WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 15, 1022-1032. Research Article ISSN 2277–7105

DEVELOPMENT AND VALIDATION OF TWO UV-SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF EMPAGLIFLOZIN AND LINAGLIPTIN IN ITS PURE AND PHARMACEUTICAL DOSAGE FORM

Priyanka K.*, K. Vinod Kumar[1], T. Snehalatha[1], Md. Abdul Sufiyan[1], N. Kavya[1], K. Akhila[1]

*Assistant Professor, Department of Pharmaceutical Analysis, Smt. Sarojini Ramulamma College of Pharmacy, Mahabubnagar, Telangana, India.

[1]Smt. Sarojini Ramulamma College of Pharmacy, Mahabubnagar, Telangana, India.

Article Received on
09 June 2025,

Revised on 29 June 2025,
Accepted on 19 July 2025

DOI: 10.20959/wjpr202515-37729

***Corresponding Author**
Priyanka K.
India.

ABSTRACT

The proposed research suggests two simple, sensitive, precise and accurate UV-Spectrophotometric methods for simultaneous estimation of Empagliflozin and Linagliptin in its pure and tablet dosageform. Method A involves Simultaneous Equation Method, which is based on measurement of absorption at 224nm and 238nm for Empagliflozin and Linagliptin respectively. Method B is the Absorption Ratio Method which is based on measurement of absorption at wavelength of 224nm and 228nm i.e. λ_{max} of Empafliflozin and iso-absorptive point of Empagliflozin and Linagliptin respectively. Linearity was observed in the concentration range of 6-12µg/ml for both the drugs in both the methods. The accuracy of two methods were found to be within the range of 99.5-101.6% for both Empagliflozin and Linagliptin. The precision of Empagliflozin and Linagliptin in both methods are within the limits. The low limit of detection and limit of quantification values prove the sensitivity of the proposed methods. The developed methods were validated as per ICH Q_2 R_1 guidelines and were found to be satisfactory. The proposed two methods were found to be simple, sensitive, specific, precise and accurate. These two methods can be successfully applied for the routine analysis of Empagliflozin and Linagliptin in its pure and tablet dosage form.

KEYWORDS: Empagliflozin, Linagliptin, Simultaneous equation method and Q-Absorbance Ratio Method.

ABBREVATIONS

Empa – Empagliflozin

Lina - Linagliptin

SEM - Simultaneous Equation Method

ARM - Absorption Ratio Method

1. INTRODUCTION

Simultaneous estimation plays an important role in pharmaceutical industry because it is very feasible and time saving method. Specrophotometric methods and Chromatogaphic methods provide high degree of assurance that these techniques fit for the simultaneous estimation in the pharmaceutical dosage form.[1]

Empagliflozin chemically, (2S, 3R, 4R, 5S, 6R)-2-[4-Chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-trio) is an inhibitor of the sodium glucose co-transporter-2 (SGLT-2), which is found almost exclusively in the proximal tubules of nephronic components in the kidneys. SGLT-2 accounts for about 90 percent of glucose reabsorption into the blood. Blocking SGLT-2 reduces blood glucose by blocking glucose reabsorption in the kidney and thereby excreting glucose (i.e., blood sugar) via the urine of all the SGLT-2 Inhibitors currently available, empagliflozin has the highest degree of selectivity for SGLT-2 over SGLT-1, SGLT-4, SGLT-5 and SGLT-6.[2]

Linagliptin chemically,(8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1H-purine-2,6-dione) is a competitive, reversible DPP-4 inhibitor. Inhibition of this enzyme slows the breakdown of GLP-1 and glucose-dependant insulinotropic polypeptide (GIP). GLP-1 and GIP stimulate the release of insulin from beta cells in the pancreas while inhibiting release of glucagon from pancreatic beta cells. These effects together reduce the breakdown of glycogen in the liver and increase insulin release in response to glucose.[3]

2. MATERIALS AND METHODOLOGIES

Chemicals and reagents

Linagliptin API was received as a gift sample from Qualychrome lab, Hyderabad. Empagliflozin was purchased from Dhamtech Pharma, Mumbai. Ethanol and distilled water were supplied by SD Fine chemicals, Mumbai.

