OPTIMIZATION AND VALIDATION OF UV ANALYTICAL PROTOCOLS FOR LINAGLIPTIN USING DIVERSE CALIBRATION APPROACHES

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***Abstract*—UV spectrophotometric techniques play a crucial role in pharmaceutical analysis for the estimation of active compounds. This study presents the development and validation of a UV-Visible Spectroscopy method for Linagliptin using methanol as a solvent. The method adheres to Beer-Lambert's law and ICH guidelines, exhibiting a maximum absorbance at 297 nm. Excellent linearity (R^2 = 0.999) was observed over the tested concentration range, with precise LOD and LOQ values confirming the method's sensitivity. Additionally, the method demonstrated high precision, accuracy, and robustness, making it a reliable choice for routine pharmaceutical quality control applications.**

***Index Terms*—UV spectrophotometry, Linagliptin, Methanol, Validation, ICH, Pharmaceutical Analysis.**

I. INTRODUCTION

Linagliptin is a very effective oral hypoglycemic medication. Dipeptidyl peptidase-4 (DPP-4), which is selective and has a long half-life, is known as 8-[(3R)-3-aminopiperidin-1-yl]-1-[(4-methylquinazolin-2-yl)methyl]-3-methyl-7-(but-2-yn-1-yl)purine-2,6-dione. The chemical formula is $C_{25}H_{28}N_8O_2$, and the molecular weight is 472.54 g/mol. It works by reversibly and competitively blocking dipeptidyl peptidase (DPP)-4 enzyme that breaks down incretin hormones — glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) — that promote insulin release.

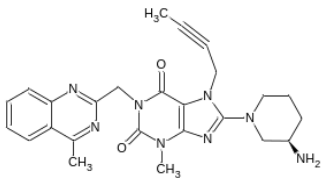

Fig. 1 ***Linagliptin***

Incretin hormones have an inhibitory effect on pancreatic alpha cells' secretion of glucagon, leading to increased pancreatic beta cell insulin synthesis. Linagliptin (BI-1356) was approved by the US FDA on May 2, 2011, for the monotherapy treatment of type 2 diabetes mellitus, or in conjunction with other medicines (metformin, etc.), in combination with changes in diet and physical activity. According to a literature review, Linagliptin was analyzed using RP-HPLC, UV spectroscopy, and degradation studies using a straightforward solvent system (distilled water). Consequently, the current study's goal was to create and validate a reliable, straightforward and precise spectrophotometric technique (UV/Visible) for the identification of linagliptin in its pure form and medicinal preparation using three different methods.

II. MATERIALS AND METHODS

2.1 Instrumentation

The study was performed using a Systronics (India) Limited UV-visible spectrophotometer, Model AU-270, featuring a double beam and double detector arrangement with a 1 cm matched cell. The software used was UV Professional Double Beam. The mobile phase was degassed using an ultrasonicator cleaner, and an electronic balance was used for weighing.

2.2 Chemicals and Reagents

MPS standard was obtained from CTX Lifesciences Pvt. Limited, batch no. 24MS000014, with a manufacturing date of February 2024 and an expiration date of 2029. Analytical-grade reagents were gifted by Ajanta Pharmaceutical PPIC department, Mumbai. Calibrated glassware from Borosil Company was used throughout the analysis.

III. METHODOLOGY

3.1 Selection of Solvent

Methanol was chosen as the solvent due to its superior solubility properties for Linagliptin.

3.2 Preparation of Standard Stock Solution

A 50 mg quantity of pure Linagliptin was accurately weighed and dissolved in methanol in a 50 ml volumetric flask to obtain a final concentration of

1000 ppm. A working standard of 100 ppm was prepared from this stock solution using distilled water.

3.3 Determination of Lambda Max

The UV spectrum of MPS was scanned between 200 and 400 nm at a concentration of 100 ppm. The maximum absorbance wavelength was observed at 297 nm.

3.4 Estimation of Linagliptin

Table No. 1: Absorbance

Concentration	Absorbance
10 ppm	0.280
20 ppm	0.543
30 ppm	0.812
40 ppm	1.083
50 ppm	1.357
Tablet 20 ppm	0.506

3.4.1 Standard Absorptivity Method

Prepare standard solutions of Linagliptin in methanol at concentrations ranging from 10 ppm to 50 ppm. Measure the absorbance of each solution at 297 nm using a UV-visible spectrophotometer. Calculate the absorptivity (a) using Beer-Lambert's law:

$$A = abc$$

Where: A = Absorbance, a = Molar Absorptivity, b = Path length (1 cm), c = Concentration in ppm.

