Development, Validation and FTIR Characterization of Sitagliptin and Empagliflozin Using a Novel Analytical Method

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***Abstract*- A simple, accurate, and precise UV spectrophotometric method was developed and validated for the estimation of Sitagliptin and Empagliflozin in physical mixture. The method was successfully evaluated for key validation parameters such as accuracy, precision, and linearity in accordance with ICH guidelines. The linearity was found to be satisfactory for both drugs, with correlation coefficients (R^2) indicating a strong linear relationship. Accuracy was confirmed through recovery studies, with mean percentage recoveries falling within the acceptable range of 98–102%. Precision studies yielded low %RSD values, indicating good reproducibility. The method also demonstrated robustness and practicality, making it well-suited for routine quality control analysis.**

In addition, FTIR spectroscopy was employed to assess the functional groups and structural integrity of Sitagliptin and Empagliflozin. The FTIR spectra confirmed the presence of characteristic peaks, indicating no significant drug-excipient interaction and supporting the compatibility of the active pharmaceutical ingredients. The combination of UV spectrophotometric and FTIR analyses provides a comprehensive and efficient approach for the quality evaluation of Sitagliptin and Empagliflozin in pharmaceutical formulations.

***Index Terms*- Sitagliptin, Empagliflozin, Development, Validation, FTIR, UV Spectroscopy, Analytical.**

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and progressive β-cell dysfunction, leading to hyperglycemia. The management of T2DM often requires a combination of therapeutic agents targeting different physiological pathways to achieve glycaemic control. Among such agents, **Sitagliptin** and **Empagliflozin** have emerged as effective oral antidiabetic drugs with distinct mechanisms of action.

Sitagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances the body's natural ability to regulate blood glucose by prolonging the activity of incretin hormones. Chemically, it is known as **(R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro [1,2,4] triazolo [4,3-a] pyrazin -7(8H)-yl]-1-(2,4,5-trifluorophenyl) butan-2-amine**. The structure of Sitagliptin includes a triazolopyrazine ring system, a trifluorophenyl group, and an amide linkage, contributing to its high selectivity and potency as a DPP-4 inhibitor.

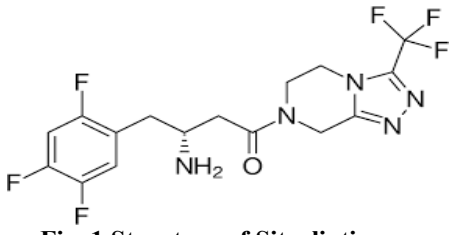

Fig. 1 Structure of Sitagliptin

Empagliflozin, on the other hand, is a sodium-glucose co-transporter 2 (SGLT2) inhibitor that reduces blood glucose levels by inhibiting glucose reabsorption in the kidneys, thereby promoting urinary glucose excretion. Its chemical name is **(1S)-1,5-anhydro-1-C-[4-chloro-3-[[4-[(3S)-oxolan-3-yl] oxyphenyl] methyl] phenyl]-D-glucitol**. Structurally, Empagliflozin consists of a glucose-derived moiety bonded to a substituted phenyl ring, which is essential for its SGLT2 inhibitory activity.

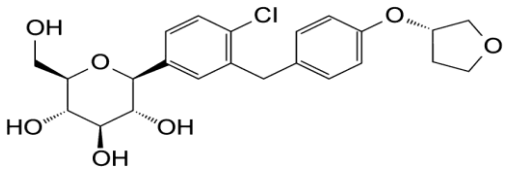

Fig. 2 Structure of Empagliflozin

The combination therapy of Sitagliptin and Empagliflozin is gaining importance due to their

complementary mechanisms, resulting in improved glycaemic control without significant risk of hypoglycaemia. As these drugs are increasingly used in fixed-dose combinations (FDCs), it becomes crucial to develop and validate analytical methods that are efficient, cost-effective, and suitable for routine quality control.

