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Development of Analytical Methods for Glyxambi Estimation

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ABSTRACT

The study's objective was to create RP-HPLC and UV-spectrophotometric techniques for the simultaneous analysis of Empagliflozin and Linagliptin in commercially available tablets called Glyxambi. The methodologies' linearity, accuracy (% Recovery), precision (interday, intraday, and repeatability), and resilience have all been validated. Both techniques were accurate (% recovery was 99.19%–100.14%) and linear (R^2 =0.997–0.999 for the UV method and 0.999 for the RP-HPLC method). The approach was also discovered to be reliable (% RSD> 2%) and precise. Both methods were used to determine the potency of three commercially available brands, and no statistically significant difference was found between the potencies produced by the two approaches. Glyxambi tablets marketed as Empagliflozin and Linagliptin can be analyzed using any of the recognized techniques.

INTRODUCTION:

Glyxambi 25 mg/5 mg Tablet is a type of anti-diabetic medication. The main purpose of it is to cure Type 2 diabetes. Empagliflozin and linagliptin are both ingredients in Glyxambi 25 mg/5 mg Tablet 10's. It is used to manage high blood sugar in persons with type 2 diabetes together with a healthy diet and exercise regimen. Keeping blood sugar levels under control helps prevent blindness, kidney damage, limb loss, nerve damage, and issues with sexual function. Your chance of having a heart attack or stroke may be reduced if your diabetes is well controlled. The way empagliflozin works is by improving your kidneys' ability to remove sugar. In addition, Linagliptin functions by raising levels of incretins, which are naturally occurring molecules. By boosting insulin release, incretins aid in blood sugar regulation, especially after a meal. They also decrease the amount of sugar your liver makes. [1]

Due to its strong demand in the international market, we chose the aforementioned combination for HPLC method development. According to a literature review, analytical techniques such as UV, [2, 3, 4] RP-HPLC [5, 6, 7] have been employed to estimate these medicines either alone or in combination with other pharmaceuticals. However, there are only a few methods available for the simultaneous quantification of these medications, including UV [8, 3, 9] and RP-HPLC [10, 11, 12]. Additionally, it has been found that the retention durations for the aforementioned medications are long, which raises the expense of the approach due to the need for more solvent and time. Although Vadloori et al. reported good resolution between the two medicines, the linearity range showed that their approach was less sensitive. Additionally, some of the approaches required a more expensive mobile phase with a pH adjusted below 2.5, which could potentially harm the column [13]. To ensure the identity, purity, potency, and effectiveness of the medicine in the dosage forms, it is important to create newer analytical method(s) for routine analysis of these drugs. The developed methods were validated following the standards for validating analytical processes established by the International Conference on Harmonization. [14]

2. MATERIALS AND METHODS

2.1 Chemicals and reagents: Empagliflozin and Linagliptin were gifted by Yarrow Chem Pvt. Ltd. Mumbai. Glyxambi table was purchased for Medical Shop, Kanpur. HPLC-grade Acetonitrile and Methanol were purchased from Merck Industry, Mumbai. Analytical grade

Potassium dihydrogen phosphate and o-phosphoric acid were of Merck Industry, Mumbai. All the chemicals for the analysis were freshly prepared, analyzed, and used.

2.2. Instrumentation: UV-Visible double beam spectrophotometer LAB INDIA, 3000 Plus, UVWinin Japan with 1 cm matched pair quartz cell and spectral bandwidth of 1 nm was used. HPLC system (Waters,715. DataAce) with variable wavelength detector (SPD-20 A) was used. Analysis was performed on Hypersil - C18 (250mm×4.6×5micron) with an internal particle size of 5 µm. All weighing operations were performed by using an electronic balance (Model Shimadzu AUW-220D).

2.3. Chromatographic condition: The mobile phase containing Acetonitrile: Methanol maintained at pH 3.5 was selected as the optimum composition of the mobile phase, as this solvent system ideally resolved the components. C18 (4.6 × 250 mm) Hypersil BDS was used as a stationary phase for the selected method. The flow rate was set to 1.0 mL min−1 and UV detection was carried out at 254 nm. The mobile phase and the sample were degassed by sonication for 10 min and filtered through 0.4 µm membrane filter paper. All the determinations were performed at a constant column temperature (25 °C).

