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Stability Indicating Assay of Empagliflozin and Linagliptin

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KEYWORDS
RP-HPLC;
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Validation;
t test

ABSTRACT

The newly developed stability-indicating RP-HPLC method is simple, robust, and validated on the basis of ICH guidelines for simultaneous determination of Empagliflozin and Linagliptin in tablets. Retention times under the optimized condition were 1.86 and 2.61 minutes for Empagliflozin and Linagliptin, respectively. This research article indicates the best separation of Empagliflozin and Linagliptin from their degradation products. Separation was achieved on a Sunniest ECO C_{18}, 250 mm x 4.6 mm, 5 µm analytical column at a wavelength of 275nm, using a mobile phase: phosphate buffer (pH-5) and methanol (20:80) in an isocratic elution mode at a flow rate of 1.5 ml/min, an injection volume of 10 µl, and a run time of 4 minutes. The RSDs for the precision studies were less than 1.5% for both drugs. The %RSD was less than 1.5 in all the parameters of robustness. The forced degradation studies were carried out using 0.1N HCl, 0.1N NaOH, 3% H_2O_2 and exposure to temperature (60^0 C). Stress study indicates the Empagliflozin is completely degraded in an oxidative medium and 39.22% for Linagliptin. The degradation of the drugs in acidic medium and exposure to temperature are within the ICH acceptable limits. The two API chromatographic peaks in basic degradation study are not well resolved. The observed students 't' test values are within the acceptable range in the tablet assay and recovery study. So this method is a fast, sensitive, robust, and efficient high-performance liquid chromatographic method for the concurrent determination of Empagliflozin and Linagliptin

Introduction:

Worldwide, 463 million adults suffer with diabetes. In most nations, the percentage of individuals with type-2 diabetes is rising [1]. Type-1 diabetes is brought on by insufficient insulin production, but type2 diabetes (T2DM) is brought on by cells that are resistant to insulin.Anti-diabetic medications are required for lifelong management of type 2 diabetes mellitus.

Empagliflozin (EMPA) (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-Chloro-3-[[4-[(3*S*)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol is a potent and selective sodium–glucose cotransporter 2 (SGLT2) inhibitor used for the treatment of type 2 diabetes. Empagliflozin reduces renal glucose reabsorption, thereby increasing urinary glucose excretion, leading to a reduction in plasma glucose levels in subjects with type 2 diabetes in an insulin-independent patient and EMPA had the highest selectivity for other hypoglycemic drugs in the same class[1].

Linagliptin (LINA) 8-[(3*R*)-3-Aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro -1*H*-purine-2,6-dione is a potent and selective dipeptidyl peptidase-4 (DPP-4) inhibitor used for the treatment of patients with type 2 diabetes.Linagliptin prevents the inactivation of incretin peptides such as glucagon-like peptide 1 (GLP-1), stimulates insulin release, and inhibits glucagon secretion.

Guidelines for the management of type 2 diabetes recommend metformin combination as first-line pharmacological treatment; however, contraindications to metformin, such as renal impairment, are common among patients with type 2 diabetes and metformin

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intolerance owing to gastrointestinal side effects. The combination of empagliflozin and linagliptin led to significantly greater reductions in glycated haemoglobin (HbA1c) and had superior efficacy compared with either drug alone[1]. Both the drugs are not official in any Pharmacopoeia.

The fixed dose combination of empagliflozin 25mg and linagliptin 5 mg is an approved formulation. Literature survey indicates few HPLC, UPLC and LC-MS methods[2-22] for quantification of both drugs and they are summarized in table 1. The present work is to develop stability-indicating RP-HPLC method for simultaneous determination of Empagliflozin and Linagliptin in tablets which is more accurate with better resolution and less time consuming than existing methods.

