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Development of Analytical Methods for Glyxambi Estimation by FTIR

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ABSTRACT

A new analytical method has been developed for the simultaneous estimation of Glyxambi tablet by using Fourier Transform Infrared (FTIR) Spectroscopy. This method involves the extraction of EMP and LNT from tablets using chloroform and direct measurement in liquid phase mode using reduced path length cell. The spectra were measured in absorbance mode, equipment was configured to collect spectra at 8 cm^{-1} resolution, and the spectra were collected between 4000 cm^{-1} and 400 cm^{-1}. The infrared spectra showed different peaks in the IR spectrum of EMP at 1765 cm^{-1}, 32345 cm^{-1}, and 3455 cm^{-1}, respectively. Peaks in the LNT FTIR spectra were found at 1065 cm^{-1}, 1675 cm^{-1}, and 3286 cm^{-1}, respectively, which correspond to NH_2 asymmetric stretching, NH_2 symmetric stretching, and the N-H group. Beer-Lambert's law was obeyed over the concentration range of 3-15 µg/ml for EMP and 2-10 µg/ml for LNT. All the results were found to be within the limits. Therefore, it can be used for routine quality control of these two drugs in bulk and formulations.

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. [1]

The World Health Organization (WHO) is very concerned about pharmaceutical drug quality control because any health service is put at risk if there is no assurance that these products meet the standards for quality, safety, and efficacy [2]. Pharmaceutical businesses are required by the Food and Drug Administration (FDA) to guarantee the complete efficacy and safety of the marketed product for the duration of its defined shelf life, provided that it is kept in its original, undamaged packaging and under approved circumstances [3]. As a result, one of the major problems in the pharmaceutical industry is the management of drug quality and stability [4, 5]. The production process, which includes suitable packaging and formulation, is directly linked to the quality of drug goods and affects it in addition to time and storage conditions [6]. Excipients and tablet coatings do, in fact, play a crucial role in shielding active pharmaceutical ingredients (APIs) from environmental conditions such temperature, humidity, light, and oxygen exposure. Therefore, stability tests are necessary to establish the expiration date but they are also useful in guiding formulation stabilization tactics and informing crucial information on how to handle and store drug items. [7, 8]

Numerous studies have been published in the literature that evaluate the stability of various EMP/LNT formulations; the majority of these studies adhere to the International Council for Harmonization (ICH) and United States Pharmacopeia (USP) guidelines, which call for the validation of quantitative high-performance liquid chromatography (HPLC) methods for the quality control of pharmaceutical products [9, 10, 11, 12]. After being kept on the international space station for several months, Du and colleagues described the degradation patterns of various drugs. After classifying the drugs according to the (USP) criteria and using ultra- and high-performance liquid chromatography to determine the APIs, they discovered that the only instance in which the rate of deterioration was greater on space than in space was with clavulanate. This evidence was probably due to the susceptibility of the compound to uncontrolled relative humidity [13].

MATERIALS AND METHODS

Chemicals and reagents: Empagliflozin and Linagliptin were gifted by Yarrow Chem Pvt. Ltd. Mumbai. Glyxambi table was purchase for Medical Shop, Kanpur. HPLC grade Acetonitrile and Methanol was purchased from Merck Industry, Mumbai. Analytical grade Potassium dihydrogen phosphate, o-phosphoric acid was of Merck Industry, Mumbai. All the chemicals for the analysis were freshly prepared, analyzed and used.

Instrumentation: The analysis used a Shimadzu 8400S FTIR apparatus with a DLATGS detector and IR solution software.

Method Development: A liquid sample methodology was employed in the development of the FTIR spectroscopic technology. During the technique development process, the following variables were optimized.

Selection of Measurement Mode: Empagliflozin and Linagliptin IR spectra were captured in absorbance mode for quantitative investigation. At various wavenumbers, absorbances of functional peaks were determined.