2.4. For the UV method

2.4.1. Preparation of standard solutions: 20 Glyxambi pills that were precisely weighed were transferred separately into a suitable standard conical flask, dissolved, and diluted with water until the mark was reached. The concentration of both medication solutions was lowered to 10 µg/ml. The solution was run in a 1 cm cell and scanned in the UV region between 200 and 400, with distilled water serving as the reference. The superimposed spectra were recorded. [15]

2.4.2 Choose of analytical wavelength for estimation: Empagliflozin and Linagliptin's spectral properties were seen in the overlay spectra and simultaneously computed using the simultaneous equation approach. The maximum wavelengths for both medicines, 261 nm, and 243 runs, were chosen. For the absorption ratio approach, the absorbance is measured at two wavelengths, one of which is the λmax of one of the components of linagliptin, λ2, 243 nm, and the other of which is a point of equal absorption for the two components of empagliflozin, 254 nm. The wavelengths chosen for Empagliflozin and Linagliptin, respectively, for the Area under Curve technique were 253 nm and 269 nm and 274 run and 284 nm, respectively.

2.4.3 Quantification of formulation: For all analytical studies, the tablet Glyxambi-A containing 25 mg of empagliflozin and 5 mg of linagliptin was obtained. The dosage of twenty tablets was weighed precisely. The typical pill weight was discovered and pulverized. The powder containing 25 mg of Glyxambi was weighed and deposited into a 100 ml conical flask. Distilled water was then added, and the required amount was used to make up the volume after the substance had been ultrasonically dissolved for 15 minutes. Whatman filter paper No. 41 was used to filter the content. To get a final conc and 5 µg mL-1 of empagliflozin, which theoretically comprises 1 µg mL-1 of linagliptin, the filtrate was appropriately diluted. At each of the chosen wavelengths, the sample solution's absorbance was measured. It was determined how much Empagliflozin and Linagliptin were in the sample tablet solution. Six times this surgery was carried out. [16, 17]

2.4.4 Recovery studies: Recovery experiments were conducted to make sure the proposed approach was reliable and appropriate. The procedure involved combining a known amount of standard medicine with a formulation sample, and the content was then pre-analyzed using the suggested approach. Empagliflozin and Linagliptin were added at concentrations of 80%, 100%, and 120% to a formulation quantity comparable to 30 mg of empagliflozin and normal doses.

2.5 For HPLC: Empagliflozin and Linagliptin solutions "(10 µg mL-1) were produced in the mobile phase [Methanol: Acetonitrile (50:50 v/v)], scanned in the UV range of 200-400 nm, and the spectra were recorded It was discovered that both medications show distinct absorbance at 254 nm" and may be used to accurately and uninhibitedly estimate the presence of two cups. As a result, 254 nm was chosen as the detection wavelength for the RP-HPLC method's isocratic elution technique's estimate of two medicines.

2.5.1 Sample solution preparation: Using a volumetric flask with a 25 ml capacity, 30 mg of tablet powder "(consisting of 25 mg of empagliflozin and 5 mg of linagliptin) was accurately weighed, dissolved in methanol, and the volume was topped off with methanol (1000 µg mL-1) After further dilution, the final concentrations were 40 mg mL-1 and 20 mg mL-1, respectively.