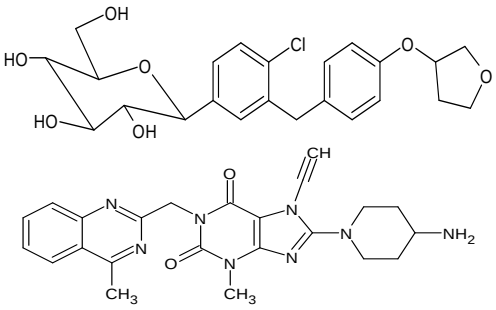

Fig 1: (a) Empagliflozin, (b) Linagliptin

Table 1: Reported Analytical Methods for Empagliflozin and Linagliptin

Analyte	Method	Column	Mobile Phase and Flow Rate	Retention Time (min)	Reference citation
LINA, EMPA	HPLC	Discovery C18 250 mm×4.6 mm, 5 µm	Buffer (4.5): Acetonitrile (68:32); 1 ml/min	6.447, 4.716	2
EMPA, LINA	HPLC	g C-18 column BDS 250 mm × 4.6 mm, 5 µm; 30 ºC	0.1% O-Phosphoric acid and Acetonitrile (60:40 v/v); 1 ml/min	2.05, 4.10	3
EMPA, LINA	HPLC	ODS-3 Inertsil C18 150 mm×4.6 mm, 5 µm; 25±2°C	Phosphate buffer and Acetonitrile (65:35 v/v); 1 ml/min	3.276, 6.966	6
EMPA, LINA	HPLC	Thermo C18 250 mm × 4.6, 5µm	Acetonitrile: methanol (50:50 v/v); 1 ml/min	3.357, 4.085	7
EMPA, LINA, MET	HPLC	Kromosil 250 mm x 4.6 mm, 5µm; 30°C	Buffer: Acetonitrile (45:55v/v)	2.370, 2.787, 3.419	8
EMPA, LINA, MET	HPLC	RP-Inertsil-Octa-Decyl S-3V-C18 :250 mm x 4.6 mm, 5 µm; 40^0 C	Buffer Di-potassium hydrogen phosphate (0.03M) and a pH 3.0 adjusted with O-phosphoric acid; 1 ml/min	8.187, 11.71	9
EMPA, LINA, MET	HPLC	Phenomenex C18 250 mm×4.6 mm, 5 µm	Acetonitrile: Methanol: Water (27: 20: 53, v/v/v); 1 ml/min	14.5, 3.4, 2.01	11
EMPA, LINA	HPLC	kromosil 250mm x 4.6 mm, 5 µm; 30°C	Buffer (pH4.8) : Acetonitrile(70:30); 1 ml/min	1.92, 3.69	13
EMPA, LINA	RP-UPLC	Phenomenex Luna omega polar C18, 00mmx2.1mm, 1.6µ; 35°C	Mobile phase A: 10mM Potassium dihydrogen orthophosphate (pH-3.0) and Mobile phase B: acetonitrile and methanol (55:45%v/v); 0.5 ml/min	6.588, 15.758	14
EMPA, LINA	UPLC, HPTLC	1) Zorbax eclipse plus C-18: 4.6 mm X 100 mm, 3.5 µm 2) Pre-coated TLC plates and silica gel 60 GF254 (20 × 20 cm;FlukaChemie, Switzerland	1) Phosphate buffer (pH 4): Acetonitrile (65:35) 2) ethyl acetate–chloroform–acetonitrile (55:25:20); 1.5 ml/min	1.417, 2.453	15
EMPA, LINA	HPLC	Shimadzu C18 250 mm × 4.6 mm, 5 µm	Acetonitrile: Buffer (pH 3) (80:20 v/v; 0.80 ml/min	3.714, 3.064	16
EMPA	HPLC	ZORBAX C18 250 x 4.6mm, 5µm	Acetate buffer (pH 3.4): Acetonitrile (60:40% v/v); 1.0 ml/min	2.57	17
EMPA, LINA, MET	UPLC	Acclaim™ RSLC 120 C18 100 mm × 2.1 mm, 2.2 mm; 50^0C	Potassium dihydrogen phosphate buffer (pH 4): methanol (50: 50 v/v); 0.4 ml/min	1.6, 2.3, 4.7	18
EMPA, LINA,	HPLC	Discovery column C18 150mm× 4.6 mm, 5 µm; 30^0C	Acetonitrile: phosphate buffer (pH 5) (38:62% v/v); 1 ml/min	3.04, 3.93,	19