Selection of Apodization: Through the interferogram's Fourier transform, the "apodization" function is utilized to calculate the power spectral density. The resolution and SIN ratio of spectra are affected by the apodization function. Noise on the balance also grows as resolution does. The "Happ-Genzel" apodization function was chosen.

Selection of Beam: The instrument's beam operation can be changed using the beam parameter. For the main unit's sample measurement, the parameter "internal" was chosen.

Selection of Detector: It was decided to use the common pyroelectric detector with the DLATGS element.

Selection of Mirror Speed: Mirror speed 2.8 (mm/sec) was selected for the standard DLATGS element of the detector.

Choice of Sampling Method: Because the KBr-pressed pellet approach makes it difficult to gather samples with tiny quantities, Glyxambi tablets were used for quantitative estimation. Consequently, a liquid sampling technique was used. A suitable IR solvent was used to create the solutions, and fixed path length liquid cells were employed for the analysis. Between the two plates, the liquid drop deposits a thin sodium chloride layer. When selecting a solvent,

the solubility profile of the drug and the solvent's IR transparency window were taken into account. They were shown to be readily soluble in non-polar solvents such dimethyl sulfoxide, chloroform, dimethylformamide, and cyclohexane (DMSO). In the range of 800-5000 cm^{-1}, chloroform is transparent. Cyclohexane is transparent in the IR windows of 1500-2500 cm^{-1}, 1100-1200 cm^{-1}, and 700-840 cm^{-1}. For the quantitative determination of empagliflozin and linagliptin, chloroform was used as a suitable solvent. The two chugs' primary stock solutions were made in DMSO. [14]

Assessment of Functional Groups Using IR Spectra: In order to test the two drugs, functional group peaks whose absorbance demonstrated a proportionate rise in absorbance with an increase in cone were selected from the IR spectra of empagliflozin and linagliptin taken in absorbance mode.

Preparation of Standard Solutions: Empagliflozin and linagliptin, each weighing 25 mg, were separately transferred into a 25 ml volumetric flask with 2 mL of DMSO. Chloroform was added to the volume after the contents had been sonicated for five minutes. The mixture was centrifuged for 15 minutes at 3000 rpm with Whatman filter paper.

Validation of the Developed Methods The developed approach underwent validation in accordance with ICH Guidelines. As tests for method validation, variables like linearity, accuracy, precision, specificity, robustness, LODs, and LOQs were used. [15]

RESULTS AND DISCUSSION:

The new FTIR method was developed to quantify EMP and LNT in the tablet formulation using a liquid sample approach (Glyxambi). The FTIR spectra of pure EMP and LNT using a pressed pellet method revealed a number of unique bands. However, due to the high amounts of lactose and cellulose, using a pressed pellet technique to directly identify medications was not feasible (excipients used in pharmaceutical formulations).

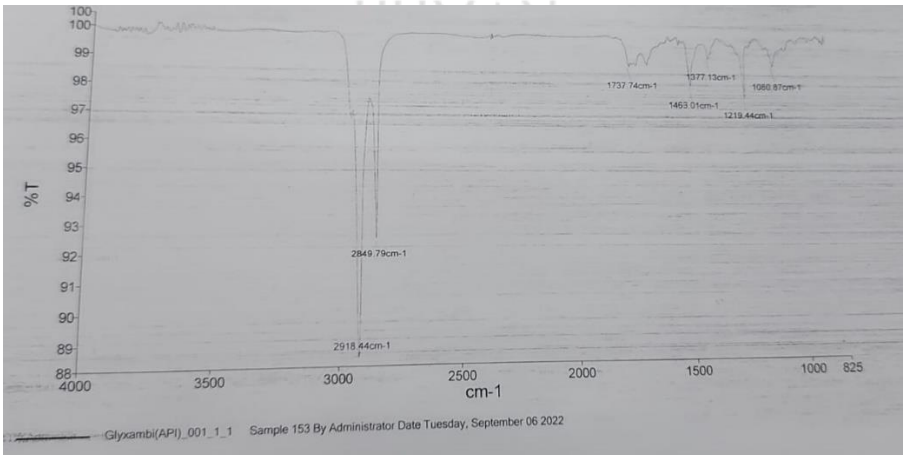
The active principle must therefore first be distinguished from the samples. The drug was extracted from the tablets using chloroform, and an IR analysis employing a liquid sample technique was carried out in the chloroform solution. Utilizing the instrumentation parameters mentioned in Table 1, the FTIR technique was improved.