INSTRUMENTS

For the experiment, a Lab India Analytical UV-Visible spectrophotometer(double-beam)-with a pair quartz cell having a 1cm length was used. Weighing was carried out using CONTECH electronic balance.

Preparation of Standard solution of Empagliflozin and Linagliptin

Both Empagliflozin(10mg) and Linagliptin (10mg) were weighed carefully and transferred to separate 10ml volumetric flask and diluted with ethanol to get the concentration of 1000µg/ml. This concentration is diluted in a separate volumetric flask to attain 100µg/ml. This solution is used as a stock solution. The stock solution is further diluted with ethanol to attain desired concentrations.

Preparation of sample solutions

Marketed formulation (Glyxambi 25/5 Boehringer Ingelheim, Germany) were broken into powder and separately transfer 10mg equivalent weight of tablet powder into 10ml volumetric flask and dissolve it in ethanol and make up the solution up to 10 ml with ethanol. From this further dilutions were made to get the desired concentrations for both the drugs.

METHODOLOGY

A) Simultaneous Equation Method

The simultaneous equation was applied to evaluate Empagliflozin and Linagliptin Concentrations in the tablet formulation. The UV Spectra were recorded between 200-400 nm. Maximum Absorbances were seen at 224nm and 238nm respectively and isobestic point was observed at 228nm. The below mentioned formula were used to determine the exact amount of medication in each tablet.

$$C_x = (A_2\, ay_1 - A_1\, ay_2) / (ax_2\, ay_1 - ax_1\, ay_2)$$

$$C_y = (A_1\, ax_2 - A_2\, ax_1) / (ax_2\, ay_1 - ax_1\, ay_2)$$

C_x and C_y represent the amounts of Empagliflozin and Linagliptin in the sample solutions respectively. The absorbance of sample solution at 224nm is A_1 and at 238nm is A_2. Absorptivity of Empa at 224nm and 238 nm were denoted by ax_1 and ax_2. Absorptivity of Lina at 224nm and 238 nm were denoted by ay_1 and ay_2.

B) Absorbance Ratio Method

Excellent linearity was found between the wavelengths of 224nm (λ_{max} of Empa) and 228nm(the isobestic point). Hence these two wavelengths were chosen for simultaneous determination using AR method. The formulas below were used to determine the concentrations of Empa and Lina in the test solutions using the AR method.

$C_x = \{(Q_m - Q_y)/ (Q_x - Q_y)\} * (A_1/ax_1)$

$C_y = \{(Q_m - Q_x)/ (Q_y - Q_x)\} * (A_1/ay_1)$

ax_1 and ax_2 stands for the absorptivities of empa at 224nm and 228nm respectively. ay_1 and ay_2 are absorptivities of Lina at 224nm and 228nm. A_1 and A_2 were measured at absorbances of sample solution at 224nm and 228nm. C_x and C_y represents, concentration of Empa and Lina in the sample solution.

$Q_m = A_2/A_1$ $Q_x = ax_2/ax_1$ $Q_y = ay_2/ay_1$

3. RESULTS AND DISCUSSIONS

Selection of wavelength

The absorbance of the solutions containing Empagliflozin10µg/ml and Linagliptin 10µg/ml were scanned separately in the UV range 200-400nm using ethanol as blank.

