Development And Validation of Analytical Methods for The Simultaneous Estimation of Empagliflozin and Linagliptin In Bulk And In Pharmaceutical Dosage Form

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Abstract-
Simple, precise and economical UV Spectrophotometric methods have been developed for the simultaneous estimation of Empagliflozin and Linagliptin in bulk and pharmaceutical dosage forms. The simultaneous equation (Vierodt's Method), which is based on measurement of absorption at 233nm and 277nm i.e. λmax of Empagliflozin and Linagliptin respectively. Linearity was observed in the concentration range of 5-15µg/ml for Empagliflozin and 2-6µg/ml for Linagliptin. The accuracy of methods was assessed by recovery studies and was found to be within range of 98-101% for both Empagliflozin and Linagliptin. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents. The results were validated statistically as per ICH Q2 R1 guidelines and were found to be satisfactory.

Keywords: Diabetes, Empagliflozin, Linagliptin, analytical validation.

INTRODUCTION

Analytical chemistry is the science and practise of determining the composition of materials in terms of the elements that make up that composition. Pharmaceutical analysis is a branch of science that deals with the methods for determining the purity, safety, and quality of medications and chemicals. Procedures for determining the identity, strength, quality, and purity of novel compounds are included. Separating, identifying, and determining the relative amounts of the components in a sample of matter are also part of the technique.

The importance of quality assurance in determining the safety and efficacy of medications cannot be overstated. For the design, development, standardization, and quality control of pharmaceutical products, it has very specific and sensitive analytical methods. They're equally vital in pharmacokinetics and drug metabolism investigations, which are both crucial for determining bioavailability and clinical response.

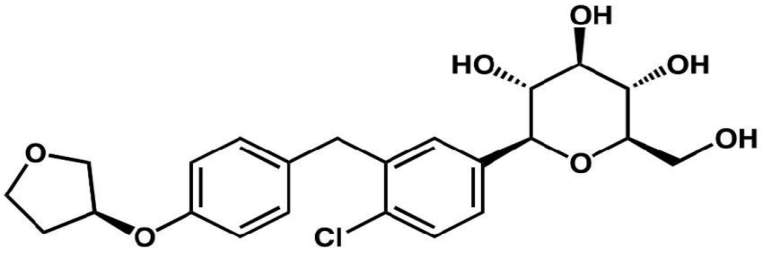
In the present-day situation in India, one of the most prevalent health concerns among the general population is diabetes. Recent research has highlighted a significant uptick in the incidence of type-2 diabetes within society. Type-2 diabetes is a progressive condition characterized by a continuous rise in glycosylated hemoglobin (HbA1C) levels over time, which is associated with an elevated risk of both micro- and macrovascular complications. Additionally, it substantially diminishes life expectancy.

A combination of linagliptin and empagliflozin Is available in tablet form for oral use, designed for treating type 2 diabetes and managing cardiovascular risk. Empagliflozin (EMPA) acts as a sodium glucose cotransporter-2 (SGLT-2) inhibitor, improving blood glucose control in adults with type 2 diabetes. SGLT-2 co-transporters typically reabsorb glucose from the kidney filtrate, but EMPA inhibits this process, reducing renal glucose reabsorption and lowering the renal glucose threshold. This leads to increased glucose excretion, which helps reduce hyperglycemia and also contributes to blood pressure reduction.

On the other hand, Linagliptin (LINA) serves as a competitive, reversible DPP-4 inhibitor. Its role is to prevent the degradation of GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulin tropic polypeptide). GLP-1 and GIP play pivotal roles in stimulating insulin release from pancreatic beta cells and inhibiting glucagon release from these cells. This combined action leads to decreased glycogen breakdown in the liver and an enhanced insulin response to glucose level.

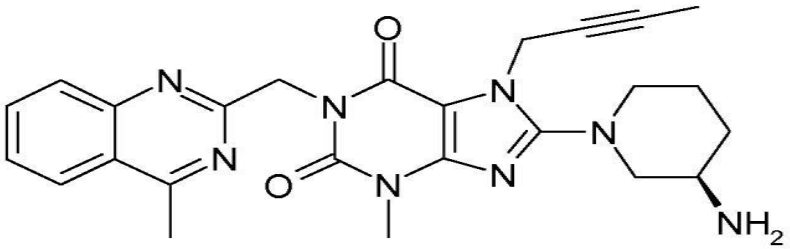
Empagliflozin

Empagliflozin is recognized as an inhibitor of sodium-glucose co-transporter-2 (SGLT2), which plays a crucial role in the kidney's glucose reabsorption. In clinical practice, it is commonly employed as a supplementary treatment alongside dietary and exercise interventions. It is frequently prescribed in combination with other pharmaceutical treatments to effectively manage type 2 diabetes mellitus.