Table No. 1: Optimized FTIR Conditions"

Measurement mode	Absorbance mode
Apodization	Happ-Genzel
Solvent	Chloroform
Frequency	Range 400-4000 cm-1
Number of Scans	30
Resolution	8.0 cm-1

Based on the medicines' solubility, dimethyl chloride and chloroform were selected as the solvents for collecting IR spectra using the liquid sampling approach. With chloroform, the peak intensity was improved (as chloroform is transparent throughout the region of 800-5000 cm^{-1}). Chloroform was decided upon as the solvent as a result. To cover the mid-IR region, the frequency range (400–4000 cm^{-1}) was selected. Since the S/N ratio gets better with more scans, 45 scans were selected for the best S/N ratio. A resolution of 8cm-1 was employed to increase peak-to-peak separation.

FTIR spectra of a normal Glyxambi tablet produced utilising the KBr-pressed pellet process and the liquid sampling technique.



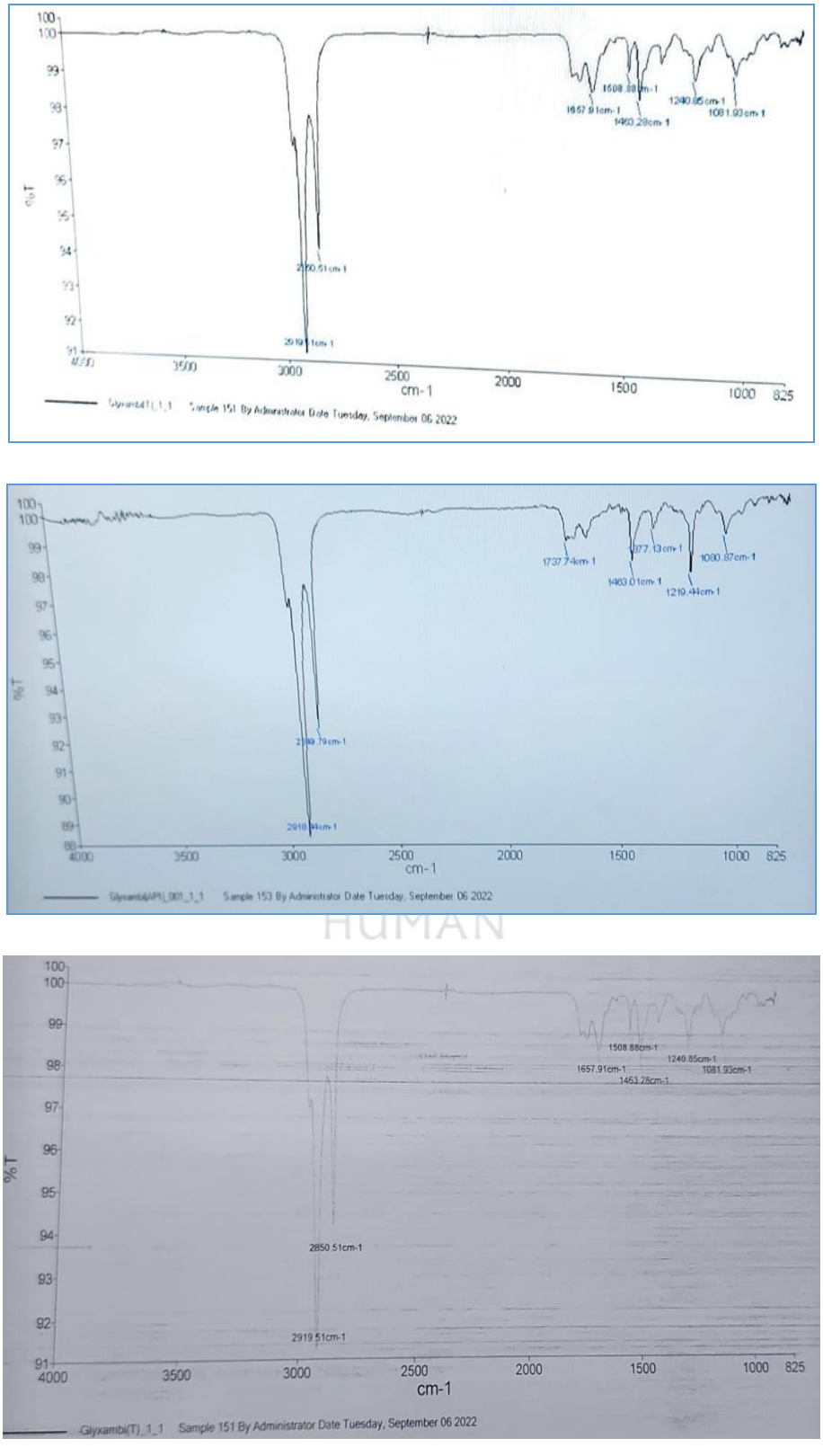


Figure 1: FTIR spectra of Glyxambi tablet Liquid sample

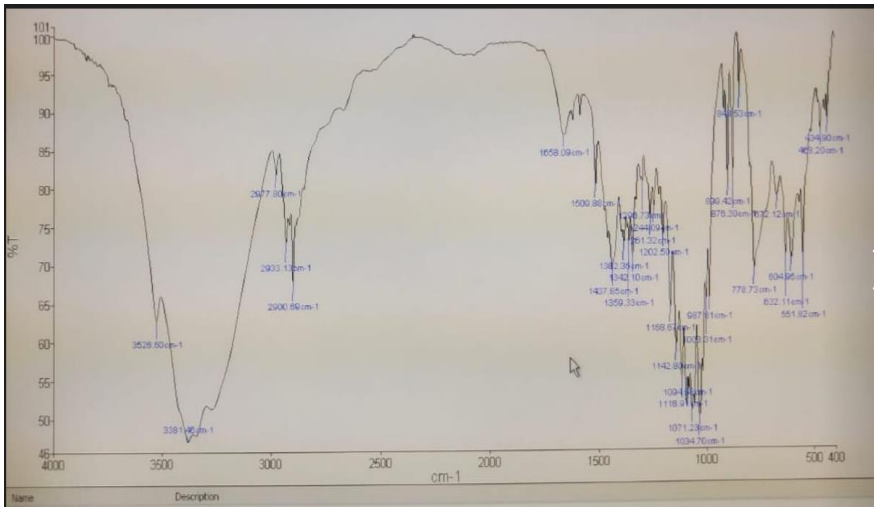


Figure 2: FTIR spectra of emphagliflozin solid sample

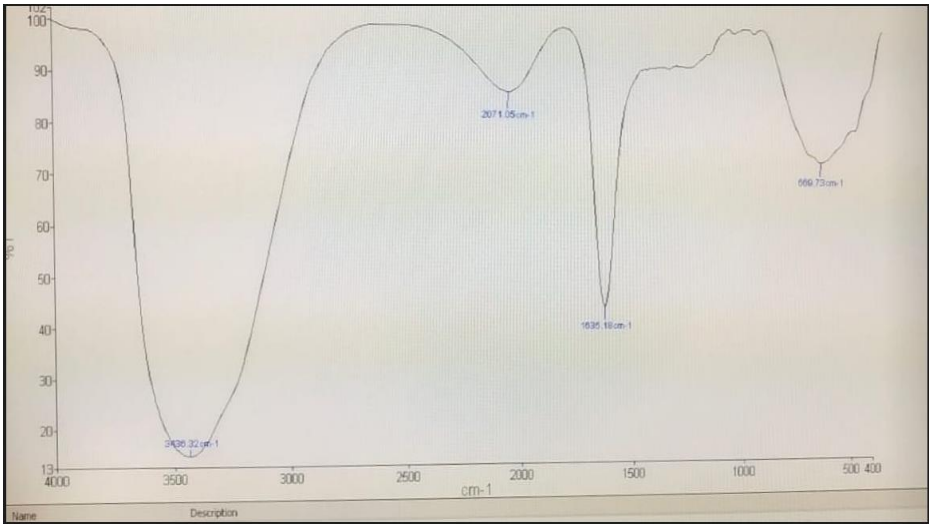


Figure 3: FTIR spectra of emphagliflozin liquid sample

