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# DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF LINAGLIPTIN IN TABLET DOSAGE FORM

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## ABSTRACT

**Objective:** To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Linagliptin in tablet dosage form. **Methods:** The drug is soluble in Analytical Grade Methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in Analytical Grade Methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Linagliptin in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in Methanol, Sparingly soluble in Ethanol and very slightly soluble in Water and Isopropanol. So, the analytical grade Methanol is used as a diluent in method. The melting point of Linagliptin was found to be 192-193˚C (uncorrected). It showed absorption maxima 295 nm in analytical grade Methanol. On the basis of absorption spectrum the working concentration was set on 15 µg/ml (PPM). The linearity was observed between 5-25 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.8, 99 and 98.6% for three levels respectively. The % RSD for precision and ruggedness was found to be 0.82% and 0.52% respectively. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Linagliptin in tablet dosage form. The method could be considered for the determination of Linagliptin in quality control laboratories.

**KEYWORDS:** Linagliptin, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

## INTRODUCTION

Linagliptin is a white to yellowish or only slightly hygroscopic solid substance. Linagliptin is a dipeptidyl peptidase 4, also DPP- 4 inhibitors developed by Boehringer Ingelheim, which can be used to treat diabetes mellitus type-2. Linagliptin was approved by the U.S. Food and Drug Administration (FDA) on 2 May 2011 for treatment of type II diabetes.[1] Linagliptin or 8 [(3R)-3-aminopiperidin-1-yl]-7- but-2-ynyl-3-methyl-1-[(4-methyl-Quinazolin-2- yl) methyl] purine-2,6-dione (fig 1.) is an antidiabetic drug acting through inhibiting dipeptidyl peptidase-4, an enzyme that degrades the incretin hormones glucagon-like peptide-1 and glucose-dependent insulinotropic hormone.[2] The empirical formula is $C_{25}H_{28}N_8O_2$ and the molecular weight is 472.5. When the produced insulin is not adequately use by the body or if pancreas is unable to produce insulin, a chronic disease occurs called diabetes. It refers to various metabolic disorders and if left untreated, characterized by hyperglycemia.[3] Diabetes may causes serious complications like vision loss, nerve damage, kidney failure, stroke and heart attack. With the increase in diabetes cases in the world, the usage of antidiabetic drugs is also increasing. Therefore, the use of Linagliptin, as an antidiabetic agent, is increasing.[4]

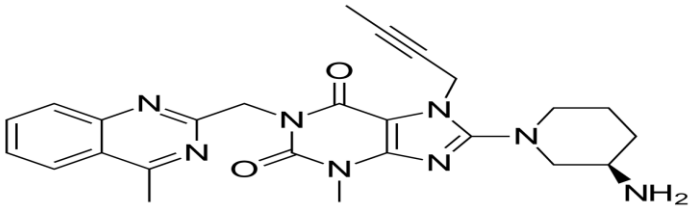

**Fig. 1: Chemical structure of linagliptin.**

From literature review it's found that few method was reported on spectrophotometry for simultaneous estimation Linagliptin,[4-6] one chemical derivatization[7] method reported for linagliptin. Also UV Ion pair complexation[8] method, Capillary electrophoresis method[9] available for linagliptin. Lot of work was done on HPLC method development for Linagliptin drugs.[10-13] But these method has lack of sensitivity and accuracy so, new rapid, sensitive, accurate and precise method was planned to developed for estimation of Linagliptin in tablet dosage form for UV spectroscopic method.

## MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair

Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance. Melting point apparatus of Equiptronics were used.

- **Materials**

Linagliptin was obtained as a gift sample from Micro Labs Pvt. Ltd. Company. Linagliptin tablets were procured from local pharmacy. Analytical Grade Methanol was used throughout the experiment as a diluent. Freshly prepared solutions were employed.

### Method development

**A. Determination of ƛ max (15 PPM)[14,15]**

50 mg weighed amount of Linagliptin was dissolved into 100 ml of volumetric flask with Methanol. Pipette out 1.5 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with Methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

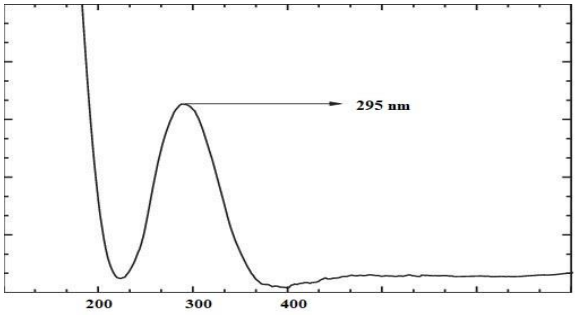


**Fig. 2: Calibration curve.**

**B. Preparation of working concentration[15]**

**Preparation of standard stock solution**

Standard stock was prepared by dissolving 50 mg of Linagliptin in 100 ml of Methanol to get concentration of 500 µg/ml (PPM).

**Preparation of standard solution**

Pipette out 1.5 ml from standard stock solution and diluted up to 50 ml with Methanol to get concentration of 15 µg/ml (PPM).

