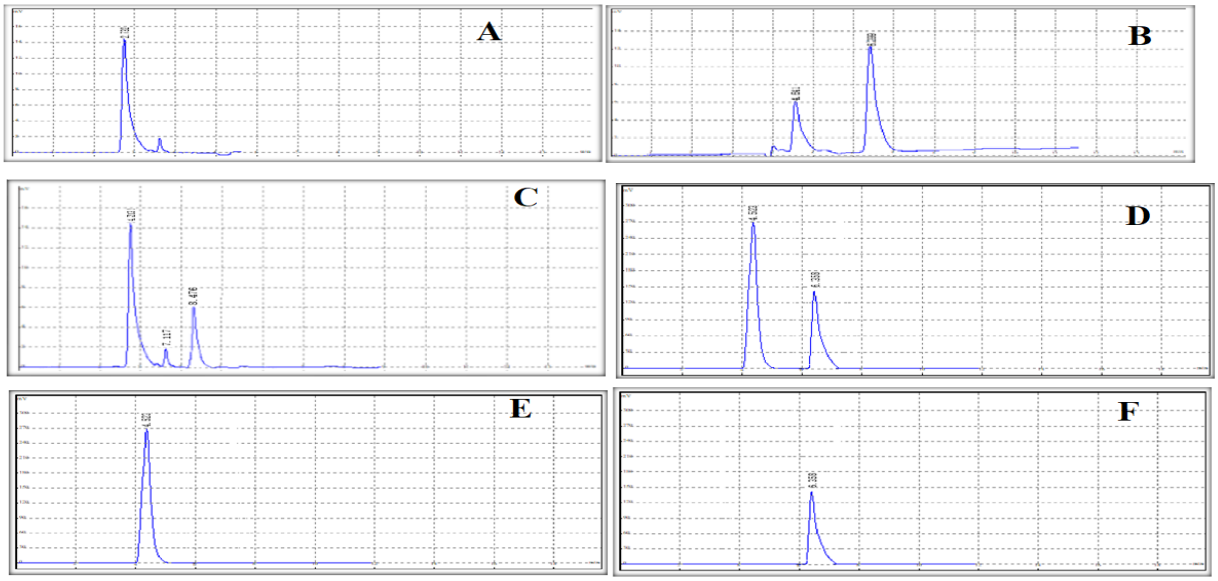

Figure 4. TrialChromatogramobtainedbyusingA)Methanol:water(90:10)as mobile phase B) Acetonitrile:Water(80:20)as mobile phase, C) Methanol:Buffer(80:20)pH5.5 as mobile phase, D) Methanol:Buffer(60:40)pH3.5 as mobile phase, E) Methanol:Buffer(60:40)pH4as mobile phase of LIN and F) Methanol:Buffer(60:40)pH3.5as mobile phase of EMP

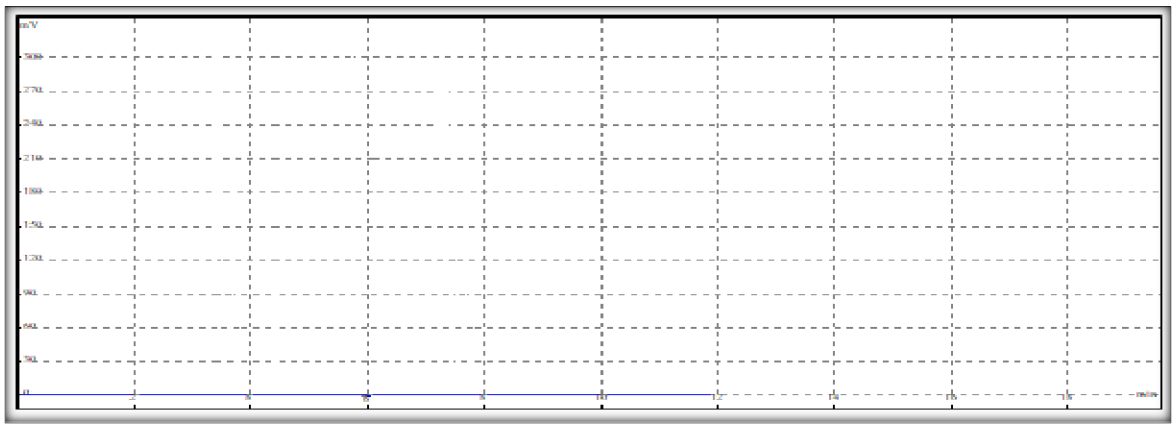

Figure5. BlankChromatogramobtainedbyMethanol:Buffer(60:40)pH3.5as mobile phase.