2.5.2 Linearity and calibration curve: Transferred into 10 ml volumetric flasks, "the primary stock solutions (1–5 ml of 20 µg mL-1) were then made up to the required volume with the mobile phase, which contained concentrations of 2, 4, 6, 8 and 10 µg mL-1 of empagliflozin The primary stock solution (1–5 ml of 40 µg/ml) was put into 10 ml volumetric

flasks and diluted with mobile phase to the proper volume, which included Linagliptin at the nominal concentrations of 4, 8, 12, 16, and 20" µg mL-1. At a flow rate of 1 ml/min, 20 microliters of this solution were injected into a column each time. At 254 nm, the method's detection was observed. In triplets, the process was repeated. [18, 19]

2.5.3 Recovery Studies

i) Preparation of raw material stock solutions of EMP and LNT: Accurately measured doses of 25 mg EMP and 5 mg LNT were put into a 100 ml volumetric flask, dissolved in methanol, and the capacity was topped off with methanol (240 µg mL-1). Empagliflozin and linagliptin were further diluted to achieve concentrations of 48 and 96 mg/mL, respectively.

ii) System suitability: Before each validation run, the chromatographic system underwent a test to determine its appropriateness. There were five replicate injections of the normal preparation. The resolution, theoretical plate, asymmetry, and percent RSD of peak area were calculated. Acceptance criteria for the system, including Asymmetry, not exceeding 2.0, Theoretical Plate not below 1800, and% RSD of Peak Area not exceeding 2.0, were met for all validation parameters.

2.6 Validation of developed method: The process of confirming through laboratory tests that a technique's performance characteristic satisfies the criteria for the intended analytical application is known as validation of the analytical method. Analytical parameters are used to express performance characteristics. [20]

i) Linearity: The capacity of the method to produce test findings that are inversely proportional to the concentration of the analyte in samples is known as linearity; Linagliptin and empagliflozin solutions in concentration ranges of 0.5 to 2.5 µg mL-1 and 3 to 15 µg mL-1 were made in six independent series.

ii) Precision: The formulation analysis, which was performed six times with the same concentration, proved the method's repeatability. Calculations were used to determine how much of each component was in the pill formulation. RSD was calculated as a percentage. The intraday and interday analyses, in which the formulation analysis was repeated three times on the same day and three days in a row, respectively, validated the method's intermediate precision. Calculating the percentage RSD and the number of chugs. [21, 22]

iii) Ruggedness: The method's tenacity was demonstrated by the analysis of the formulation carried out using various tools and analyzers. The sum and percent RSD were calculated.

iv) Accuracy: Recovery studies verified the method's accuracy. Linagliptin and empagliflozin were added to the previously examined formulation in known quantities, and the process was carried out following the analysis of the formulation. Each recovered mug's weight was calculated. Each concentration underwent three iterations of this process. Calculated as the percent RSD.

v) LOD and LOQ: Six times the linearity study was conducted. According to ICH Guidelines, the LODs and LOQs of the new approach were investigated. Depending on the strategy, such as a non-instrumental or instrumental approach, there are various methods for determining the LODs and LOQs.

$$LODs = 3.3\ \sigma/S$$

$$LOQs = 10\ \sigma\ /S$$

Where σ = standard deviation of response, s = slope of calibration curve

The "LOD and LOQ were calculated by using the average of slope and standard deviation of response" (Intercept). [23, 24, 25, 26]

3. RESULTS AND DISCUSSION"

The simultaneous estimation of two pharmaceuticals in a formulation provides greater benefits than the individual estimation of two drugs, including accuracy, reduced reagent use, and reduced time requirements. To validate the procedures following ICH requirements and apply them for its estimation in marketed formulations, new, straightforward, precise, and accurate analytical techniques were created for the following combinations. The techniques comprise:

1. "UV spectroscopic method
2. RP-HPLC method

3.1 UV SPECTROSCOPIC METHOD

The "sample solution of 10 µg/ml of Glyxambi was prepared individually and the solutions were scanned between 200 – 400 nm by using water as blank as shown in Figure 1 From the overlaid spectra by observing the spectral characteristics λmax of Empagliflozin at 235 nm and λmax of Linagliptin at 285 nm was selected for simultaneous equation" method.