Linagliptin

Linagliptin, created by Boehringer Ingelheim, is a DPP-4 inhibitor designed for managing type II diabetes. What sets Linagliptin apart from other DPP-4 inhibitors is its unique non-linear pharmacokinetic profile, limited reliance on renal elimination, and adherence to concentration-dependent protein binding. The FDA granted approval for Linagliptin on May 2, 2012.



MATERIALS AND METHOD

MATERIALS

Chemicals

All chemicals used during the project work were either AR grade or UV grade. The
Reagents and chemicals used during experimental work are as follows,

1. Water (Distilled Water)
2. Ethanol (AR Grade)
3. Hydrochloride Acid (AR Grade)
4. Sodium Hydroxide (AR Grade)

Name of Drug Pure & their Supplier

1. Linagliptin (Bakul Finechem Research Centre)
2. Empagliflozin (Bakul Finechem Research Centre)

Instruments & Apparatus

1. UV-Visible spectrophotometer (UV-1800 Shimadzu Double Beam Spectrophotometer).
2. Incubator (Gayatri Scientific Mumbai).
3. Dissolution test Apparatus (Electrolab EDT 08Lx, 08 Station).
4. Sonicator. (Leela sonic)
5. Electronic weight balances

METHODOLOGY

Preparation of standard stock solution: Accurately weigh about 10 mg of pure drug of Linagliptin and Empagliflozin was transferred to 100 ml of volumetric flask dissolved separately in 0.1 M NaOH than give final concentration of 100 µg/ml both the drug.

Selection of analytical wavelengths: Appropriate dilutions were prepared for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. LIN and EMP showed absorbance maxima at 294.40 nm and 268.60 nm .

Preparation of working solution: Appropriate volume 1 ml and 1 ml of standard stock solution of Linagliptin and Empagliflozin was transferred into 10 ml of volumetric flask diluted to mark with 0.1 M NaOH to give concentration of each drug. The resulting solutions were then scanned in UV spectrophotometer from 400 to 200 nm. From the resulting spectra λmax for Linagliptin and Empagliflozin were calculated separately. The spectrums were record.

Preparation of calibration curve: From the standard stock solution of Linagliptin and Empagliflozin appropriate aliquot was pipetted out into 10 ml volumetric flask and dilution was made with0.1 M NaOH to obtain concentration 100 µg/ml. Appropriate aliquots were pipetted out into 10 ml volumetric flasks and dilutions were made to obtain working standard solutions of concentrations 1, 2, 3, 4, 5 µg/ml. and 5, 10, 15, 20, 25 µg/ml respectively. Absorbance for these solutions were measured at294.4 nm and 268.60 nm respectively (Table) 1-2

RESULTS AND DISCUSSION

Table 1: Standard calibration of Linagliptin

Sr. No	**Conc. (µg/ml)**	**294.40 nm**	**268.60 nm**
1	**1 µg/ml**	0.115	0.08
2	**2 µg/ml**	0.224	0.172
3	**3 µg/ml**	0.332	0.254
4	**4 µg/ml**	0.443	0.338
5	**5 µg/ml**	0.545	0.423

Table 2: Standard calibration of empagliflozin

Sr. No	**Conc. (µg/ml)**	**294.40 nm**	**268.60 nm**
1	**5 µg/ml**	0.134	0.192
2	**10 µg/ml**	0.263	0.345
3	**15 µg/ml**	0.381	0.495
4	**20 µg/ml**	0.513	0.645
5	**25 µg/ml**	0.642	0.812

Sr. No	**Amount Added (µg/ml) LIN**	**Absorbance At 294 nm**	**Amount Recovered**	**% Recovery**
1	3.2	0.215	3.12	99.50
2	4.0	0.219	3.88	99.00
3	4.8	0.221	4.70	99.91
Mean				99.47
SD				