Chromatographicconditions

The chromatographic conditions were optimized through a series of preliminary trials to achieve efficient separation of Empagliflozin and Linagliptin. Chromatographic analysis was performed on a Grace C18 column (4.6 × 250 mm, 5 µm) using a mobile phase consisting of methanol and phosphate buffer (60:40, v/v; pH 3.5). The mobile phase was delivered at a flow rate of 1.0 mL/min, and the eluents were monitored at a detection wavelength of 228 nm. The analysis was carried out at ambient temperature with an injection volume of 20 µL. These optimized conditions provided well-resolved, symmetrical peaks with satisfactory system suitability parameters for the simultaneous estimation of both drugs.

Preparationofcalibration curve

Themobilephasewasallowedtoequilibratewiththestationaryphaseuntilsteadybaseline wasobtained.Theseriesofconcentrationfromµg/mlfor2-20µg/mlEMPand2-20µg/ml for LIN drug solutions were injected and peak area was recorded.

Table 2. Observationof standardcurvesofEMPand LIN

Conc.(µg/ml) EMP	Conc.(µg/ml) LIN	Peak Area	
		EMP	LIN
2	2	18497	17942
4	4	36994	35885
6	6	55491	53827
8	8	73988	71770
10	10	92485	89712
12	12	110982	107654
14	14	129479	125597
16	16	147976	143539
18	18	166473	161482
20	20	184970	185424

Systemsuitability test

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions.

Table 3. Result of System Suitability Study

Sr. No	Peakarea		Retention Time		Asymmetry		Efficiency	
	EMP	LIN	EMP	LIN	EMP	LIN	EMP	LIN
1	92485	44856	4.523	6.358	1.112	1.898	132478.57	55389.33
2	91560	44542	4.482	6.307	1.095	1.877	130491.39	54724.66
3	92023	44183	4.455	6.263	1.109	1.873	132081.13	54946.22

4	90820	44766	4.509	6.333	1.102	1.889	131286.26	54724.66
5	91098	44363	4.473	6.275	1.098	1.887	130623.87	55333.94
Mean	91597.144	44542.008	4.489	6.307	1.103	1.885	131392.25	55023.76
±S.D	675.20384	278.32416	0.02743	0.03945	0.00712	0.00977	875.76251	322.021
C.V	0.0073714	0.0062485	0.00611	0.00625	0.00645	0.00518	0.0067	0.0059

Application of proposed method for estimation of EMP and LIN Laboratory mixture:

Thepeakareaofstandardlaboratorymixtureandsamplelaboratorymixturewascompared to obtain the concentration.

Table 4.ResultsandstatisticaldataforestimationofEMPand LINin lab. Mix

Sr. No.	Weightofstd. (mg)		Weightof sample(mg)		PeakareaofStd.		Peakareaofsample		%Drug estimation	
	EMP	**LIN**	**EMP**	**LIN**	**EMP**	**LIN**	**EMP**	**LIN**	**EMP**	**LIN**
1.			10	10			92762	44677	100.3	99.6
2.			9.9	10			92855	44901	100.4	100.1
3.	10	10	10	10.1	92485	44856	91930	44946	99.4	100.2
								Mean	100.03	99.97
								±S.D.	0.551	0.321
								C.V.	0.006	0.003

Application of proposed method for estimation of EMP and LIN in formulation:

Equalvolume(20µL)ofstandardandsamplesolutionwereinjectedseparatelyafter equilibrium of stationary phase. The chromatograms were recorded and the response i.e.peak area of major peaks were measured. The content of EMP and LIN was calculated by comparing a sample peak with that of standard.