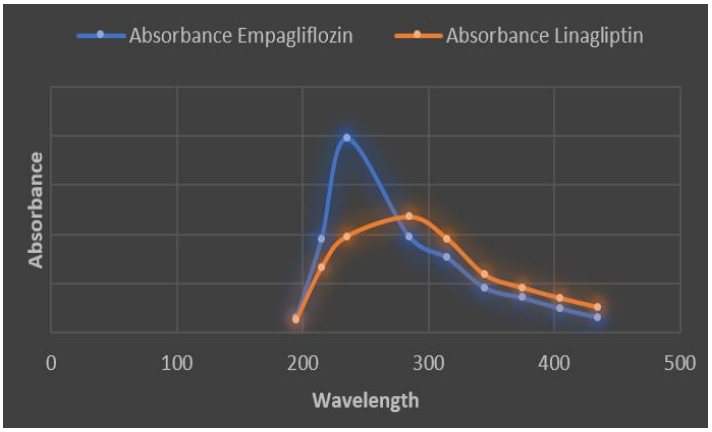

Figure no 1: Overlain Spectra of Empagliflozin and Linagliptin

The "stability of the drug solution was observed at different time intervals. Empagliflozin was stable for 5 hours and Linagliptin was stable for 4" hours. Concentrations (0.5-2.5 µg/ml, 3-15 µg/ml) of the stock solutions of EMP and LGT were produced from aliquots of the stock solutions. For the two medicines, the absorbance versus concentration calibration curve was plotted.

EMP at 235 nm and LGT at 285 nm were found to have correlation coefficients of 0.999846 and 0.998965, respectively. The LOD and LOQ for empagliflozin at 235 nm were determined to be 0.3865 µg/ml and 1.186433 µg/ml, respectively. The LOD and LOQ for linagliptin were determined to be 0.0227644 µg/ ml and 0.0688533 µg/ml at 285 nm, respectively. All of the specified wavelengths' correlation coefficient values are discovered to be more than 0.999. The chosen concentrations, therefore, follow Beer's law and are linear. In Figures 2 and 3, respectively, the calibration graphs for EMP and LGT at 235 nm and 285 nm are displayed.

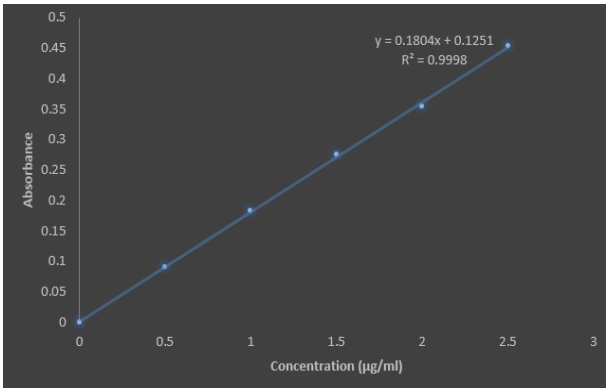

Figure 2: Calibration Curve of Empagliflozin AT 235 nm

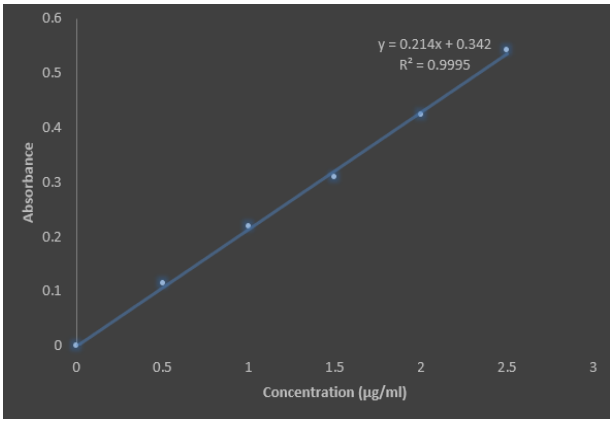

Figure 3: Calibration Curve of Linagliptin At 285 nm

3.1.1 Simultaneous Equation Method

Sample solutions of 10 g/ml of EMP and LNT were produced separately, and the solutions were scanned between 200 and 400 nm while using water as a blank. The simultaneous equation technique was chosen from the overlay spectra by looking at the spectral features, particularly the maximums of the EMP at 2351 nm and the LNT at 285 nm. In Tables 1 and 2, respectively, the optical properties at 235 nm and 285 nm are displayed. To determine whether the devised procedure was accurate or not, it was used for the examination of a synthetic mixture. For EMP and LNT, the average percent of the synthetic mixture was determined to be 100.104. The quantity found and the anticipated concentration was in good accord. Therefore, it was intended to apply for the formulation analysis.