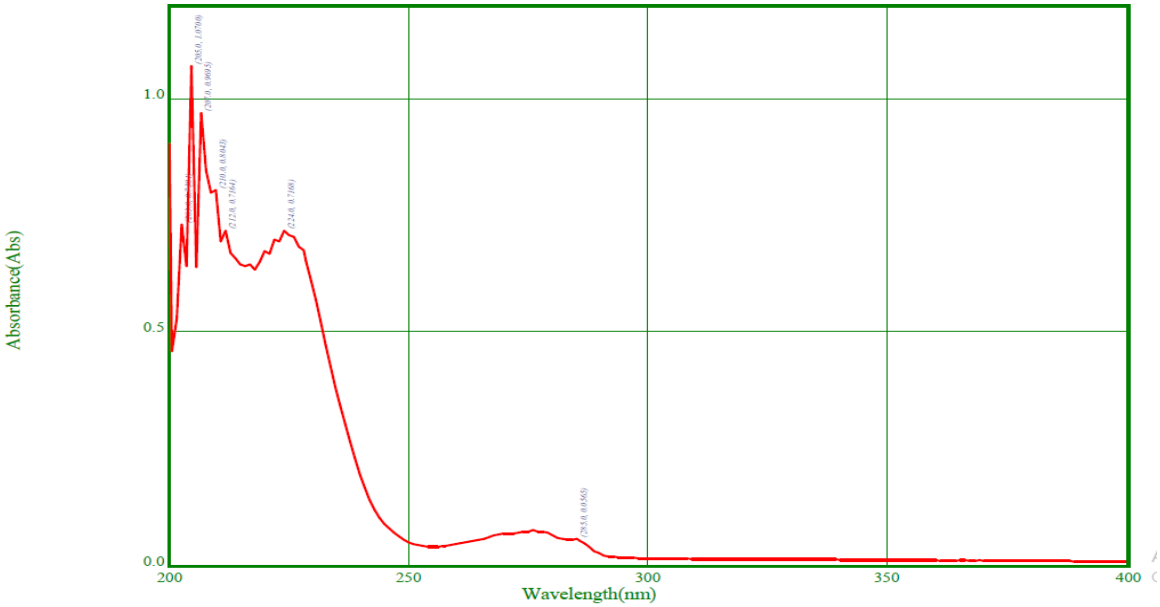

Figure no 1: Figure showing λ_{max} of Empagliflozin.

For the Empagliflozin maximum wavelength was found at 224 nm, and maximum absorbance was found to be 0.7168.

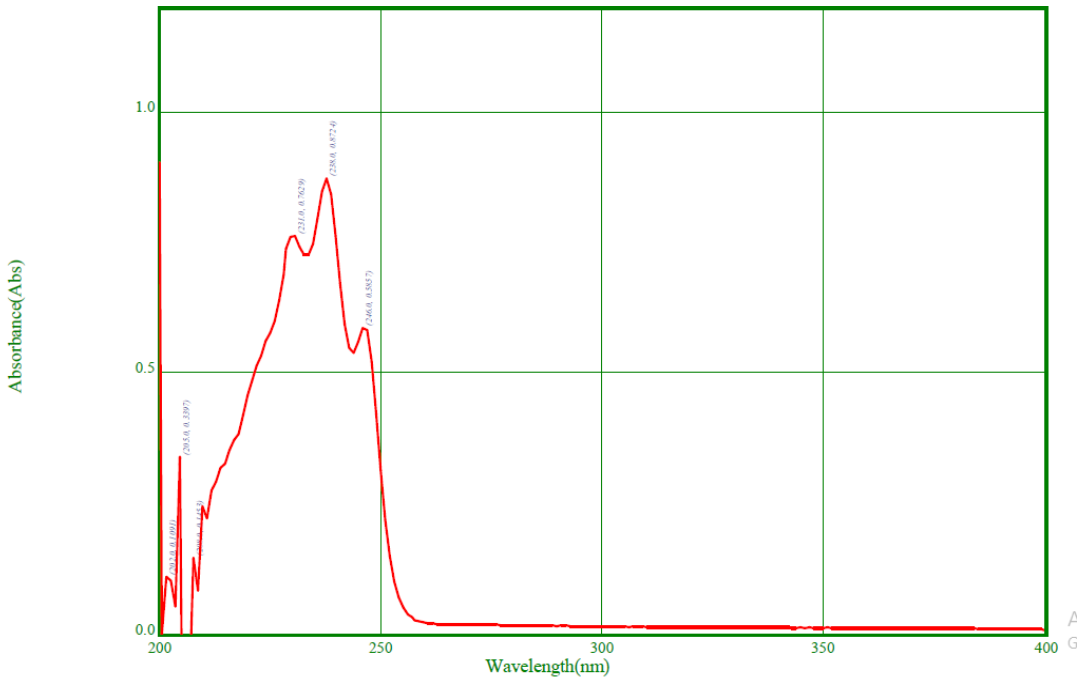

Figure no. 2: Figure showing λ_{max} of Linagliptin.

For the Linagliptin, maximum wavelength was found at 238 nm, and maximum absorbance was found to be 0.8724.