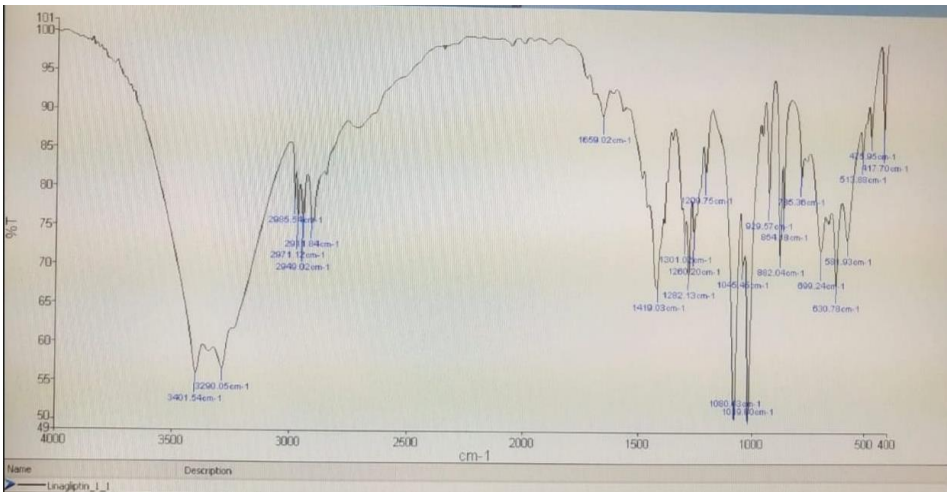


Figure 4: FTIR spectra of linagliptin solid sample

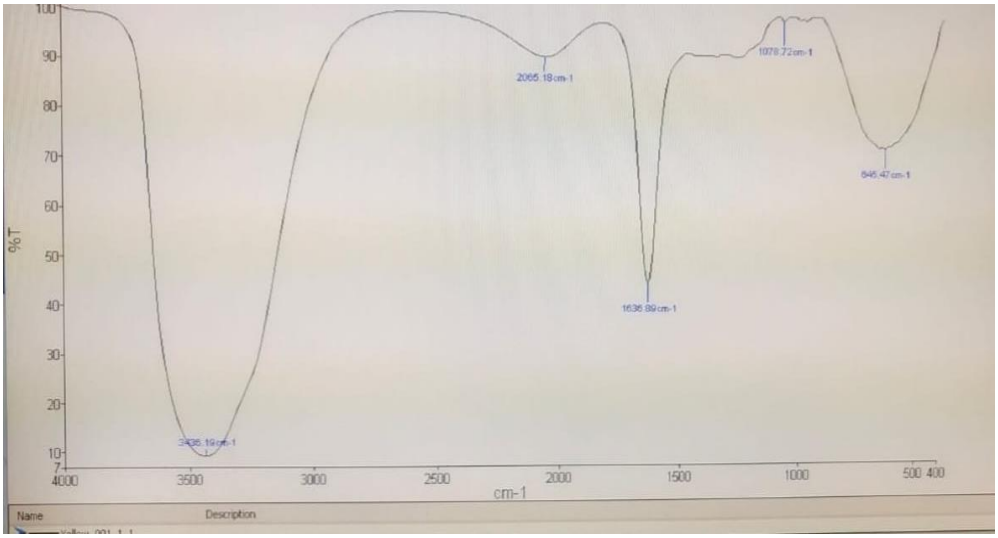


Figure 5: FTIR spectra of linagliptin liquid sample

The carbonyl group, N-H group, and O-H group are each represented as peaks in the IR spectrum of EMP at 1765 cm^{-1}, 32345 cm^{-1}, and 3455 cm^{-1}, respectively. The O-H group among which displayed a distinct, strong peak that grew linearly with concentration. It was chosen for the EMP quantitative analysis.

Peaks in the LNT FTIR spectra were found at 1065 cm^{-1}, 1675 cm^{-1}, and 3286 cm^{-1}, respectively, which correspond to NH_2 asymmetric stretching, NH_2 symmetric stretching, and the N-H group. The NH_2 group stood out among them with a distinct, strong peak that grew linearly with concentration.

It was discovered that all of the estimated values for the validation parameters fell within allowable bounds.

A “linear and proportional relationship was observed between the concentration and absorbance in the range of 3-15 µg/mL for EMP and 2-10 µg/ml for LNT This is used for the quantitation of drugs in pure and formulations The linearity of the method was established by performing linear regression analysis for the calibration curve constructed between concentration and absorbance as shown in” Table 2 and 3.