UV spectrophotometry is one such technique that offers simplicity, rapidity, and cost-efficiency in pharmaceutical analysis, especially for bulk drug and formulation assessment. The present study focuses on the development and validation of a UV spectrophotometric method for the estimation of Sitagliptin and Empagliflozin in physical mixture, following ICH Q2(R1) guidelines.

In addition to spectrophotometric analysis, **Fourier Transform Infrared (FTIR) Spectroscopy** was employed to study the functional groups of the drugs and assess their compatibility. FTIR analysis serves as a non-destructive tool to evaluate the presence of characteristic functional groups and detect any possible interaction between active pharmaceutical ingredients (APIs) and excipients. The combined use of UV and FTIR methods ensures a comprehensive analytical approach for the qualitative and quantitative evaluation of these antidiabetic agents.

II. MATERIALS AND METHODS

Spectrophotometric analysis was performed using a **Lab India UV-Visible Spectrophotometer** with matched quartz cells. Pure drug samples of **Sitagliptin** and **Empagliflozin** were obtained as gift samples from **Alkem Laboratories Pvt. Ltd., Mumbai, India**. No marketed formulation or fixed-dose combination product was used in this study; the analysis was conducted solely on the bulk drug substances.

The FTIR analysis was carried out using a **BRUKER Alpha ATR** spectrometer. The resolution was set to **4 cm^{-1}**, and the spectra were recorded over the wavenumber range of **4000–400 cm^{-1}**. The samples were analysed in the **ATR (Attenuated Total Reflectance)** mode, where the sample was directly placed onto the crystal surface for analysis.

UV SPECTROPHOTOMETRIC METHOD:

Selection of Common Solvent

The selection of a common solvent was made after assessing the solubility of both drugs in different solvents. Sitagliptin was found to be soluble in methanol, while Empagliflozin showed good solubility in methanol as well. Therefore, a mixture of methanol and water (50:50 v/v) was chosen as the common solvent for the analysis.

Preparation of Standard Stock Solutions

Standard stock solutions of Sitagliptin and Empagliflozin were prepared separately by dissolving 10 mg of each drug in 100 mL of the solvent mixture (methanol and water, 50:50 v/v) to obtain standard stock solutions of 100 ppm each.

III. METHOD DEVELOPMENT

After the preparation of the standard stock solutions, the next step involved developing a method for the simultaneous estimation of Sitagliptin and Empagliflozin using UV-Visible spectrophotometry. The method was developed by optimizing key parameters such as the choice of solvent, wavelength selection, and concentration range for both drugs.

Wavelength Selection

The UV-Visible spectra of Sitagliptin and Empagliflozin were scanned in the wavelength range of 200–400 nm using the Lab India UV-Visible Spectrophotometer. The wavelength corresponding to the maximum absorbance (λmax) for Sitagliptin was found to be 267 nm and for Empagliflozin at 236 nm. These wavelengths were selected for further analysis, as they showed clear and distinct peaks for both drugs.

Preparation of Calibration Curves

For the determination of the drugs, calibration curves for both Sitagliptin and Empagliflozin were constructed by diluting the stock solutions to different concentrations (50ppm for Sitagliptin and 50ppm for Empagliflozin). The absorbance of these solutions was measured at the respective λmax values. The linearity of the method was tested by plotting the absorbance against concentration. Both calibration curves exhibited a linear relationship with correlation coefficients (R^2) close to 1 for both drugs, indicating the reliability of the method**.**

IV. VALIDATION OF THE METHOD

The developed method was validated as per the ICH Q2(R1) guidelines for parameters such as accuracy, precision, linearity, limit of detection (LOD), and limit of quantification (LOQ).

Accuracy:

Recovery studies were performed by spiking the sample solutions with known amounts of Sitagliptin and Empagliflozin. The recovery rates were found to be within the acceptable range of 98–102%, confirming the accuracy of the method.