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MET				5.99	
EMPA, LINA, MET	HPLC	ThermoHypersil octa decyl silane 250 mm × 4.6 mm, 5 µm; 40°C	0.043 M Potassium dihydrogen O-Phosphate buffer (pH 3.79 adjusted using O-phosphoric acid pre mixed with 0.05%v/v TEA: methanol (34.4:65.6, v/v); 1 ml/min	3.4, 4.6, 5.7	20
EMPA, LINA	LC-MS	C18 50 mm × 2.1 mm, 1.7 µm	0.1 % aqueous formic acid and acetonitrile (50:50, v/v) ; 0.2 ml/min	--	21

MATERIALS AND METHOD

Chemicals and Reagents: Reference samples of Empagliflozin and Linagliptin, Water (HPLC grade) KH_2PO_4 (AR Grade), HCl (AR Grade), NaOH (AR Grade), CH_3OH(HPLC Grade) was used in the analysis.

Instruments and Software: A Schmidazu LC 2010 CHT with quaternary constant flow system and equipped with auto sample injector was utilized in the study. Schimadzu LC Solution software was employed to monitor and integrate the output signals. Other instrument and apparatus such Contech Analytical balance, Eutech pH meter, Leela Sonic Ultrasonicates were used in this study.

Diluent: Water and methanol were mixed in a ratio of 50:50 (v/v) and it was used as diluent in an entire investigation of method development and validation of stress study.

Mobile Phase: It was prepared in the ratio of Buffer (pH 5) and Methanol in the ratio of 20:80

Buffer Solution:0.01 M KH_2PO_4 prepared with HPLC grade water and pH adjusted to 5 with dilute NaOH

Optimized Chromatographic conditions

The separation of Empagliflozin and Linagliptin was achieved by using Column: Sunniest ECO C_{18}, 250 mm X 4.6 mm, 5µm at a Column temperature of 25°C with Buffer: Methanol (20:80) as mobile Phase, at a Flow rate of 1.5 ml/minute. Elution effected in isocratic mode with Injection volume of the analyte sample of 10 µl and detection at 275 nm with run time 4.0 minutes

Standard Stock Solutions: Empagliflozin and Linagliptin standard stock solutions were made independently by weighing 50 mg of Empagliflozin and 10 mg of Linagliptin into two distinct 100 ml volumetric flasks and then adding 40 ml of diluent to each flask. Subsequently, the medication was dissolved in the flasks using an ultrasonicator. Subsequently, the flask was diluted using the diluent upto volume to get 500µg/ml of Empagliflozin and 100µg/ml of Linagliptin.

Linearity and Range

Preparation of Working Standard Solution: Seven distinct 10 ml volumetric flasks were filled with 1, 2, 3, 4, 5, 6, and 7 ml of the standard stock solutions of Empagliflozin and Linagliptin inorder to create the working standard solution. Each concentration displayed in the linearity table was then obtained by adding diluents upto the volume. Each concentration was injected in the proposed optimized chromatographic condition to record the chromatogram. Linear regression equation for Empagliflozinin the concentration range of 0-350 µg/mlis found to be **Y = 2,062.7771 X – 1712.125** and **r^2 = 0.9998**. Linear regression equation for Linagliptin in the concentration range of 0-70µg/mlis found to be **Y = 8282.411 X + 1632.5833 andr^2 = 0.9999.** The linearity curves of Empagliflozin and Linagliptin are shown in the below Fig 2 & 3 respectively. The linearity data of both the drugs are shown in the below Table 1. The overlay chromatogram of both drugs is shown in the Fig: 4.