Table no 1: Optical Characteristics Of EMP

Parameters	**Empagliflozin at 235 nm**	**Linagliptin at 285 nm**
"Beer's law limit (µg/ml)"	0.5-2.5	0.5-2.5
Molar absorptivity (L mol^{-1} cm^{-1})	17734.8721	8113.9875
"Sandell's sensitivity (µg/cm^2/0.001 A.U)"	0.01567433	0.3450973
"Correlation coefficient (r)"	0.999898	0.999876
"Regression equation"	Y=0.0623476x+0.000128	Y=0.0256326x+0.000533
"Slope (m)"	0.0632632	0.0529282
"Intercept (c)"	0.00010452	0.00010343
"LOD"	0.854383	0.89765
"LOQ"	2.987354	1.987662
Standard error	0.000324322	0.000321336

Table no 2: Optical Characteristics of LNT

Parameters	Empagliflozin at 235 nm	Linagliptin at 285 nm
"Beer's law limit (µg/ml)"	3-15	3-15
Molar absorptivity (L mol^{-1} cm^{-1})	5788.976	10173.955
"Sandell's sensitivity (µg/cm^2/0.001 A.U)"	0.025874	0.0345421
"Correlation coefficient (r)"	0.998664	0.9987954
"Regression equation"	Y=0.052554x+0.000043	Y=0.0275367x+0.000153
"Slope (m)"	0.0238544	0.0328222
"Intercept (c)"	0.0021087	0.0046722
"LOD"	0.858537	0.89932
"LOQ"	2.983156	1.97535
"Standard error"	0.00032987	0.0003288

Table no 3: Synthetic Mixtures of EMP and LNT

Drug	Sample No.	Conc. (µg/ ml)	Amount found (µg/ ml)	Percentage obtained	Average (%)	S.D	% R.S.D.	S.E.
EMP	1	0.5	0.4788	99.012	100.02	0.23678	0.23111	0.00023
	2	1.0	0.9743	100.02				
	3	1.5	1.6855	99.997				
	4	2.0	2.0322	99.95				
	5	2.5	2.4088	100.01				
LNT	1	3	2.912	99.98	100. 01	0.19664	0.19855	0.00896
	2	6	5.598	99.01				
	3	9	8.787	100.03				
	4	12	10.964	100.03				
	5	15	14.075	100.31				

LNT and EMP were determined to have percentage purity values of 100.02 ±0.23111 and 100.01± 0.19664, respectively, in the formulation. The repeated analysis of the formulation six times served as confirmation of the method's accuracy. RSD was calculated as a

percentage. For LNT and EMP, the % RSD was determined to be 0.23111 and 0.19855, respectively. The approach appears to have good precision based on the low% RSD values. Table 4 presents the outcomes.

Table no 4: Quantification Study of formulation

Drug	Sample no.	Labeled amount (mg/tab)	Amount found (mg/tab)	% Obtained	Avg. (%)	S.D	% RSD	SE
EMP	1	25	24.45	97.80	99.56	0.47642	0.48432	0.01265
	2	25	24.76	99.04				
	3	25	24.98	99.92				
	4	25	24.85	99.40				
	5	25	24.56	98.24				
	6	25	24.89	99.56				
LNT	1	5	4.87	97.4	98.89	0.04521	0.41677	0.01157
	2	5	4.56	98.2				
	3	5	4.87	97.4				
	4	5	4.98	99.6				
	5	5	4.65	93.0				
	6	5	4.99	99.8				

* Mean of Six Observations

Furthermore, intraday and interday analysis supported the method's accuracy. The formulation underwent intraday and interday analysis three times on the same day and once over three days. EMP's intraday and interday precision RSD was determined to be 0.01265, whereas LNT's was found to be 0.01157. (Table 5). The low% RSD readings imply that the method's accuracy was further verified.