Precision:
The method's precision was assessed by performing intra-day and inter-day precision studies. The % RSD (Relative Standard Deviation) was calculated, with values below 2%, indicating high precision and reproducibility of the method.

Linearity:
As mentioned, linearity was confirmed for both drugs within the concentration ranges of X–Y µg/mL for Sitagliptin and A–B µg/mL for Empagliflozin.

LOD and LOQ:
The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the standard deviation of the response and the slope of the calibration curve, confirming the method's sensitivity.

V. FTIR SPECTROSCOPY METHOD

Sample Preparation:
For both pure drug samples and their mixtures, the drugs were triturated with KBr in a 1:100 ratio and pressed into pellets using a hydraulic press. However, for the FTIR study, the ATR mode was employed by directly placing the sample on the ATR crystal, which provides a more straightforward and rapid analysis without the need for sample preparation like pellet formation.

Identification of Functional Groups:
The FTIR spectra of Sitagliptin and Empagliflozin were recorded to identify characteristic functional groups. The following key functional groups were identified for both drugs:

Sitagliptin:

- N-H Stretching: A strong, broad peak around 3350 cm^{-1} corresponds to the N-H stretch, characteristic of amines.
- C=O Stretching: A strong peak around 1680 cm^{-1} indicates the presence of a carbonyl group (C=O), typical of amides or esters.
- Aromatic C-H Bending: A peak around 750–850 cm^{-1} corresponds to C-H bending in the aromatic ring.

Empagliflozin:

- O-H Stretching: A broad, strong peak around 3400 cm^{-1} indicates the presence of an O-H group, typically associated with hydroxyl groups.
- C-H Stretching: A peak around 2900 cm^{-1} is characteristic of C-H stretching in alkyl groups.
- C-O Stretching: A peak around 1050 cm^{-1} corresponds to C-O stretching, characteristic of ether or alcohol groups.

VI. RESULTS AND DISCUSSION

UV SPECTROPHOTOMETRIC METHOD:
The method showed excellent performance in terms of linearity, accuracy, and precision. Calibration curves of both drugs were linear in the concentration range of 5–30 µg/mL The recovery values ranged between 98% and 102%, which confirms the accuracy of the method. Precision studies showed relative standard deviation (RSD) values less than 2%, indicating high reproducibility.

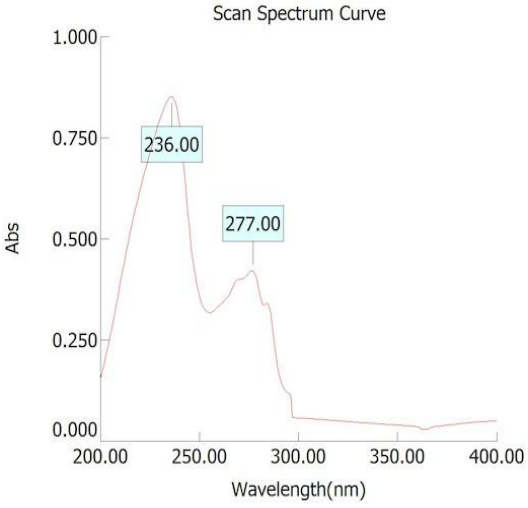

Fig 3. λmax Curve Sitagliptin (236 nm)

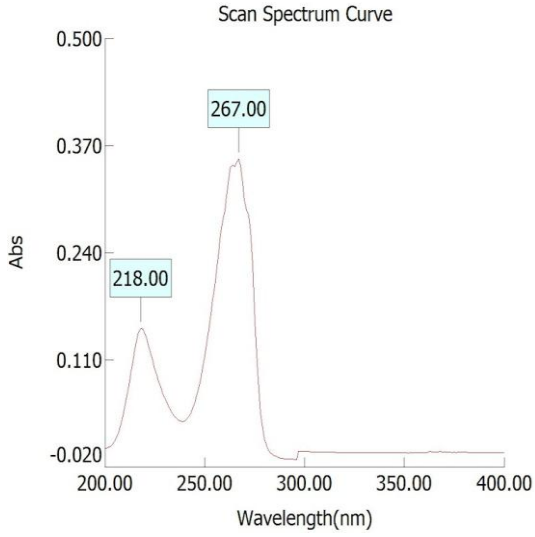

Fig 4. λmax Curve Empagliflozin (267 nm)