Table 2: The linearity data of EMPA and LINA

Sl. No.	Empagliflozin		Linagliptin	
	Conc. (µg/ml)	**Area**	**Conc.** (µg/ml)	**Area**
1	0	0	0	0
2	50	101340	10	84085
2	100	201070	20	169184
3	150	310526.5	30	251520
4	200	410010	40	330047.5
5	250	511896.5	50	419048.5
6	300	612643.5	60	499113.5
7	350	725725.5	70	579137.5

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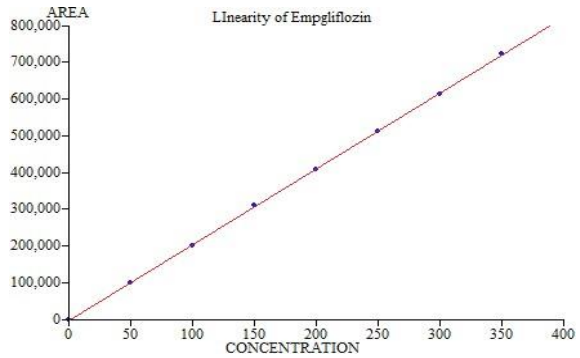

Fig: 2.The linearity curves of Empagliflozin

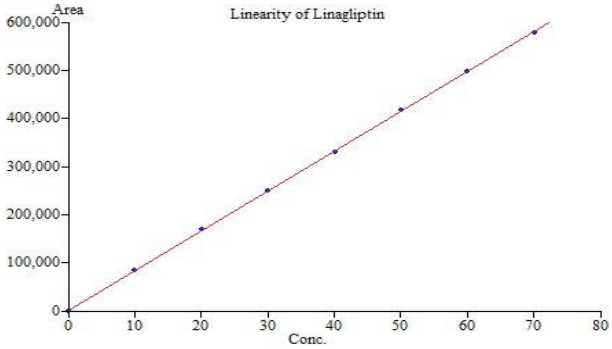

Fig: 3.The linearity curve of Linagliptin

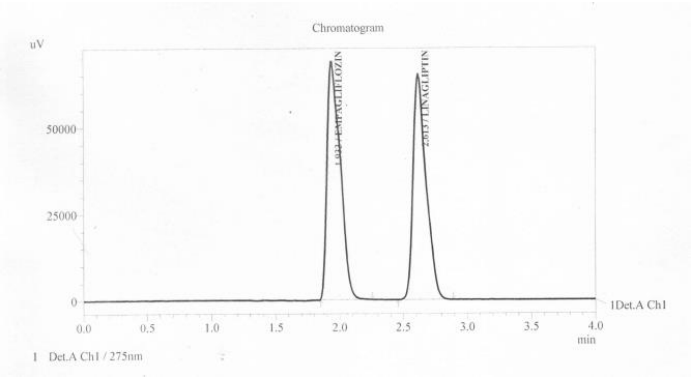

Fig 4: Representative chromatogram of Standard sample EMPA 250µg/ml and LINA 50µg/ml

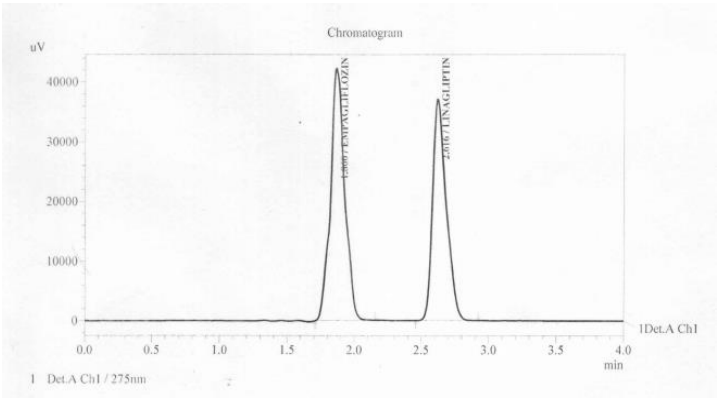

Fig 5:Representative chromatogram of Tablet sample: EMPA 150µg/ml and LINA 30µg/ml

Table 3: Results of Tablets analysis (n=5)

Analyte	Labelclaim (mg/Tablet)	**AmountFound (mg/Tablet)**	C.I.	% RSD	SE	t
Empagliflozin	25	25.026	100.04±0.387	1.084	0.488	1.065
Linagliptin	5	5.024	100.480±1.295	1.035	0.472	1.027

Preparation of Sample Solution from Marketed Tablets: The study involved obtaining tablets from the local market, determining the average weight of twenty tablets, and then finely powdering them in a mortar. After precisely weighing, a powdered tablet equivalent to 50 mg of Empagliflozin and 10 mg of Linagliptin, it was transferred into a 100 ml clean, dry volumetric flask, dissolved with diluent, sonicated, and make up to

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volume with the diluents before being filtered with the help of 0.25µm filter paper. Subsequently, transfer 3 milliliters of each filtrate into five different 10 ml volumetric flasks and dilute the mixture to the appropriate level. Table 3 displays the findings from the tablet analysis. The representative chromatogram of tablet samples is shown in Fig 5.