	0.3721
% RSD	0.47

Accuracy: Accuracy of an analytical method is the closeness of the test results obtained by that of the true value. It was ascertained on the basis of recovery studies performed at different levels of concentrations.
Table 5: Result of Accuracy

Precision: It is expressed as ± S.D. or % RSD of series of measurements. Precision of the methodwas verified by using stock solutions in the ratio of 1:5 containing 1µg/ml LIN and 5µg/ml EMP. System repeatability was done by repeating the assay three times of tworeplicate dilutions of the same concentration after every two hours on the same day forintraday precision. Interday precision was carried out by performing the assay of two sample sets after 24 hours and 48 hours, results are reported in Table3-4.

Table 3: Results of Precision Studies (Intra-day)

Session	**Absorbance at**		**% Estimation**	
	294 nm	**268.60 nm**	**LIN**	**EMP**
Morning	0.6346	0.357	99.71	99.16
Afternoon	0.6299	0.356	99.38	99.10
Evening	0.6243	0.357	99.61	99.15
MEAN			**99.90**	**99.14**
S.D.			**0.51**	**0.032**
% RSD			**0.64**	**0.033**

Table 4: Results of Precision Studies (Inter-day)

Session	**Absorbance at**		**% Estimation**	
	294 nm	**268.60 nm**	**LIN**	**EMP**
Morning	0.6243	0.357	99.65	99.16
Afternoon	0.6087	0.356	99.31	99.10
Evening	0.6075	0.357	99.99	99.15
MEAN			**99.64**	**99.14**
S.D.			**0.27**	**0.032**
% RSD			**0.34**	**0.033**

Robustness: It expresses the precision within laboratories, Variation like different solvent. Robustness of the methods was assessed by carrying out assay 3 times with different solvent by using same equipment. The

results of the same are presented in Table .

Table 5: Result of Robustness

Session	**Absorbance at**		**% Estimation**	
	294 nm	**268.60 nm**	**LIN**	**EMP**
$CH_3OH + H_2O$ (1:9)	0.419	0.369	99.90	99.96
0.1N NaOH	0.432	0.348	99.11	99.85
MEAN			**99.47**	**99.90**
S.D.			**0.105**	**0.70**
% RSD			**0.15**	**0.20**

Ruggedness Study: The Ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of same samples under the variety of normal test conditions such as different laboratories, different analysts using operational and environmental conditions. The ruggedness study is carried out using the different analyst.

Table 6: Ruggedness study

Sr. No.	**Absorbance At**		**% Label Claim**	
	λ1	**λ2**	**X**	**Y**
Analyst 1	0.489	1.031	99.43	98.78
Analyst 2	0.512	1.526	99.84	98.89
		Mean	**99.63**	**98.83**
		S.D.	**0.205**	**0.055**
		C.V.	**0.205**	**0.055**

Method validation:

The UV spectrophotometric method was validated as per ICH guidelines for method validation. The performance parameters like linearity, precision and accuracy were evaluated.

Linearity: Linearity was studied by diluting standard stock solution of LIN 1-5µg/ml and EMP 525 µg/ml concentrations (n=3). Calibration curves with concentration verses absorbance were plotted at their respective wavelengths and the obtained data was subjected to regression analysis using the least square method. The standard curves for LIN and EMP are shown in Fig. 13, 14, 15 & 16 and data is presented in Table 7.