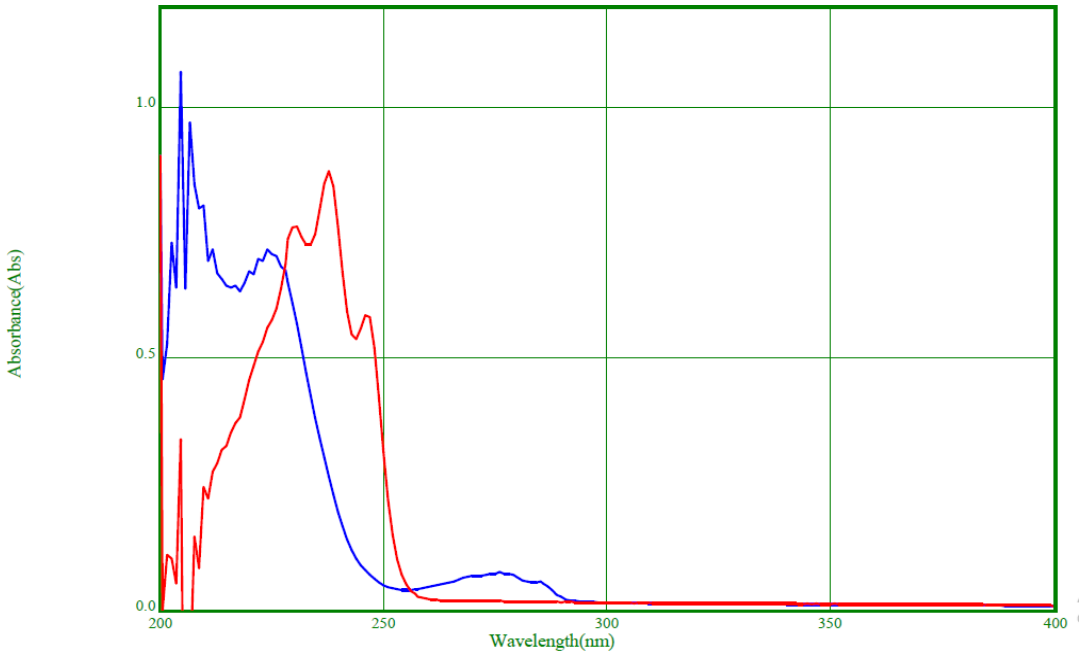

Figure no. 3: Figure showing isobestic point of Empagliflozin and Linagliptin.

The isobestic point was observed at 228nm by using overlapping method.

ASSAY

A) Simultaneous Equation Method

Table No. 1: Observation results for Assay by SEM.

S.No	Drug Name	Conc.	Absorbance		Absorptivity	
			224nm	238nm	224nm	238nm
1	Empagliflozin	10µg/ml	0.7168	0.6723	0.07168 (ax_1)	0.06723(ax_2)
2	Linagliptin	10µg/ml	0.4305	0.8724	0.04305(ay_1)	0.08724(ay_2)
3	Tablet formulation	Unknown	1.134 (A_1)	1.530 (A_2)		

$C_x = (A_2\, ay_1 - A_1\, ay_2) / (ax_2\, ay_1 - ax_1\, ay_2)$

= 9.86µg/ml

$C_y = (A_1\, ax_2 - A_2\, ax_1) / (ax_2\, ay_1 - ax_1\, ay_2)$

= 9.98µg/ml

B) Q-Absorbance Ratio Method

Table No. 2: Observation results for Assay by ARM.

S.No	Drug Name	Conc.	Absorbance		Absorptivity	
			224nm	224nm	224nm	224nm
1	Empagliflozin	10µg/ml	0.7168	0.6715	0.07168 (ax_1)	0.06715(ax_2)
2	Linagliptin	10µg/ml	0.4432	0.4640	0.04432(ay_1)	0.04640(ay_2)
3	Tablet formulation	Unknown	1.140 (A_1)	1.116 (A_2)		

$C_x = (Q_m - Q_y) * A_1 / (Q_x - Q_y) * ax_1$

= 9.82 µg/ml

$C_y = (Q_m - Q_x) * A_1 / (Q_y - Q_x) * ay_1$

= 9.9 µg/ml

Assay of Empagliflozin

= **(**Concentration x average weight in mg) / **(**Weight of powder equivalent to 10mg x label claim of Empagliflozin) X 10

Assay of Linagliptin

= **(**Concentration x average weight in mg) / **(**Weight of powder equivalent to 10mg x label claim of Linagliptin) X 100

The percentage purity of Empagliflozin and Linagliptin in tablet dosage form were found to be 98.6% and 99.8% respectively by using simultaneous equation method and 98.2% and 99% by using Q-Absorbance Ratio Method.