Method Validation

The HPLC method was validated in terms of System linearity, specificity, sensitivity, precision, and accuracy, robustness in accordance with ICH Q2 (R1) guideline and system suitability test as per USP.

Precision: The intraday precision/repeatability can be determined by injecting three working standard solutions and test sample injections. The areas of all the injections were taken and % Relative standard deviations were calculated. The % RSD was found to be less than 1.5. The inter day precision can be determined by utilizing three different concentration working standard solutions and three different test sample solutions were injected on three different days. The areas of all the injections were taken and % Relative standard deviation was calculated. The results obtained were found to be less than 1.5% in standard and test sample solutions.

System Suitability: Five replicate injections of 50µg/ml and 250µg/ml of empagliflozin and linagliptin, respectively, were used to conduct the system suitability test. System appropriateness is the chromatographic parameter conformance that guarantees the analytical system's functionality. Table No. 4 displays all the system suitability parameters.

Table 4: Results of system suitability parameter of Empagliflozin&Linagliptin

Sl. No.	Parameter	Specification	Empagliflozin	Linagliptin
1	Retention Time	----	1.86	2.61
2	Tailing factor	< 2.0	1.19	1.49
3	Theoretical Plates	> 2000	2529	2765
4	Area (% RSD)	< 2	0.56	0.44
5	Resolution (Rs)	> 2.0	---	3.866

Table 5: The results of recovery study of the drugs (n=5)

%Level of recovery	Analyte	Formulation (µg/ml)	Amount of drug added (µg/ml)	Amountof drug found*(µg/ml)	CI	% RSD	SE	t
50	EMPA	30	15	45.182	100.404±1.469	1.178	0.530	0.764
	LINA	150	75	225.248	100.110±0.279	0.224	0.101	1.096
100	EMPA	30	30	60.122	100.90±1.640	1.309	0.595	0.344
	LINA	150	150	300.39	100.130±0.457	0.368	0.165	0.788

C.I.: Confidence Interval at 95% Confidence Level within which true value maybe found = R ± ts/√n, where R is the mean percent result of the recovery study's analysis (n = 5). The following are theoretical "t" values for n - 1 degrees of freedom at a 95% confidence level: t (0.05, 4) = 2.776

Accuracy: To evaluate the suggested approach's accuracy, a spiked recovery study utilizing the traditional addition method was conducted. There weretwo performance levels: 50% and 100%. Table 5 displays the accuracy studies' findings.

Peak purity: The retention time (Rt) of standard combination of the drug and tablet samples of the two medications was used to determine the level of purity of Empagliflozin and Linagliptin. Overlay chromatogram of standard and samples of marketed formulation shows good correlations of the retention time of as shown in Fig. 6

Robustness: Carefully adjusting the chromatographic conditions—including wavelength (272nm and 278 nm), flow rate (1.3 and 1.7 ml/min), and organic phase (+5% to -5%)was used to assess the robustness of the suggested test method. % RSD was less than 1 in every

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parameter of the robustness studies. Thus, it indicates that the proposed method is reliable and robust.

Sensitivity: The sensitivity of the method was estimated by calculating LOD and LOQ. The LOD and LOQ were separately determined based on the standard calibration curve. The Lower limit of detection and limit of quantization were found to be 0.296, and 0.896µg/ml respectively.