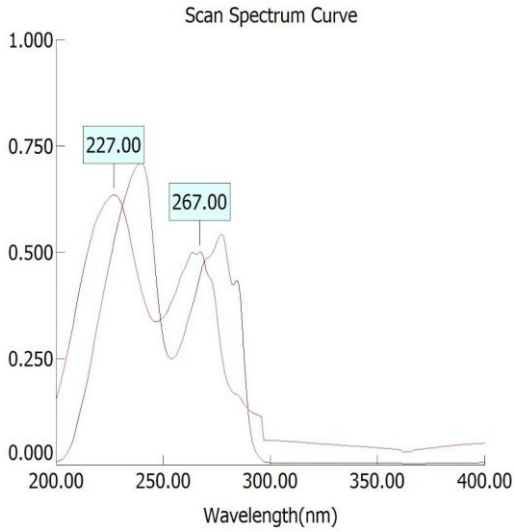

Fig.5: Overlay spectra of Empagliflozin and Linagliptin

Table no. 1 Reading of Linearity

EMPAGLIFLOZIN		SITAGLIPTIN	
conc.	**Abs.**	**conc.**	**Abs.**
0.2	0.045	0.2	0.013
0.4	0.117	0.4	0.032
0.6	0.16	0.6	0.046
0.8	0.21	0.8	0.056
1	0.28	1	0.068
1.2	0.304	1.2	0.082