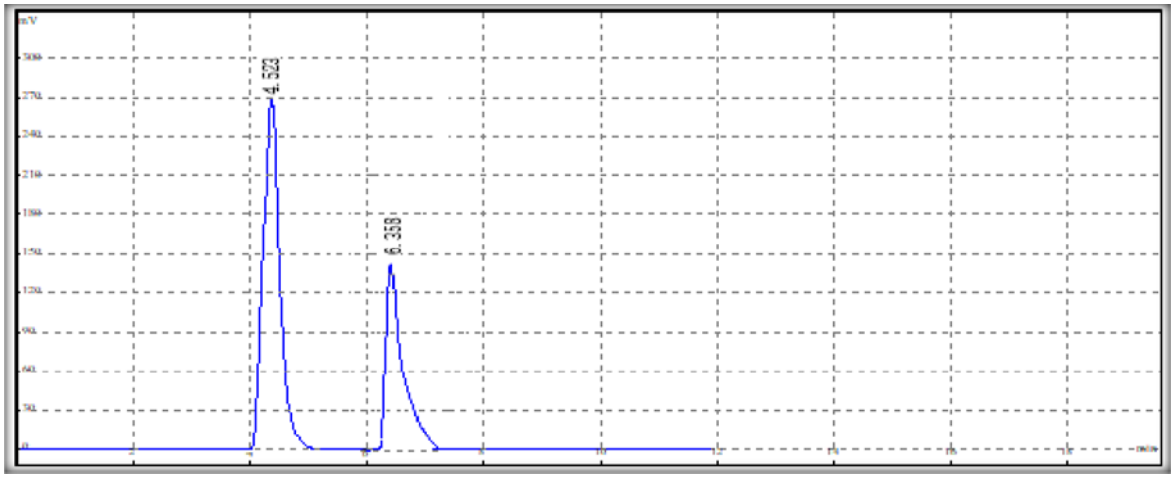

Figure6. ChromatogramobtainedbyformulationofEMPand LIN

Table 5. Resultsandstatisticaldataforestimationof EMPandLINinmarketed formulation.

Brandname:EmpazinLAvargwt :106 mg

Sr. No.	Weightof std.(mg)		Weight of sample (mg)	Peakareaof Std.		Peakareaof sample		%Labelclaim	
	EMP	**LIN**		**EMP**	**LIN**	**EMP**	**LIN**	**EMP**	**LIN**
1.			106			92115	44677	99.6	99.6
2.			106			93225	44901	100.8	100.1
3.	10	10	106	92485	44856	93317	44946	100.9	100.2
							Mean	100.43	99.97

	±S.D.	0.723	0.321
	C.V.	0.007	0.003

Validationparameters

Accuracy: Itwasascertainedonthebasisofrecovery studiesperformedbystandardadditionmethod. The results of recovery studies and statistical data are recorded in Table 6.

Table 6.Results andstatisticaldata forRecoverystudy ofEMPandLIN

Sr. No.	wt. of formulation	Amountof Drug Addedin (µg/ml).		PeakAreaof stand.		PeakAreaof sample		% Recovery	
		EMP	LIN	EMP	LIN	EMP	LIN	EMP	LIN
1	106	1	1	92485	44856	92762	44991	100.3	100.3
2		1	1			92855	45035	100.4	100.4
3		1	1			91930	45080	99.4	100.5
4		2	2			92300	45305	99.8	101
5		2	2			92208	45349	99.7	101.1
6		2	2			92115	44542	99.6	99.3
7		3	3			93225	44766	100.8	99.8
8		3	3			93317	44721	100.9	99.7
9		3	3			93502	44677	101.1	99.6
						Mean		100.22	100.19
						S.D.		0.624	0.629
						C.V		0.006	0.006

Precision: Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

Table 7.ResultsandstatisticaldataofPrecisionStudy Brand Name: Empazin L

Sr. No	Weightof std.(mg)		Wt. of sample (mg)	Peakareaof std.		Peakareaof sample		%Labelclaim	
	EMP	LIN		EMP	LIN	EMP	LIN	EMP	LIN
1.	10	10	106	92485	44856	93225	89272.3	100.8	99.8
2.			105.9			93317	89182.8	100.9	99.7
3.			106			93502	89093.4	101.1	99.6
							Mean	100.93	99.70
							±S.D.	0.153	0.100
							C.V.	0.002	0.001

Ruggedness

Thestudiesofruggednesswere carriedoutunder twodifferent conditions

1) Days
2) Analyst.

Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 8

Interday(Differentdays):

Table 8. ResultsandstatisticaldataofInterdayStudy Brand Name:Empazin L

Sr. No.	Weightof std.(mg)		Wt. of sample(mg)	PeakareaofStd.		Peakareaof sample		%Labelclaim	
	EMP	LIN		EMP	LIN	EMP	LIN	EMP	LIN

1.			106			92115	45349	99.6	101.1
2.			106			92762	44542	100.3	99.3
3.	10	10	106	92485	44856	92855	45125	100.4	100.6
							Mean	100.10	100.33
							±S.D.	0.436	0.929
							C.V.	0.004	0.009

Intraday: It was performed by using same procedure as under marketed formulation analysis and absorbancerecorded at 3 hrs interval within aday. Thepercent label claim was calculated using formula and Result and statistical data are shown in Table No. 9

Table 9. ResultsandstatisticaldataofIntradayStudy Brand Name:Empazin L

Sr.No.	Weightof sample (mg)	Peakareaof std		Peakareaof sample		%Labelclaim	
		EMP	LIN	EMP	LIN	EMP	LIN
1.	106			92393	44946	99.9	100.2
2.	106			92577	44811	100.1	99.9
3.	105.9	92485	44856	92115	44991	99.6	100.3
					Mean	99.87	100.13
					±S.D.	0.252	0.208
					C.V.	0.003	0.002

Differentanalyst: The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

Table 10.Resultandstatisticaldataof Differentanalyststudy

Sr.No.	%Labelclaim			
	ANALYST I		ANALYST II	
	EMP	LIN	EMP	LIN
1	99.8	100.8	99.4	100.9
2	99.7	100.9	99.6	101.1
3	99.6	99.4	99.6	101.5
4	99.6	99.8	100.3	101.1
5	100.8	99.7	100.4	101.5
Mean	99.9	100.12	99.86	101.22
±S.D	0.509901951	0.683373983	0.45607017	0.268328157
C.V	0.005104124	0.006825549	0.004567096	0.00265094

Specificity: Specificity was measured as ability of the proposed method to obtain well separated peak for EMP and LIN without any interference from component of matrix.
Meanretentiontimefor–

EMP– 4.523
LIN–6.358
Thevaluesobtainedwereveryclosetothatinstandardlaboratorymixtureindicatesno interference from the component of matrix.

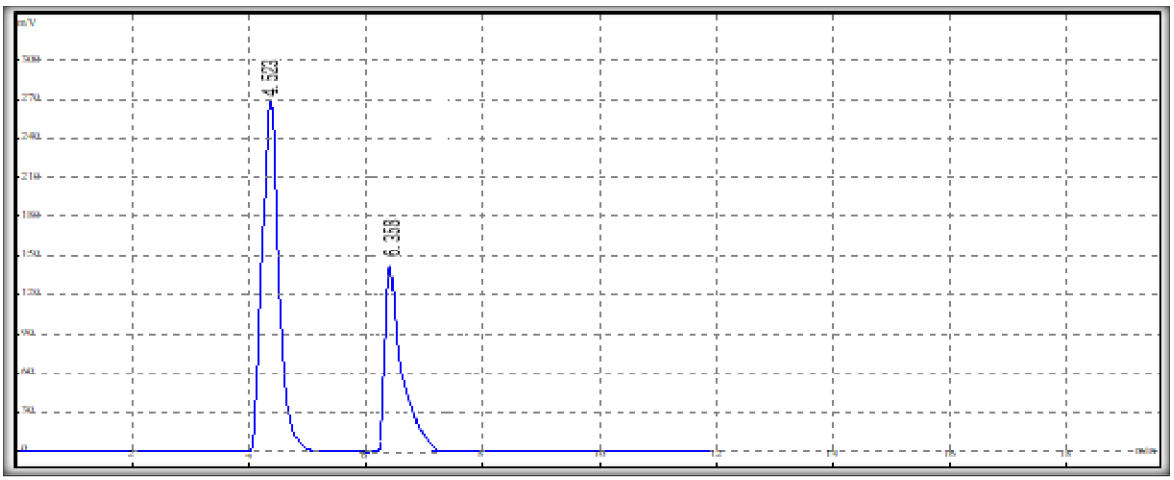

Figure7. ChromatogramobtainedbyformulationofEMPandLIN

Linearityand range

According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved anddilutedappropriatelywith mobilephaseto obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutionswererecorded. EMP and LINmarketed formulationwasfoundto belinearinthe range ± 20% of the test concentration of the respective drug.