The standard absorptivity method is based on the principle of Beer-Lambert's law, which states that absorbance is directly proportional to concentration. By determining the absorptivity coefficient (a), unknown sample concentrations can be estimated accurately. This method is widely used due to its simplicity and ability to provide reliable quantitative results. By using this formula, the percentage purity of the drug was found to be 93.70% (refer Table No. 1).

3.4.2 Single Point Standardization Method

Prepare a standard solution of Linagliptin in methanol at a known concentration (e.g., 100 ppm). Prepare the test sample solution in methanol. Measure the absorbance of both the standard and test sample at 297 nm using a UV-visible spectrophotometer. Calculate the concentration of the test sample using the equation:

$$C_t^{es}{}_t = (A_t^{es}{}_t \times C_t^{s}{}_t^{d}) / A_t^{s}{}_t^{d}$$

Where $C_t^{es}{}_t$ = concentration of sample, $C_t^{s}{}_t^{d}$ = concentration of standard, $A_t^{es}{}_t$ = absorbance of sample, $A_t^{s}{}_t^{d}$ = absorbance of standard.

The Single Point Standardization Method is a direct and simple approach for estimating the concentration of an unknown sample by comparing its absorbance with that of a standard solution of known concentration. It is particularly useful for quick and routine analysis where high precision is not required. By using this formula, the percentage purity of the drug was found to be 93.70% (refer Table No. 1).

3.4.3 Calibration Curve Method

Prepare standard solutions of Linagliptin in methanol at concentrations ranging from 10 ppm to 50 ppm. Measure the absorbance of each solution at 297 nm using a UV-visible spectrophotometer. Plot a calibration curve by graphing absorbance against concentration. Determine the equation of the calibration curve using linear regression analysis. Use the calibration curve equation to estimate the concentration of unknown samples by interpolating their absorbance values.

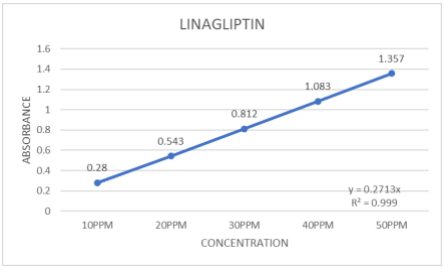

Fig. 2: Calibration Curve

The Calibration Curve Method is based on Beer-Lambert's law, where absorbance is directly proportional to concentration. By constructing a standard calibration curve, the concentration of unknown samples can be accurately determined. This method ensures high precision and reliability in quantitative analysis. By using the equation of line, the percentage purity of the drug was found to be 93.70%.

IV. METHOD VALIDATION

4.1 Linearity

The closeness of the test results obtained by the method to the true value or an accepted reference value. It is usually expressed as a percentage recovery. The regression coefficient (R^2) from the linearity curve was found to be 0.999, indicating excellent linearity.

Table No. 2: Linearity

Concentration	Absorbance	Calculations	
10	0.280	MEAN	0.815
20	0.543	SD	0.381
30	0.812	RSD	0.003617
40	1.083	%RSD	0.361685

50	1.357	Slope	0.2713
Tablet 20 ppm	0.506	Intercept	0

** Result of RSD & %RSD is Mean of Three Readings*

4.2 Precision

The degree of repeatability of an analytical method under normal operating conditions. It is divided into intraday and interday precision. Results showed minimal variation in absorbance values over different time intervals and on different days, demonstrating the method's precision.

Table No. 3: Intraday Precision

Time	Concentration	Absorbance	Calculations
0 Hrs	30 PPM	0.812	Mean SD RSD %RSD 0.810667 0.009172 0.002433 0.24326
2 Hrs	30 PPM	0.809	
4 Hrs	30 PPM	0.814	
8 Hrs	30 PPM	0.811	
12 Hrs	30 PPM	0.810	
24 Hrs	30 PPM	0.808	

Table No. 4: Interday Precision

Time	Conc.	Day 1	Day 2	Day 3
0 Hrs	30 PPM	0.808	0.810	0.814
2 Hrs	30 PPM	0.814	0.812	0.811
4 Hrs	30 PPM	0.810	0.814	0.809
8 Hrs	30 PPM	0.816	0.808	0.816
12 Hrs	30 PPM	0.812	0.806	0.818
24 Hrs	30 PPM	0.818	0.816	0.815
Mean		0.813	0.811	0.81383
SD		0.003416	0.003416	0.003023
RSD		0.004201	0.004212	0.003715
%RSD		0.420129	0.421165	0.371459

4.3 Accuracy

Accuracy is often assessed at different levels, typically 80%, 100%, and 120% of the target concentration in validation studies. This ensures that the analytical method provides reliable results across a range of concentrations. The accuracy is determined by recovery studies, where known amounts of the analyte are added to the sample, and the percentage recovery is calculated.