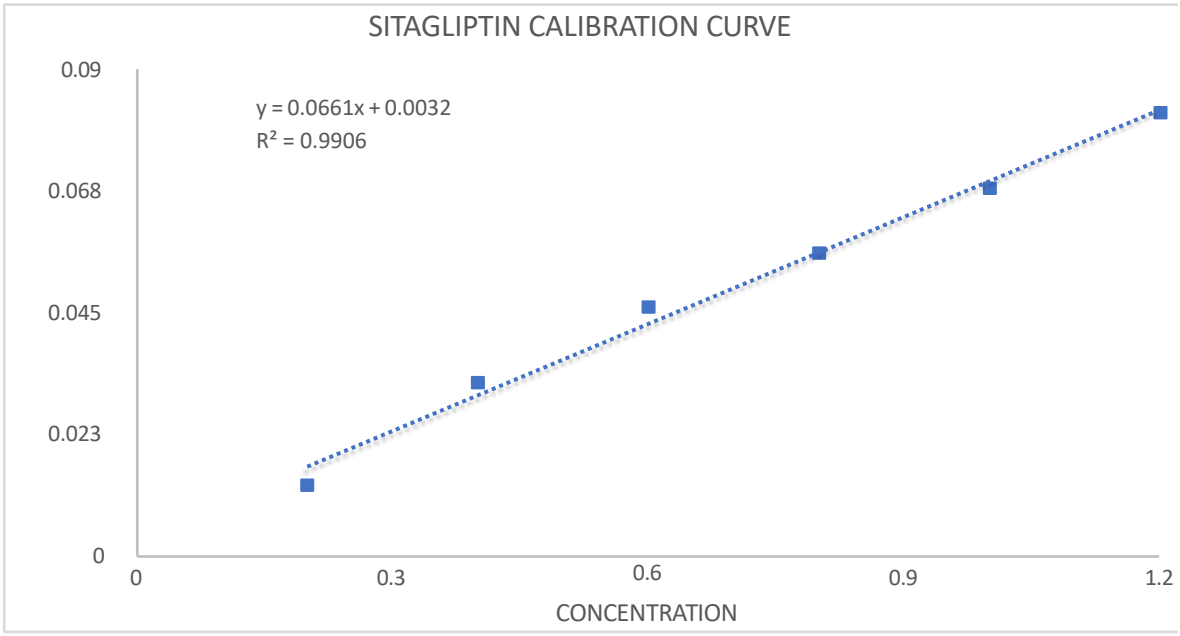

Fig. 6 Standard curve of Sitagliptin at 236 nm

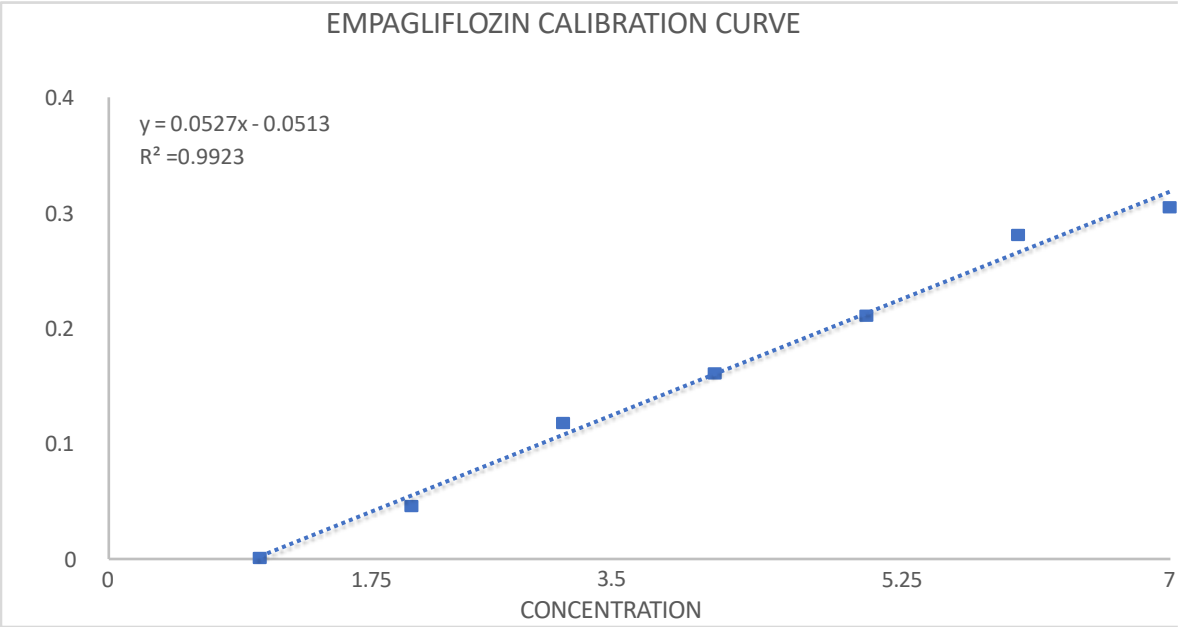

Fig. 7 Standard curve of empagliflozin at 267 nm

Table no. 2 Summary of Validated Parameters

Parameters	Sitagliptin	Empagliflozin
Wavelength	236 nm	267 nm
Correlation Coefficient (r2)	0.9906	0.9923
Regression Coefficient (y=mx+c)	0.0661x+0.0032	0.0527x+0.0513
Slope (m)	0.0661	0.0527
Intercept (c)	0.0032	0.0513
LOD	0.7778 µg/mL	0.7778 µg/mL
LOQ	2.3571µg/mL	2.3571µg/mL

Table no. 3 Intraday Precision

Drug	Concentration (µg/ml)	Absorbance			Average	SD	%RSD
Sitagliptin	0.5	0.290	0.289	0.288	0.289	0.001	0.346
	1.5	0.629	0.615	0.612	0.619	0.0091	1.467
	2.5	0.733	0.713	0.707	0.718	0.0136	1.897