Table no 5: "Intra-Day and Inter-Day Analysis Of Formulation

Drug	Sample No.	Labeled amount (mg/tab)	"Percentage obtained"		SD		% RSD	
			"Intra-day"	"Inter day"	"Intra-day"	"Inter day"	"Intra-day"	"Inter day"
EMP	1	25	99.6442	99.5785	0.5065	0.3367	0.5043	0.3321
	2	25	99.7442	99.9652				
	3	25	99.3422	99.7558				
	Mean		99.6321	99.6443				
LNT	1	5	98.8975	98.7432	0.2031	0.3350	0.1975	0.3354
	2	5	98.7642	98.6293				
	3	5	98.7542	98.9084				
	Mean		98.6433	98.6325				

* Mean of Three Observations

The findings of the Ruggedness investigation using various instruments are displayed in Table 6. Recovery studies verified the method's accuracy. The percentage recovery was determined to be between 99.6443 ±0.08144 for LNT and 98.6325± 1.2230 for EMP.

Table no 6: Ruggedness analysis of formulation

Drug	Condition	% obtained	S.D	%RSD	S.E
EMP	"Analyst 1	99.8766	1.1677	1.1542	0.0321
	Analyst 2"	99.9976	1.0857	1.1456	0.0325
	"Instrument 1	99.9887	0.9865	0.9463	0.0149
	Instrument 2"	99.9964	1.0533	1.1032	0.0353
LNT	"Analyst 1	98.9865	0.9865	0.9965	0.0245
	Analyst 2"	98.6322	0.9586	0.9075	0.0165
	"Instrument 1	98.6433	0.5604	0.4986	0.0145
	Instrument 2"	98.9993	0.4573	0.4874	0.0217

The "percentage RSD was found to be 1.2218 for EMP and 0.08139 for LNT The low percentage RSD indicated that there was no interference due to excipients used in

formulation Hence, the accuracy of the method was confirmed. The data for recovery studies are given in" Table 7.

Table no 7: Recovery Study of Glyxambi

Drug	Sample no.	Amount present (µg/ml)	Amount added (µg/ml)	The amount estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	SD	% RSD	S.E
EMP	1	1.0402	1.02	1.0443	1.0633	99.86	1.2321	1.2223	0.1342
	2	1.0402	1.05	1.0543	1.0543	99.43			
	3	1.0402	1.08	1.0866	1.0532	100.01			
					Mean	99.98			
LNT	1	0.0668	0.04	0.0234	0.0644	99.97	0.0823	0.0867	0.0086
	2	0.0668	0.07	0.0453	0.0865	100.3			
	3	0.668	0.09	0.0653	09844	100.9			

3.2 RP-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: Conditions were improved in the RP-HPLC process to get a good separation of eluted chemicals. To elute the title component, various mobile phase compositions were initially used. Based on peak characteristics (height capacity, theoretical plates, tailing or symmetry factor), run time, resolution, mobile phase, and the flow rate was chosen.

At first, a chromatogram was recorded using a device with a mobile phase that contained acetonitrile: methanol (50:50% v/v) in equal parts. Last but not least, a mobile phase composed of 60:40 v/v Methanol: Acetonitrile with 0.1 ml of 0.1% triethylamine was attempted; Methanol: Acetonitrile with 0.1 ml of 0.1% Triethylamine in the ratio of 60: 40% v/v at a flow rate of 1.0 ml/min was chosen after calculating all system suitability parameters. It was discovered that the retention times for EMP and LNT were respectively 2.915± 0.1 min and 4.637 ±0.1 min, with a resolution of 9.087, which is a superior resolution.