VALIDATION PARAMETERS

SPECIFICITY

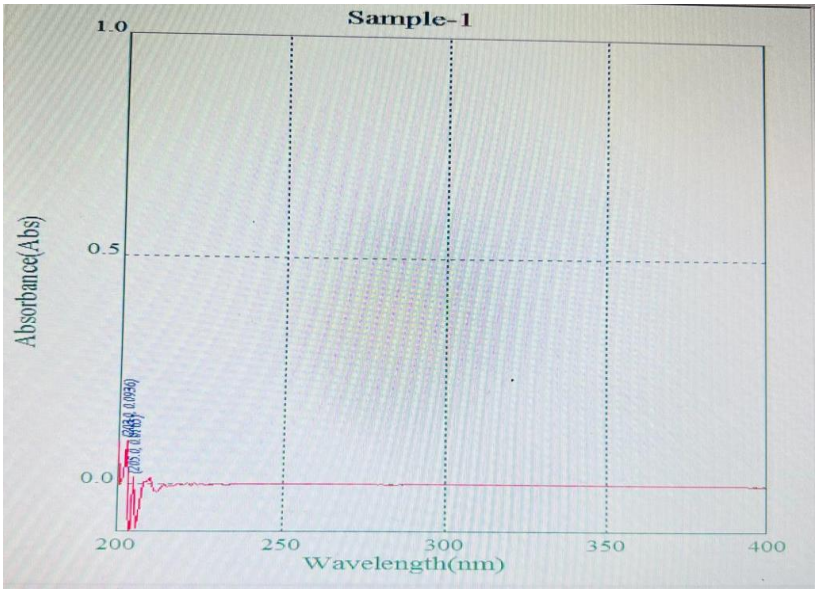

Figure no. 4: Spectrum showing blank.

Observation: From the spectrum we can conclude that excipients or solvents are not interfering the spectrum of Empagliflozin and Linagliptin.

LINEARITY

Table No. 3: Observation values for Linearity.

S.No	Conc. [µg/ml]	SEM		Q-ARM	
		Absorbance of Empa(224nm)	Absorbance of Lina(238nm)	Absorbance at 224nm	Absorbance at 228nm
1	6	0.5385	0.5409	0.5385	0.5132
2	8	0.6354	0.7189	0.6354	0.5949
3	10	0.7168	0.8724	0.7168	0.6746
4	12	0.8399	1.0041	0.8399	0.7399
5	14	0.9647	1.2082	0.9647	0.8451

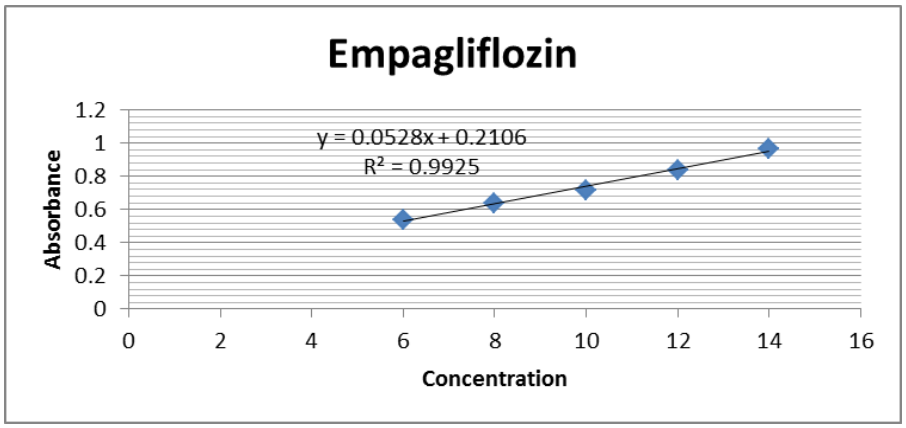

Fig no. 5: Calibration Curve of Empagliflozin.