Empagliflozin	0.5	0.549	0.545	0.544	0.546	0.0026	0.485
	1.5	0.66	0.657	0.651	0.656	0.0046	0.699
	2.5	0.664	0.661	0.655	0.66	0.0046	0.694

Table no. 4 Interday Precision

Drug	Concentration (µg/ml)	Absorbance			Average	SD	%RSD
Sitagliptin	0.5	0.223	0.222	0.22	0.222	0.0015	0.689
	1.5	0.548	0.546	0.543	0.546	0.0025	0.461
	2.5	0.704	0.697	0.695	0.699	0.0047	0.676
Empagliflozin	0.5	0.623	0.616	0.613	0.6173	0.0051	0.831
	1.5	0.67	0.667	0.662	0.6663	0.0040	0.607
	2.5	0.673	0.671	0.666	0.6700	0.0036	0.538

Table no. 5 Accuracy

Drug	Level (%)	% Recovery Values			Average Recovery	SD	% RSD
Sitagliptin	80%	99.65	99.56	99.68	99.63	0.0624	0.0627
	100%	100.65	100.45	100.62	100.57	0.1135	0.1112
	120%	100.58	100.73	100.69	100.66	0.0776	0.0772
Empagliflozin	80%	99.42	99.58	99.51	99.50	0.0800	0.0804
	100%	100.28	100.49	100.33	100.37	0.1061	0.1057
	120%	100.74	100.61	100.88	100.74	0.1350	0.1339

Table no. 6 Repeatability

Drug	Concentration (µg/ml)	Absorbance	Average	SD	%RSD
Sitagliptin	2.5	0.734	0.734	0.0010	0.1340
		0.734			
		0.733			
		0.734			
		0.736			
		0.734			
Empagliflozin	2.5	0.664	0.664	0.0004	0.0610
		0.664			
		0.664			
		0.665			
		0.664			
		0.664			

VII. FTIR SPECTROSCOPIC METHOD

The FTIR spectra of Sitagliptin and Empagliflozin showed clear and sharp peaks corresponding to their functional groups, enabling identification and quantification. Linearity was confirmed with correlation coefficients above 0.998. The recovery results also fell within the acceptable range, confirming the accuracy of the method. The non-destructive nature and minimal sample preparation make FTIR suitable for rapid screening.