Table 11. ObservationsofLinearityand rangestudyforEMPandLIN

%Labelclaim	Peakarea	
	EMP	LIN
80	73988	35884
90	83236	40370
100	92485	44856
110	101731	49341
120	111982	54427

Robustness

Robustnessisameasureofhowwellananalyticalmethodcanproducereliableresults whentherearesmallchangestotheexperimentalconditions. Therobustnessstudyfor EMP and LIN shown in following table 12.

Table 12.ResultofRobustnessstudyofEMP

Condition	Parameter	Peak Area	RT
Change of wavelength	226 nm	92476	4.521
	228 nm	92494	4.523
	230 nm	92485	4.532
Change in Temperature	30 °C	92448	4.531
	25 °C	92476	4.523
	20°C	92374	4.554
ChangeinFlowrate	0.8 ml/min	92420	4.587
	1 ml/min	92484	4.525
	1.2 ml/min	92439	4.503
ChangeinMobile Phase	65:35	92577	4.515
	60:40	92485	4.528
	55:45	92484	4.543

Table 13.ResultofRobustnessstudyofLIN

Condition	Parameter	Peak Area	RT
Change of wavelength	226 nm	44852	6.358
	228 nm	44860	6.369
	230 nm	44856	6.351
Change in Temperature	30 °C	44838	6.352
	25 °C	44852	6.355
	20°C	44802	6.365
ChangeinFlowrate	0.8 ml/min	44825	6.378
	1 ml/min	44856	6.361
	1.2 ml/min	44834	6.342
ChangeinMobile Phase	65:35	44901	6.335
	60:40	44856	6.356
	55:45	44856	6.361

Limitof Detection(LOD)andLimitof Quantitation(LOQ):

The sensitivity of the developed RP-HPLC method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). The LOD represents the lowest concentration of analyte that can be detected but not necessarily quantified, whereas the LOQ represents the lowest concentration that can be quantified with acceptable accuracy and precision. The LOD values were found to be **0.871 µg/mL** for Empagliflozin and **0.178 µg/mL** for Linagliptin, while the corresponding LOQ values were **2.89 µg/mL** and **1.03 µg/mL**, respectively. These results indicate that the developed method possesses adequate sensitivity for the simultaneous estimation of both drugs.

Conclusion

A simple, rapid, accurate, precise, and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and tablet dosage forms. The optimized chromatographic conditions provided excellent peak resolution with acceptable retention times and satisfactory system suitability characteristics. The method exhibited excellent linearity over the selected concentration range and demonstrated high accuracy, precision, specificity, ruggedness, and robustness in accordance with ICH Q2(R2) guidelines. Furthermore, the low LOD and LOQ values indicated adequate sensitivity for routine analysis. The developed method was successfully applied to the quantitative estimation of Empagliflozin and Linagliptin in marketed tablet formulations, producing assay values within acceptable pharmacopeial limits. Therefore, the proposed RP-HPLC method is reliable, economical, and suitable for routine quality control, stability studies, and assay of combined pharmaceutical dosage forms containing Empagliflozin and Linagliptin.

REFERENCES

1. El Sheikh R, Hassan WS, Elzanfaly ES, Zaazaa HE. Development and validation of rapid stability-indicating high-performance liquid chromatography method for the determination of linagliptin and empagliflozin in pure and dosage forms. Asian J Pharm Clin Res. 2020;13(4):172-177. doi:10.22159/ajpcr.2020.v13i4.36939.
2. Vankalapati KR, Alegete P, Boodida S. Stability-indicating ultra performance liquid chromatography method development and validation for simultaneous estimation of metformin, linagliptin, and empagliflozin in bulk and pharmaceutical dosage form. Biomed Chromatogr. 2021;35(4):e5019. doi:10.1002/bmc.5019.
3. Patel IM, Chhalotiya UK, Jani HD, Kansara DJ, Kachhiya HM, Shah DA. Simultaneous quantification of empagliflozin, linagliptin and metformin hydrochloride in bulk and synthetic mixture by RP-LC method. Future J Pharm Sci. 2021;7:182. doi:10.1186/s43094-021-00332-1.
4. El-Desouky EA, Abdel-Raoof AM, Abdel-Fattah A, Abdel-Zaher A, Osman AOE, Abdel-Monem AH. Determination of linagliptin and empagliflozin by UPLC and HPTLC techniques aided by Lean Six Sigma approach. Biomed Chromatogr. 2021;35(7):e5102. doi:10.1002/bmc.5102.
5. Kiran TNR, Parvathi P, Suresh Kumar JN. Development and validation of RP-HPLC method for simultaneous estimation of