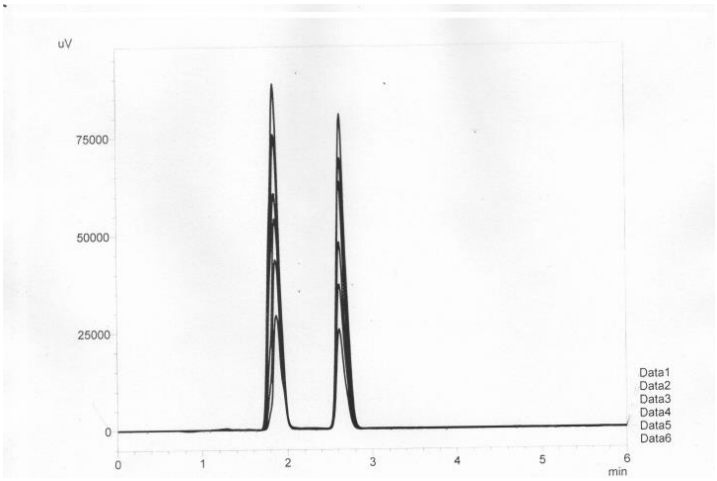

Fig: 6: Overlay Chromatogram of Empagliflozin and Linagliptin

Forced degradation study:

Force degradation studies have done to develop a stability-indicating assay by involving the proposed optimized RP-HPLC conditions by utilizing acidic, basic & oxidative, stressed temperature conditions at final concentration of 250µg/mlof Empagliflozin and 50µg/ml of Linagliptin from tablet under investigation. The results of forced degradation study of the tablet sample is shown in the Table 5. The degradation chromatogram of both the drugs are shown in the Fig 7, 8, 9 and 10.

Table 5: The results of forced degradation study of the tablet sample Empagliflozin&Linagliptin

Stress conditions	Duration (minutes)	Empagliflozin		Linagliptin	
		%Assay	%Degradation	%Assay	%Degradation
Acid (0.1 N HCl)	90	97.78	2.22	95.18	4.82
Base (0.1 N NaOH)	90	75.62	24.38	88.038	11.962
Hydrogen Peroxide (% 3)	90	4.86	95.14	60.78	39.22
Exposure to Temp 60^0 C	90	96.57	3.43	97.18	2.82

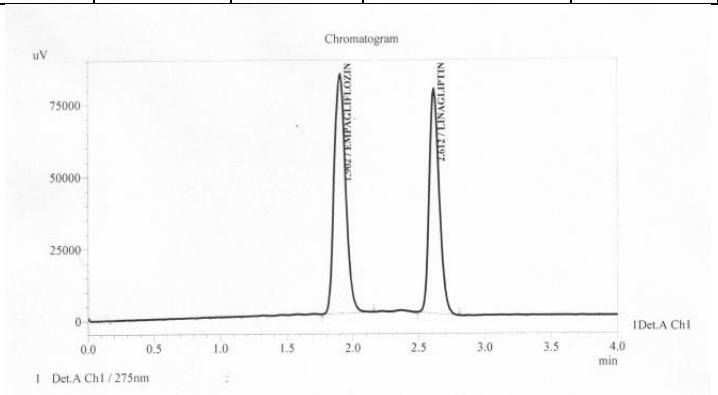

Fig: 7:Chromatogram showing EMPA and LINA in acidic stress study medium.

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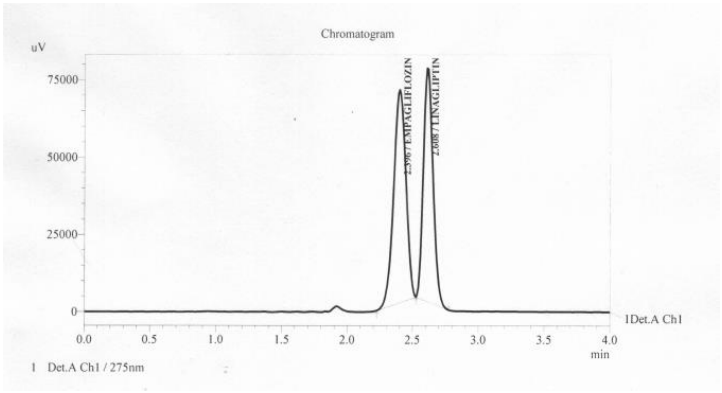