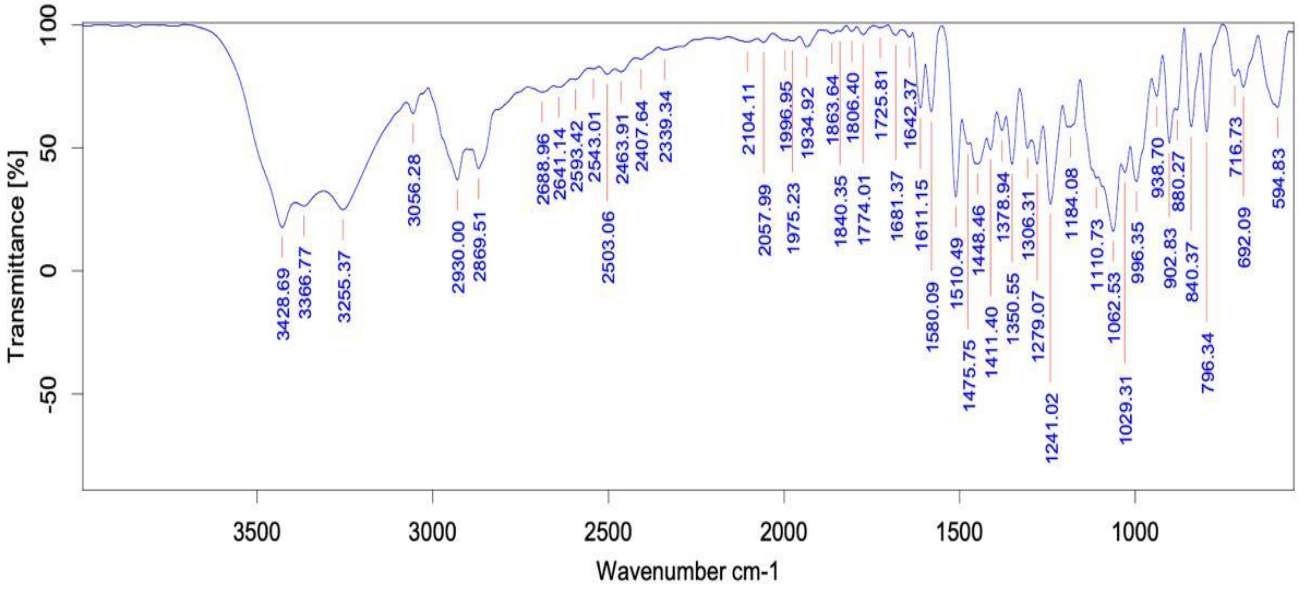

Fig. 8. FTIR Spectra of Empagliflozin

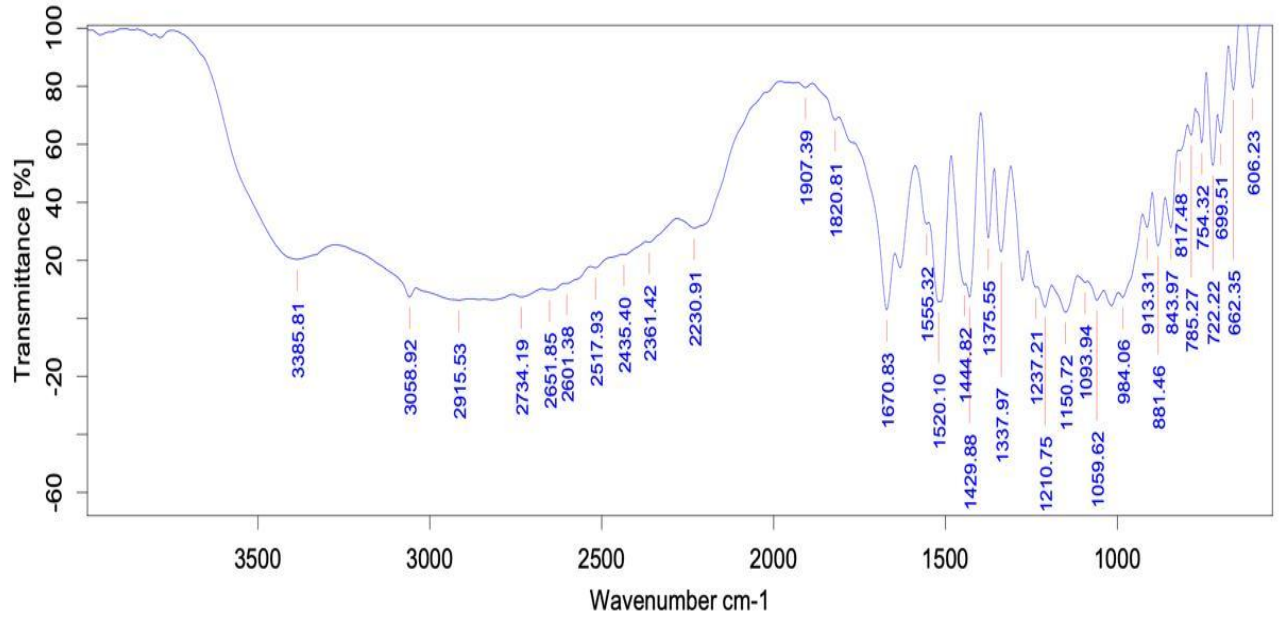

Fig. 9. FTIR Spectra of Sitagliptin

Table no. 7 Frequencies of FTIR Spectra of Sitagliptin

TYPE OF BOND		EXPECTED FREQUENCIES	OBTAINED FREQUENIES
AROMATIC	C=C-H	3000 Cm^{-1}	3058.92 Cm^{-1}
	C=C-H	900-700 Cm^{-1}	913.31 Cm^{-1}
			881.46 Cm^{-1}
			843.97 Cm^{-1}
			817.48 Cm^{-1}
			785.27 Cm^{-1}
			754.32 Cm^{-1}
			722.22 Cm^{-1}
			699.5 Cm^{-1}
	C=C	1600-1475 Cm^{-1}	1670.83 Cm^{-1}
			1444.82 Cm^{-1}
			1429.88 Cm^{-1}
	C-F	1400-1000 Cm^{-1}	1059.62 Cm^{-1}
			1093.94 Cm^{-1}
			1150.72 Cm^{-1}
			1210.75 Cm^{-1}
			1237.21 Cm^{-1}
			1337.97 Cm^{-1}
			1375.55 Cm^{-1}
	CH_2	1465 Cm^{-1}	1444.82 Cm^{-1}
			1429.88 Cm^{-1}
	C-N	1350-1000 Cm^{-1}	1059.62 Cm^{-1}
			1093.94 Cm^{-1}
			1150.72 Cm^{-1}
			1210.75 Cm^{-1}
			1237.21 Cm^{-1}
			1337.97 Cm^{-1}
	C=N	1690-1640 Cm^{-1}	1670.83 Cm^{-1}
			1670.83 Cm^{-1}
	N-N	1640-1550 Cm^{-1}	1555.32 Cm^{-1}
			1631.91 Cm^{-1}
AMIDE	N-C=O	1680-1630 Cm^{-1}	1670.83 Cm^{-1}
			1631.91 Cm^{-1}

Table no. 8 Frequencies of FTIR Spectra of Empagliflozin

TYPE OF BOND		EXPECTED FREQUENCIES	OBTAINED FREQUENIES
ETHER	C-O	1300-1000 Cm^{-1}	1306.31 Cm^{-1}