linagliptin, empagliflozin and metformin in solid dosage forms. Asian J Pharm Anal. 2020;10(3):117-124. doi:10.5958/2231-5675.2020.00021.6.
6. Abdel Sattar OI, Abuseada HHM, Emara MS, Selim I, Ali MA. Green evaluation of human plasma levels of metformin, linagliptin, and empagliflozin using HPLC and HPTLC methods: a pharmacokinetic study. BMC Chem. 2026;20:40. doi:10.1186/s13065-026-01726-z.
7. Satapathy S, Barman K, Kumar S, Singh N, Kurmi BD, Patel P. A green and QbD-optimized RP-HPLC method for simultaneous estimation of linagliptin and dapagliflozin. Int J Chem Kinet. 2026. doi:10.1002/kin.70032.
8. Adnan M, Khan MN, Ali R, Ullah Z, Ahmed I, Salman SM. RP-HPLC method development and validation for the studies of sodium-glucose co-transporter 2 (SGLT2) and dipeptidyl peptidase 4 (DPP-4) inhibitors empagliflozin and linagliptin in pharmaceutical dosage form. J Popul Ther Clin Pharmacol. 2024.
9. El-Sayed AA, El-Bagary RI, Elkady EF, Ayoub BM. Fluorimetric study on antidiabetic combined drugs: empagliflozin and linagliptin in their pharmaceutical formulation and human plasma. Spectrochim Acta A Mol Biomol Spectrosc. 2021;248:119258. doi:10.1016/j.saa.2020.119258.
10. Pandya DJ, Patel SG, Shah UA, Patel CN, Patel K. Stability indicating RP-HPLC method development and validation of dapagliflozin and linagliptin in tablet dosage form by QbD approach. Res J Pharm Technol. 2026. doi:10.52711/0974-360X.2026.00323.
11. Jung S, Bosch A, Kannenkeril D, Karg MV, Striepe K, Bramlage P, et al. Combination of empagliflozin and linagliptin improves blood pressure and vascular function in type 2 diabetes. Eur Heart J Cardiovasc Pharmacother. 2020;6(6):364-371. doi:10.1093/ehjcvp/pvz078.
12. Katsiki N, Ofori-Asenso R, Ferrannini E, Mazidi M. Fixed-dose combination of empagliflozin and linagliptin for the treatment of patients with type 2 diabetes mellitus: A systematic review and meta-analysis. Diabetes Obes Metab. 2020;22(6):1001-1005. doi:10.1111/dom.13989.
13. Liu SC, Lee CC, Chuang SM, Sun FJ, Zeng YH. Comparison of efficacy and safety of empagliflozin vs linagliptin added to premixed insulin in patients with uncontrolled type 2 diabetes: A randomized, open-label study. Diabetes Metab. 2021;47(3):101184. doi:10.1016/j.diabet.2020.08.001.
14. Lingvay I, Beetz N, Sennewald R, Schuler-Metz A, Bertulis J, Loley C, et al. Triple fixed-dose combination empagliflozin, linagliptin, and metformin for patients with type 2 diabetes. Postgrad Med. 2020;132(4):337-345. doi:10.1080/00325481.2020.1750228.
15. Watada H, Yamauchi T, Yamamoto F, Taniguchi A, Yarush L, Heilmann C, et al. Safety and tolerability of empagliflozin and linagliptin combination therapy in patients with type 2 diabetes mellitus: A pooled analysis of data from five randomized, controlled clinical trials. Expert Opin Drug Saf. 2020;19(9):1193-1202. doi:10.1080/14740338.2020.1782884.
16. Laxman B, Yojana K, Chaitali K, Shivam K, Sagar M, Godge RK. A rapid and sensitive stability-indicating RP-HPLC method development for the quantitative analysis of empagliflozin and linagliptin in bulk and synthetic mixture. Int J Health Sci. 2022;6(S3):5526-5538. doi:10.53730/ijhs.v6nS3.7166.
17. Kharwade SB, Mohite PB. QbD-driven analytical method development and validation for linagliptin and empagliflozin by RP-HPLC. J Neonatal Surg. 2025;13(1). doi:10.63682/jns.v13i1.8641.

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