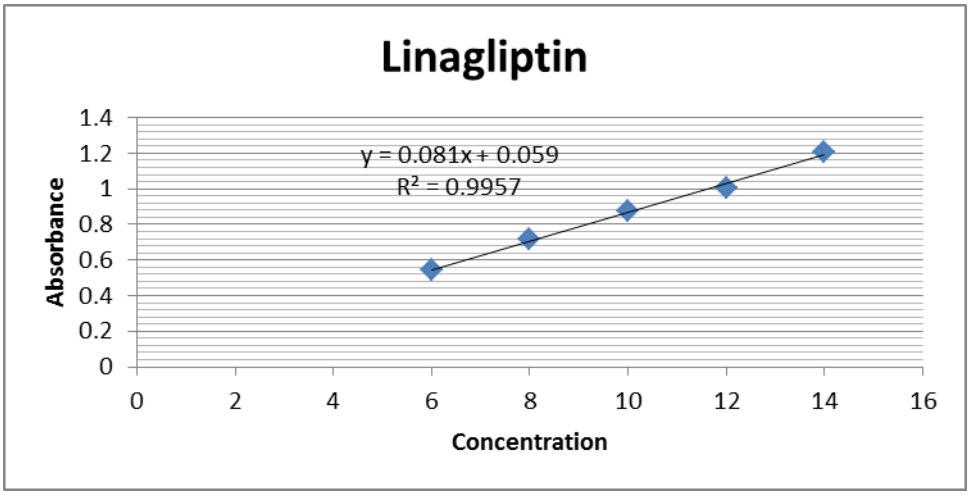

Fig no.6: Calibration Curve of Linagliptin.

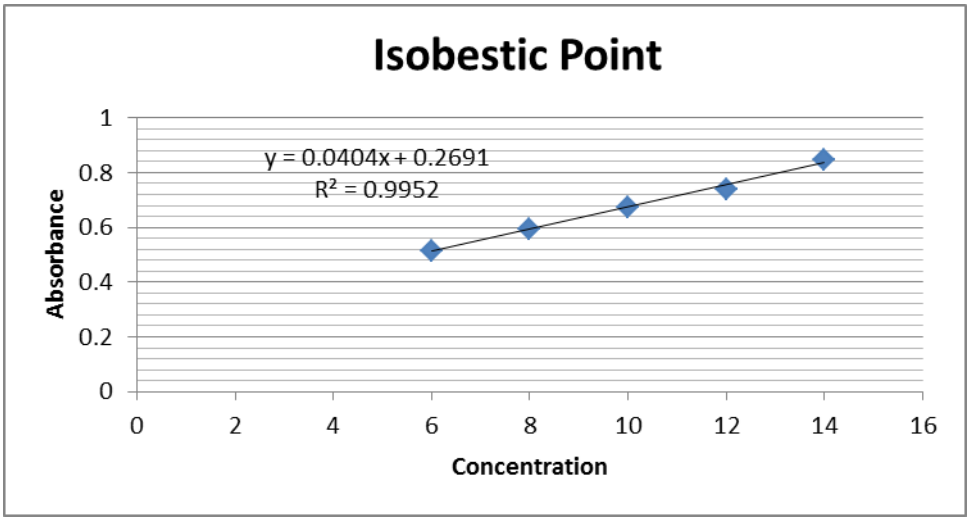

Fig no. 7: Calibration Curve at Isobestic point.

ACCURACY

Table No. 4: Observation values for Accuracy.

S. No	Accuracy level	Conc.taken (ug/ml)	SEM		Q-ARM	
			Empagliflozin Recovery	Linagliptin Recovery	Empagliflozin Recovery	Linagliptin Recovery
1	80	18	100.5	102.7	100.5	101.1
2	80	18	100.3	100.1	100.3	99.4
3	80	18	100	99.4	100	99.4
4	100	20	101.5	99.5	101.5	101.5
5	100	20	100.71	103	100.71	101.5
6	100	20	100.45	102.5	100.45	101.5
7	120	22	97.7	100.4	97.7	102.2
8	120	22	101.3	99.5	101.3	99.5
9	120	22	99.5	100	99.5	102.2

The results obtained for recovery at 80%, 100%.120% are within the limits. Hence method is accurate.