			1279.07 Cm^{-1}
			1241.02 Cm^{-1}
			1184.08 Cm^{-1}
			1110.73 Cm^{-1}
			1062.53 Cm^{-1}
			1029.31 Cm^{-1}
	CH_2 (Blending)	1465 Cm^{-1}	1475.75 Cm^{-1}
			1448.46 Cm^{-1}
AROMATIC	C=C-H	>3000 Cm^{-1}	3056.28 Cm^{-1}
	C=C-H (out of plane)	900-700 Cm^{-1}	902.83 Cm^{-1}
			880.27 Cm^{-1}
			840.37 Cm^{-1}
			796.34 Cm^{-1}
			716.73 Cm^{-1}
			692.09 Cm^{-1}
	C=C	1600 And 1475 Cm^{-1}	1475.75 Cm^{-1}
			1611.15 Cm^{-1}
	C-Cl	785-540 Cm^{-1}	716.73 Cm^{-1}
			692.79 Cm^{-1}
	OH	3400-3200 Cm^{-1}	3428.69 Cm^{-1}
			3366.77 Cm^{-1}
			3255.37 Cm^{-1}

VIII. CONCLUSION

A simple, accurate, and precise UV spectrophotometric method was successfully developed and validated for Sitagliptin and Empagliflozin in physical mixture, as per ICH guidelines. The method showed excellent linearity, accuracy, precision, and sensitivity, making it suitable for routine quality control. FTIR analysis confirmed the presence of characteristic functional groups and revealed no drug–drug interactions, indicating good compatibility. Overall, the combined UV and FTIR approach provides a reliable and cost-effective method for quality evaluation of these antidiabetic agents.

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