System appropriateness tests are a crucial component of chromatographic methods, according to ICH Guidelines. They are used to confirm the chromatographic method's repeatability. "Effective system suitability experiments are conducted on newly made stock solutions of EMP and LNT using methanol (for first dilution only), and a mobile phase of different

concentrations was made in the range of 2-10 µg/ ml of EMP and 4-20 µg/ ml of LNT, respectively Each solution was separately injected with 20 µl, and the chromatograms were" captured at 254 nm.

Concentration versus peak area was used to plot the calibration curve. Three times the surgery was carried out. For two medicines, the correlation coefficient was approximately 0.999. It suggests that the linearity of the EMP and LNT concentrations was good.

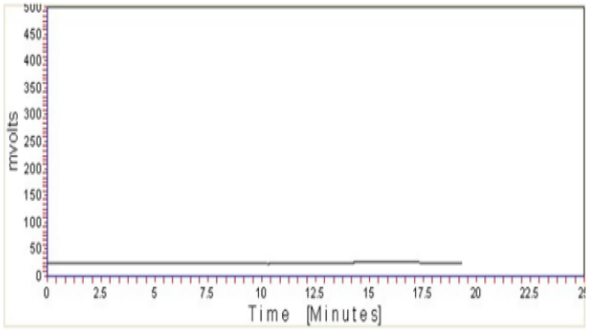

Figure no 4: chromatogram of Blank Mobile Phase

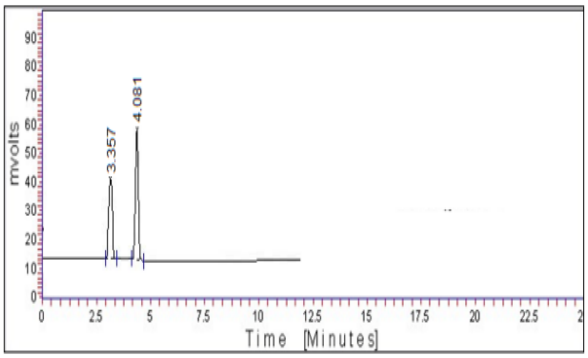

Figure no 5: Chromatogram of EMP and LNT

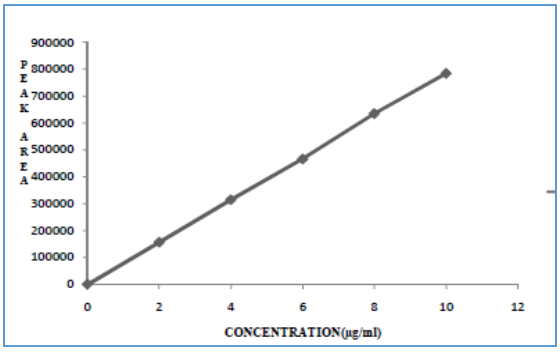

Figure no 6: Calibration curve of EMP

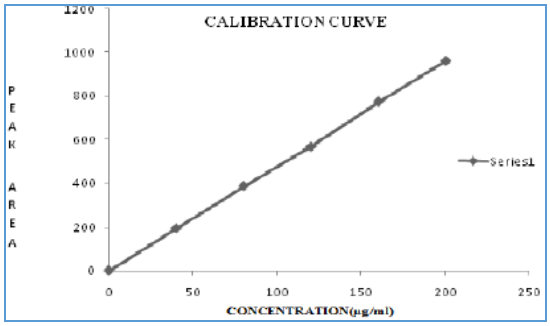

Figure no 7: Calibration Curve of LNT

Table no 8: Optical Characteristics of EMP (RP- HPLC Method)

PARAMETERS	EMP	LNT
"Beer's law limit (µg/ml)"	2- 10	4-20
"Detection wavelength"	254 nm	254 nm
"Correlation coefficient (r)"	0.99765	0.99876
"Regression equation"	Y = 8765.541X+132.295	Y = 100544.432X+1343.322
"Slope (m)"	875645.5755	10654.321
"Intercept (c)"	15866.0754	3224.76866
"LOD (µg/ml)"	0.17653	0.25433
"LOQ (µg/ml)"	0.394765	0.72533
"Standard error"	25322.08655	5422.1777

*Mean of three observations

Glyxambi's tablet dosage form was chosen for the analysis. The formulation has a 1:5 ratio between the drugs EMP and LNT. The mobile phase was produced with a 10 g/ml concentration of LNT that also contains 2 µg/ml of EMP. After injecting 20 µl of each solution, chromatograms were captured. For EMP and LNT, respectively, the percentage purity was found to be 99.83 ±0.28304 and 99.93± 0.00460.