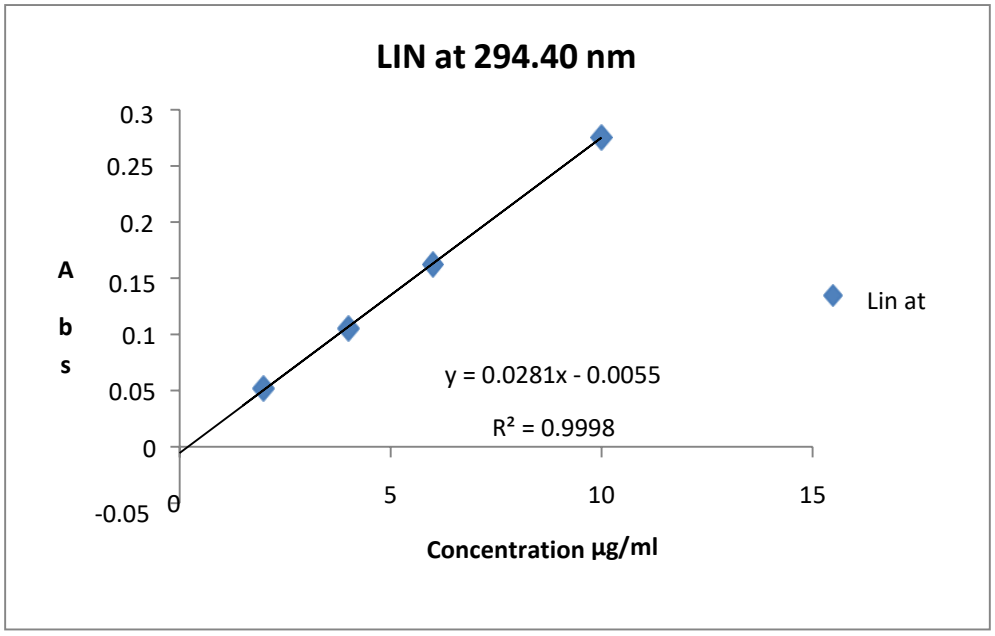


Figure 13: Calibration curve for LIN at 294.40 nm in 0.1N NaOH

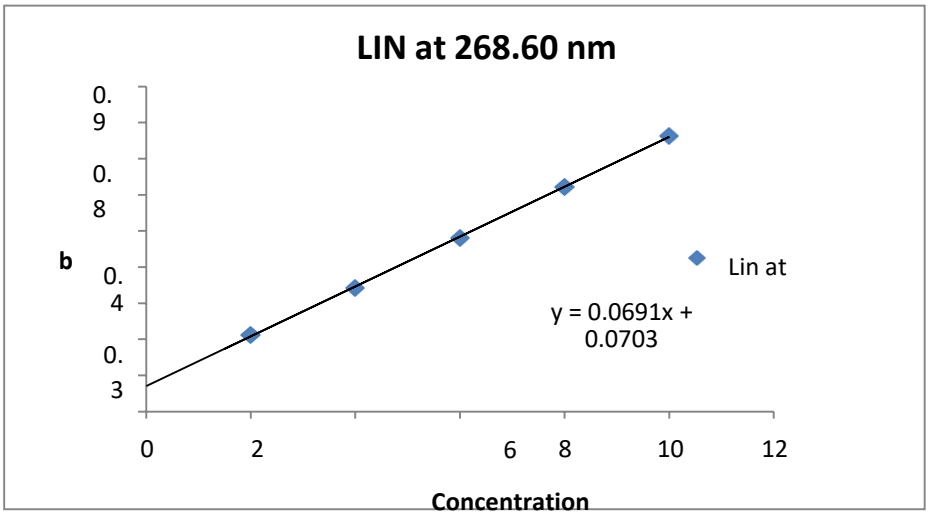


Figure 14: Calibration curve for LIN at 268.60 nm in 0.1N NaOH

SUMMARY

Recently, fixed dose of EMP and LIN was approved as Diabetes type 2 is linked to a high rate of cardiovascular morbidity and mortality. When you have diabetes, you have elevated cholesterol, which is known as diabetic hyperlipidemia.

Linagliptin is an oral anti-hyperglycaemic (Antidiabetic drug) of the Dipeptidyl peptidase-4 (DPP-4) inhibitor class.

Method A: Simultaneous Equation Method

Method B: Absorbance Ratio Method

Table 7: Summary data for optical characteristics and validation data of developed

Method	Sample	Wavelength	Range	LOD	LOQ	Precision
Method	LIN	294.40	1-5	0.247	0.750	99.64
A	EMP	268.60	5-25	0.124	0.375	99.14
Method	LIN	294.40	1-5	0.587	1.77	100.3

B	EMP	268.60	5-25	0.445	1.34	100.9

CONCLUSION

The new UV-spectrophotometric processes are simple, accurate, precise, repeatable, sensitive, and cost-effective, according to the findings. These can become an outstanding analytical tool for routine quality control of Linagliptin and Empagliflozin and their combined pharmaceutical dosage form without any prior separation of components. As a result, we believe the technology can be used to routinely analyses pharmaceutical dosage forms including Linagliptin and Empagliflozin.

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