**C. Procedure for UV reading**

**Blank solution:** (For Auto zero)

Fill the cuvette with Methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**Standard solution**

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**Sample solution**

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

**D. Procedure for sample preparations[14,15]**

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Linagliptin was accurately weighed and transferred into the 100 ml of volumetric flask, added 60 ml Methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with Methanol. Filtered the solution through whatmann filter paper. Pipette out 1.5 ml of the above

solution and diluted up to 50 ml with Methanol. The absorbance was measured at 295 nm. The absorbance was recorded:

**Table 1: Absorbance of dosage form.**

| Xenon pharmaceutical limited linasmart® 5 (5 mg) | | |
|---|---|---|
| **Sr. no.** | **Sample** | **Absorbance** |
| 1 | Blank | 0.0000 |
| 2 | Standard | 0.6527 |
| 3 | Sample | 0.6414 |

**Table 2: Dosage form specifications.**

| Type | Company | M.D. | E.D. | Batch No. | Average weight (g) | Assay (%) |
|---|---|---|---|---|---|---|
| 1 | Xenon Pharma LTD | 03/2024 | 04/2026 | MT1524D | 0.0905 | 98.27 |

**E. Method of validation**[14,16-19]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

**Linearity**

The linearity of the proposed assay was studied in the concentration range 5 - 25 PPM at 295nm. The calibration data showed a linear relationship between concentrations.

**Table 3: Linearity studies.**

| Sr. no. | Sample concentration | Absorbance |
|---|---|---|
| 1 | 5 PPM | 0.2258 |
| 2 | 10 PPM | 0.4282 |
| 3 | 15 PPM | 0.6441 |
| 4 | 20 PPM | 0.8588 |
| 5 | 25 PPM | 1.0945 |
| **Correlation coefficient** | | **0.9993 ~ 0.999** |

**Accuracy**

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 50ml of volumetric flask with Analytical Grade Methanol and measuring the absorbance at 295nm.

**Table 4: Accuracy studies.**

| Spectrophotometric method | | | |
|---|---|---|---|
| **Accuracy (%)** | **Qty weighed (mg)** | **Qty found (mg)** | **Recovery (98-102%)** |
| 80 | 0.8 | 0.79 | 98.75 ~ 98.8 |
| 100 | 1 | 0.99 | 99.0 |
| 120 | 1.2 | 1.18 | 98.55 ~ 98.6 |

**Precision**

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the % RSD was calculated.

**Table 5: Precision studies.**

| Sr. No. | Sample Solution | Absorbance |
|---|---|---|
| 1 | Sample Solution 1 | 0.6541 |
| 2 | Sample Solution 2 | 0.6451 |
| 3 | Sample Solution 3 | 0.6440 |
| 4 | Sample Solution 4 | 0.6401 |
| 5 | Sample Solution 5 | 0.6427 |
| MEAN | | 0.6452 |
| SD | | 0.0053 |
| % RSD | | 0.8235 ~ 0.82 |

**Ruggedness**

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

**Table 6: Results for ruggedness studies.**

| Sr. No. | Analyst | Results | Mean | % Assay | % RSD |
|---|---|---|---|---|---|
| 1 | Analyst 1 | 0.6414 | 0.6454 | 98.88 | 0.5223 ~ 0.52 |
| | | 0.6494 | | | |
| 2 | Analyst 2 | 0.6385 | 0.6428 | 98.15 | |
| | | 0.7484 | | | |

## RESULTS

### 1. Solubility of linagliptin

Solubility test was passed as per criteria.

**Table 7: Results for solubility studies.**

| Sr. no. | Title | Result |
|---|---|---|
| 1 | Methanol | Freely Soluble |
| 2 | Ethanol | Sparingly Soluble |
| 3 | Water, Isopropanol | Very slightly soluble |

### 2. Melting point of linagliptin

The melting point of Linagliptin was found to be 192-193˚C (uncorrected).

### 3. Results for linearity for assay method of Linagliptin [Conc Vs Absorbance]

The linearity of method was determined at concentration level ranging from 5 to 25 µg/ml (PPM). The correlation coefficient value was found to be **($R^2$) 0.9993 ~ 0.999**

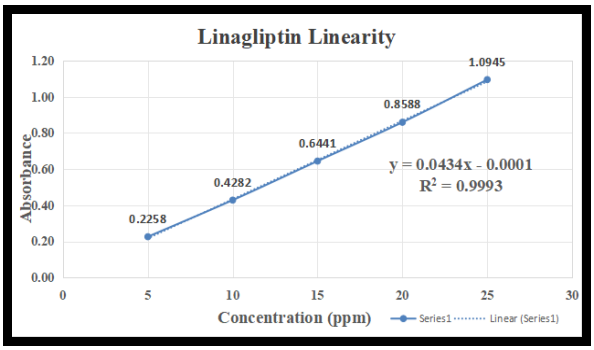


**Fig. 3: Linagliptin standard curve.**

### 4. Results for accuracy for assay method of linagliptin

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

### 5. Results for precision for assay method of linagliptin

The % RSD for different sample of precision was found to be 0.82 and it is within acceptance criteria represented in Table - 5.

### 6. Results for ruggedness for assay method of linagliptin

The % RSD for different sample of ruggedness was found to be 0.52 and it is within acceptance criteria represented in Table - 6.

## CONCLUSION

A method for the estimation of Linagliptin in tablet form has been developed. From the spectrum of Linagliptin, it was found that the maximum absorbance was 295 nm in Analytical Grade Methanol. A good linear relationship was observed in the concentration range of 5-25 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Linagliptin in solid dosage forms. Hence, the method could be considered for the determination of Linagliptin in quality control laboratories.

**ABBREVARTIONS**

1. PPM - Parts per Million
2. nm - Nanometer
3. DNA - Deoxyribonucleic Acid
4. LCMS - Liquid Chromatography and Mass Spectroscopy
5. HPLC - High Performance Liquid Chromatography
6. UV - Ultra violet
7. FDA - Food and Drug Administration
8. DPP - Dipeptidyl Peptidase
9. ICH - International Council for Harmonization
10. RSD - Relative Standard Deviation
11. SD - Standard Deviation
12. Qty - Quantity
13. C - Celsius
14. M.D. - Manufacturing Date
15. E.D. - Expiry Date

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