Table no 9: Quantification Of tablet For RP – HPLC METHOD

Drug	Sample no.	Labeled amount (mg/tab)	Amount found (mg/tab)	% Obtained	Avg. (%)	S.D	% RSD	SE
EMP	1	25	24.67	98.88	99.98	0.46443	0.40982	0.01866
	2	25	24.87	99.04				
	3	25	24.98	99.97				
	4	25	24.89	99.88				
	5	25	24.99	99.9				
	6	25	24.89	99.34				
LNT	1	5	4.99	99.9	98.93	0.04212	0.304I7	0.02107
	2	5	4.89	98.24				
	3	5	4.95	99.1				
	4	5	4.99	99.9				
	5	5	4.97	99.97				
	6	5	4.98	99.87				

Table no 10: System Suitability Parameters

Parameters	EMP	LNT
Tailing factor	1.087	1.042
Retention time	2.875	0.344
Asymmetrical factor	1.065	1.003
Capacity factor	3.98	2.01
Theoretical plates	5123	7564
Resolution	Between EMP and LNT 7.87	

Interday and Intraday analysis supported the accuracy. The formulation was subjected to intraday and interday analysis three times on the same day and once on three successive days. The %RSD for intraday and interday precision for EMP was determined to be 0.5176 and 0.3781, respectively. The lower values imply that the method's accuracy was further verified.

Table no 11: "Intra-Day and Inter-Day Analysis Of Formulation (RP-HPLC method)"

Drug	Sample No.	Labeled amount (mg/tab)	"Percentage obtained"		"SD"		"% RSD"	
			"Intra-day"	"Inter day"	"Intra-day"	"Inter day"	"Intra-day"	"Inter day"
EMP	1 2 3	25 25 25	99.7772 99.8644 99.3804	99.7433 99.8766 99.7888	0.5144	0.3177	0.5176	0.3178
	Mean		99.8777	99.7888				
LNT	1 2 3	5 5 5	99.1957 99.8942 99.9867	99.7844 98.9207 98.8988	0.2076	0.3076	0.1795	0.3023
	Mean		99.1677	99.9845				

* Mean of Three Observations

Recovery experiments were used to evaluate the method's accuracy. A known volume of EMP and LNT raw material solutions was injected at various doses into the previously assessed formulation.

Table 12: Recovery Analysis Of Glyxambi (RP - HPLC METHOD)

Drug	Sample no.	Amount present (µg/ml)	Amount added (µg/ml)	The amount estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	SD	% RSD	S.E
EMP	1	1.0312	1.01	1.0121	1.0521	99.87	1.2765	1.2073	0.1092
	2	1.0312	1.03	1.0332	1.0743	99.85			
	3	1.0312	1.05	1.0527	1.0809	100.02			
					Mean	99.99			
LNT	1	0.0593	0.03	0.0345	0.0322	99.95	0.0883	0.0977	0.0079
	2	0.0593	0.08	0.0898	0.0797	100.32			
	3	0.0593	0.11	0.1103	01144	100.05			
					Mean	100.01			

* Mean of Three Observations

CONCLUSION:

The two developed techniques, which relied on spectrophotometry and RP-HPLC, were validated following ICH recommendations. The developed methods' calculated standard deviation and percent RSD showed a high level of precision. The findings of recovery studies showed that the developed procedures were highly accurate. The experimental data allow for the conclusion that the proposed and verified methods can be used for routine analysis for the simultaneous estimate of Glyxambi in combination dosage form because they are quick, precise, sensitive, and repeatable.

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