Table No. 5: Observation values for Repeatability.

S,No	Conc. (µg/ml)	SEM		Q-ARM	
		Absorbance of Empa	Absorbance of Lina	Absorbance of Empa	Absorbance of Lina
1	10	0.7168	0.8724	0.7168	0.6746
2	10	0.7152	0.8756	0.7152	0.6752
3	10	0.7168	08792	0.7168	0.6786
4	10	0.7185	0.8699	0.7185	0.6715
5	10	0.7169	0.8710	0.7169	0.6752
	Average	0.71684	0.87362	0.71684	0.67502
	SD	0.001167	0.003783	0.001167	0.002522301
	%RSD	0.16	0.43	0.16	0.003

PRECISION

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION(LOQ)

Table No. 6: Observation values for LOD & LOQ.

Parameter	SEM		Q-ARM	
	Empa	Lina	Empa	Lina
Standard Deviation	0.001167	0.003783	0.001167	0.0025223
Slope	0.0528	0.081	0.0528	0.0025223
LOD	0.066	0.046	0.066	0.182
LOQ	0.22	0.467	0.22	0.62

ROBUSTNESS

Table No. 7: Observation values for Robustness.

Parameter	SEM		Q-ARM	
	Empa	Lina	Empa	Lina
Wavelength				
2nm less than the λ_{max}	0.7092	0.8720	0.6942	0.6695
At λ_{max}	0.7168	0.8724	0.6746	0.6710
2nm more than the λ_{max}	0.6942	0.8715	0.6615	0.6746
Temperature				
26^0C	0.7095	0.8720	0.6940	0.6692
28^0C	0.7168	0.8724	0.6746	0.6712
30^0C	0.6940	0.8719	0.6610	0.6750

4. SUMMARY AND CONCLUSION

SUMMARY

Two simple, sensitive, precise, accurate UV spectrophotometric methods have been developed and validated for the simultaneous estimation of Empagliflozin and Linagliptin in its pure and Tablet dosage form.

The process was done by using simultaneous equation method and Q-Absorbance Ratio Method with the detection wavelength set at 224nm, 238nm and 228nm for Empagliflozin, Linagliptin and isobestic respectively. The method was linear with the correlation coefficient 0.99 in the concentration range of 6-14ug/ml in both the methods. The limit detection were 0.666ug/ml and 0.046ug/ml and 0.182ug/ml respectively. The limit of Quantification were found to be 0.22ug/ml, 0.467ug/ml and 0.62 ug/ml respectively. The repeatability of inter day 1 and day 2 precision were satisfactory and the relative standard deviation did not exceed 2%. The accuracy of the method from the recovery studies is within the limits for both the drugs and tand also at isobestic point. The two method are robusted. The methods met the ICH regulatory requirements.

CONCLUSION

Two simple, accurate, precise, reproducible, robust and economical UV spectrophotometric methods for simultaneous estimation of Empagliflozin and Linagliptin in its pure and pharmaceutical dosage form have been developed. The two methods are Simultaneous Equation Method and Q-Absorbance Ratio Method. The methods were developed by using ethanol as solvent. The developed method were validated for parameters viz accuracy, precision, linearity, robustness, limit of detection and limit of quantification as per ICH guidelines. All the parameters were found to be within the acceptance limits. The results indicated that the proposed method for simultaneous estimation of Empagliflozin and Linagliptin are very accurate and cost effective and can be employed in routine sample analysis in its pure and pharmaceutical dosage form.

ACKNOWLEDGMENT

Our sincere thanks to our SSRCP management for providing us power and strength to overcome all the hurdles and hindrances that come in the way of doing project.

REFERENCES

1. https://www.researchgate.net/publication/279997529_Analytical_Techniques_in_Simulta .neous_Estimation_An_Overview.
2. https://en.wikipedia.org/wiki/Empagliflozin.
3. https://en.wikipedia.org/wiki/Linagliptin.