Table No. 2: Standard calibration curve data for EMP

Sr. No.	Concentration (µg/ml)	Absorbance at
1	3	0.1276
2	6	0.2476
3	9	0.3598
4	12	0.4789
5	15	0.5923

* Mean of Three Observations

Table No. 3: Standard calibration curve data for EMP

Sr. No.	Concentration (µg/ml)	Absorbance at
1	2	0.0753
2	4	0.1487
3	6	0.2056
4	8	0.2792
5	10	0.3502

Average of three determination

Table 4 displays the repeatability results for six replicates of the standard EMP and LNT solutions.

Table No. 4: Glyxambi tablet Repeatability data

Sr. No.	Absorbance	
	EMP	LNT
"1"	0.2543	0.1356
"2"	0.2501	0.1322
"3"	0.2499	0.1309
"4"	0.2531	0.1356
"5"	0.2503	0.1399
"6"	0.2512	0.1365
"Mean ± SD"	"0.2515 ± 0.00254"	"0.1321 ± 0.006322"
"% RSD"	"1.08"	"1.98"

* Mean of Three Observations

The proposed method is thought to be repeatably exact because "all the determined observations were close to one another, and the deviation was found to" be minimal.

By conducting recovery trials, it was stated that the procedure was accurate. Results demonstrated that the approach was reliable for measuring LNT and EMP in tablets. Tables 5 display these findings.

Table No. 5: Recovery Analysis of Glyxambi

Drug	Sample no.	Amount present (µg/ml)	Amount added (µg/ml)	Amount estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	SD	% RSD	S.E
EMP	1	1.0453	1.02	1.0103	1.0576	99.92	1.2774	1.2087	0.1081
	2	1.0453	1.04	1.0302	1.0798	99.99			
	3	1.0453	1.06	1.0502	1.0854	100.05			
					Mean	99.99			
LNT	1	0.0612	0.03	0.0208	0.0244	99.99	0.0887	0.0964	0.0079
	2	0.0612	0.05	0.0406	0.0486	100.03			
	3	0.0612	0.08	0.0705	0706	100.02			
					Mean	100.03			

* Mean of Three Observations

CONCLUSION:

A search of the literature revealed several independent techniques, such as spectrophotometric, chromatographic, and FTIR methods, for identifying Glyxambi (EMP and LNT) in commercial formulations. There are a fairly limited number of approaches for estimating drug dosage in combination dosage form. However, there is no known procedure for Glyxambi analysis. This study work has developed and validated three analytical techniques for the determination of a double component mixture of EMP and LNT in bulk and in a synthetic mixture.

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