Table No. 5: Accuracy

Spiked Level (%)	Added Conc.	Absorbance	% Recovery
80	16 ppm	0.434 0.436 0.432	79.92634
100	20 ppm	0.543 0.541 0.545	100
120	24 ppm	0.6516 0.6518 0.6514	120

** Result shows mean of 3 readings*

4.4 Ruggedness

The reproducibility of the method under normal conditions by different analysts, instruments, or laboratories. Two analysts performed the study independently, and %RSD was found to be within acceptable limits.

Table No. 6: Ruggedness

Analyst	X	Y
System	Systronics	Shimadzu
Day	Monday	Thursday
Time	Morning	Evening
Concentration	20 PPM	20 PPM
Absorbance	0.543 0.545 0.541	0.540 0.542 0.538
Mean	0.543	0.540
SD	0.001633	0.001633
RSD	0.003007	0.003024
%RSD	0.300735	0.302406

4.5 Robustness

The ability of the method to remain unaffected by small but deliberate variations in method parameters (e.g., pH, temperature, flow rate) and still provide accurate results. The method's robustness was assessed at three wavelengths (297, 299, and 295 nm), showing minimal variation in absorbance values.

Table No. 7: Robustness

Wavelength	Conc.	Absorbance	Calc.	
297 nm	20 PPM	0.543	SD	0.007483
297 nm	20 PPM	0.449	RSD	0.01404
297 nm	20 PPM	0.452	%RSD	1.403999
299 nm	20 PPM	0.567	SD	0.004497
299 nm	20 PPM	0.568	RSD	0.00788
299 nm	20 PPM	0.577	%RSD	0.78801
295 nm	20 PPM	0.455	SD	0.006944
295 nm	20 PPM	0.464	RSD	0.014977
295 nm	20 PPM	0.472	%RSD	1.497676

4.6 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is the lowest amount of analyte that can be detected but not necessarily quantified under stated conditions. LOQ is the lowest amount of analyte that can be quantitatively determined with acceptable precision and accuracy.

$$\mathbf{LOD = 3.3 \times \sigma/S} \qquad \mathbf{LOQ = 10 \times \sigma/S}$$

Where σ = Standard deviation of the response, S = Slope of the calibration curve.

LOD and LOQ were calculated as 0.0198 µg/ml and 0.0601 µg/ml, respectively.

V. ESTIMATION OF LINAGLIPTIN FROM TABLET DOSAGE FORM

Weigh and finely grind 20 tablets. Weigh a portion equivalent to 5 mg of Linagliptin. Dissolve in 10 mL methanol (sonicate if needed). Filter the solution to remove excipients. Take 1 mL of the filtrate and dilute to 10 mL (final concentration ~100 ppm). Check the absorbance of this resulting solution at 297 nm. By using the formula, the percentage purity of drug was found to be 93.70%.

Table No. 8: Assay of Marketed Formulation

Concentration	Absorbance at 297 nm	Specifications
20 ppm	0.506	Brand Name: LINAXA
20 ppm	0.508	Label Claim: 5 mg
20 ppm	0.504	Manufacturer: TORRENT PHARMACEUTICALS
Mean	0.506	Manufacturing date: Feb 2024
SD	0.001633	Expiry Date: Feb 2025
RSD	0.003227	Batch No: 2PP6M004
%RSD	0.322726	% Purity of drug: 93.70%

VI. DISCUSSION

The developed UV spectrophotometric method for Linagliptin adheres to ICH guidelines. It demonstrates high precision, accuracy, linearity, robustness, and sensitivity. The proposed method provides an efficient and cost-effective alternative for routine pharmaceutical analysis.

VII. CONCLUSION

A simple, accurate, and economical UV-spectrophotometric method for the determination of Linagliptin was successfully developed and validated according to ICH guidelines. This method offers a reliable approach for routine pharmaceutical quality control.

VIII. CONFLICT OF INTEREST

The authors declare no conflicts of interest relevant to this article.

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