Fig: 8.Chromatogram showing EMPA and LINA in Oxidative stress medium

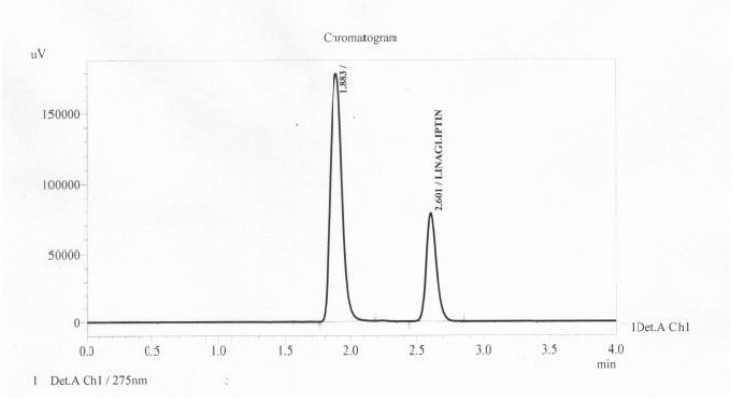

Fig 9.Chromatogram showing EMPA and LINA in basic stress medium

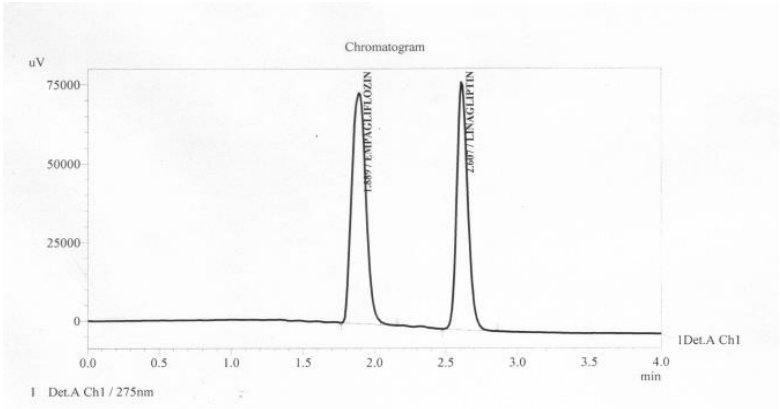

Fig. 10.Chromatogram showing EMPA and LINA in stressed temperature

Results and Discussion:

Numerous systematic trials on chromatographic parameters, such as column type and mobile phase conditions, were carried out tooptimize the suggested approach. To improve this study piece, reverse-phase solvents such as mixtures of buffer and water organic solvent were examined at various ratios on HPLC columns. The validation results show that every parameter fell between the permitted ranges specified by the ICH. The results of the system suitability test demonstrated that symmetric peaks (T < 2), well-resolved (R > 2), and more efficient separation (N > 2000) were consistently produced. Furthermore, since the RSD for the retention period of six system suitability assessments was less than 2%, the analytical output was repeatable. Because formulation excipients did not tamper with blank and placebo determinations, the approach was determined to be selective. The

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suggested approach had superior linearity, as evidenced by the r^2 value from the linear regression analysis for both medications, which was closer to 1. The RSD values for repeatability and intermediate precision measurements never surpassed 1.5%, demonstrating the higher precision of the suggested HPLC approach. Recovery results between 98–102% demonstrate the correctness of the procedure. A controlled experiment involving a minor alteration to the optimal method condition revealed no significant impact on the outcomes, indicating the approach's dependability. For the tablet assay and recovery investigation, the observed students' 't' test results were determined to be within an acceptable range.The proposed method is found simpler and faster than reported liquid chromatography methods. Stress study indicates that the degradation of the Empagliflozin is completely occurring in the basic medium whereas degradation of both the drugs are within the acceptable limit of the ICH guidelines.

Conclusion:

Empagliflozin and Linagliptin concentrations in bulk and tablet form were precisely measured using a novel, simpler and faster liquid chromatography technique that was evaluated. It was shown that the method is resilient, rapid, sensitive, accurate, precise, and stability-indicating. As a result, the created method is simple to implement for regular quality control in combination tablet dosage form and in bulk.

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AUTHORS CONTRIBUTION

All authors have contributed equally.

CONFLICT OF INTERESTS

Author declares that there have been no conflicts of interest

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