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METHOD DEVELOPMENT AND VALIDATION OF LINAGLIPTIN IN PURE FORM AND SOLID DOSAGE FORM BY USING UV SPECTROPHOTOMETRY

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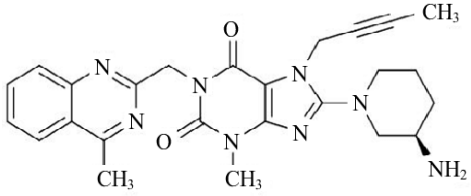
ABSTRACT

A simple, sensitive, precise, rapid and economical spectrophotometric method has been developed for quantitative analysis of Linagliptin (LIN) in Pharmaceutical formulations. Methanol was used as a solvent for development of method. The stock solution of LIN was prepared by dissolving drug in methanol. Subsequent dilution was made in methanol. The standard solution of LIN has shown absorption maxima at 243nm.The drug obeyed Beer-Lambert's law in the concentration range of 1-50 µg/mL with Regression coefficient 0.9980 at 243nm. The overall %recovery was found to be 100.5 -101.8%. %RSD from six measurements was found to be less than 2%. The analysis results were validated as per ICH guidelines.

INTRODUCTION

Linagliptin chemically is 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl) methyl]-2, 3, 6, 7-tetrahydro-1 H-purine-2,6-dione (Fig. 1) is an inhibitor of DPP-4 used for the treatment of type II diabetes. Molecular formula and molecular weight are $C_{25}H_{28}N_8O_2$ and 472.5 respectively. DPP-4 is an enzyme that degrades the incretion hormones glucagon-like Peptide (GLP-1) and glucose-dependent insulin tropic polypeptide (GLP) [1-3]. Both GLP-1 and GLP-1 increase insulin biosynthesis and secretion from pancreatic beta cells in the presence of normal and elevated blood glucose levels. GLP-1 also reduces glucagon secretion from pancreatic alpha cells, leads to reduction in hepatic glucose output [4]. Linagliptin is a hygroscopic solid substance which is available as tablet dosage form in the market. Literature survey reveals that there are very few methods based on UV [5-7] and HPLC [8-11], in bulk and dosage forms.



Linagliptin

Fig.1: Structure of Linagliptin

Since there are no more methods by UV present work has carried on the development and validation of UV Spectrophotometry which quantify the Linagliptin. The main objective of this method is to develop a simple, accurate, and precise spectrophotometric method for the estimation of Linagliptinin in bulk and pharmaceutical dosage form. International Conference on Harmonization (ICH) guidelines [12-13] was followed to validate this method.

MATERIALS AND METHODS

Materials

Bulk form of linagliptin was obtained from spectrum labs as a gift sample. Methanol obtained from Thermo Fischer Scientific Pvt Ltd was used as diluent. Commercial form of linagliptin tablets with brand name Trajenta containing 5mg of LIN was purchased from local market. Class A grade glassware was used throughout the experiment.

Instruments

Absorption spectral measurements were performed in LABINDIA (T60) double beam UV/Visible Spectrophotometer by using 1cm quartz matched cuvettes. Weighing was carried out in ELITE analytical balance.

Preparation of standard stock solution

Stock solution of 1000 µg/mL was prepared by dissolving 100 mg of LIN in methanol in a clean, dry 100 mL volumetric flask. The solution was made up to the mark with the same solvent.

From the above stock solution, 100 µg/mL working standard was prepared. It was done by transferring 10mL of standard stock into 100 mL volumetric flask and was made up to mark with methanol.

Selection of λmax

The prepared stock solution was scanned in UV Spectrophotometer between the range of 400- 200nm using methanol as blank. The maximum absorbance was found at 243nm. The spectrum scan was depicted in figure 2.

Assay: Commercial tablets of LIN 5mg per tablet was assayed by weighing and powdering 20 tablets accurately. Powder equivalent to 100mg was calculated and weighed, required amount of drug powder was transferred into dry 100mL volumetric flask which contains 50mL of methanol and kept for sonication for 10 minutes. The solution was then filtered through Whatman filter paper and made up to the mark with methanol. From this, 10mL of solution was taken into another dry 100mL volumetric flask and made up to the mark by using same solvent to obtain the concentration of 100 µg/mL solution. Sample solution of 25µg/mL was prepared by taking 2.5mL from 100 µg/mL solution and made up to the mark in 10mL volumetric flask.

METHOD VALIDATION

After method development, the method was validated for some parameters as per ICH guidelines. Parameters are Linearity, Sensitivity, Accuracy, Precision, Range, Robustness, and Ruggedness

Linearity

Linearity can be determined by taking series of dilutions of standard solutions which are used as working standards. So, working range was observed as 1-50µg/mL at 243nm. Regression data was given in Table 2 and Figure 3.

Sensitivity

Sensitivity was obtained by performing Limit of Detection (LOD) and Limit of Quantification (LOQ) calculations as per the equations given in ICH guidelines.

Limit of Detection: The lowest amount of drug present in the sample that can be detected, but not necessarily quantified is called limit of detection.

$$LOD = \frac{3.3 \times \sigma}{S}$$ Where, S = Standard deviation

Limit of Quantification: An amount of analyte that can be quantified with specified limit of accuracy and precision is called limit of quantification.

$$LOQ = \frac{10 \times \sigma}{S}$$

LOD and LOQ were calculated by placing methanol for absorbance check. The results were given in Table 3.

Accuracy

The accuracy is the degree of closeness of the test results obtained by the method to the true value. Accuracy was surveyed by using at least 9 determinations covering at least 3 focus levels of concentrations. The absorbances were measured at 243nm using blank (methanol) and the recovery from the formulation was calculated and the obtained results were given in Table 4.

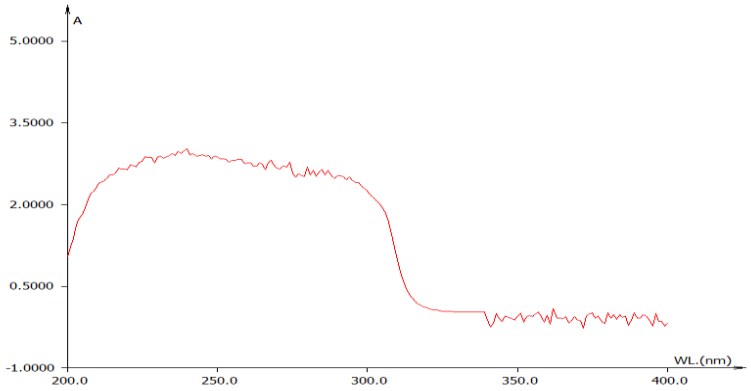


Fig. 2: λ_{max} of Linagliptin

Table 1: Assay of Linagliptin tablets

Formulation Name	Brand	Wavelength (nm)	Amount of drug taken from tablet (mg)	%Purity
Linagliptin Tablets	Trajenta 5mg	243	100	100.6%

Table 2: Results of Linearity

S. No.	Parameters	Results
1.	Absorption maxima (nm)	243
2.	Beer's law limit (µg/mL)	1- 50
3.	Correlation coefficient	0.9980
4.	Regression equation (y = mx+c)	y = 0.017+0.008
5.	Slope (m)	0.017
6.	Intercept	0.008

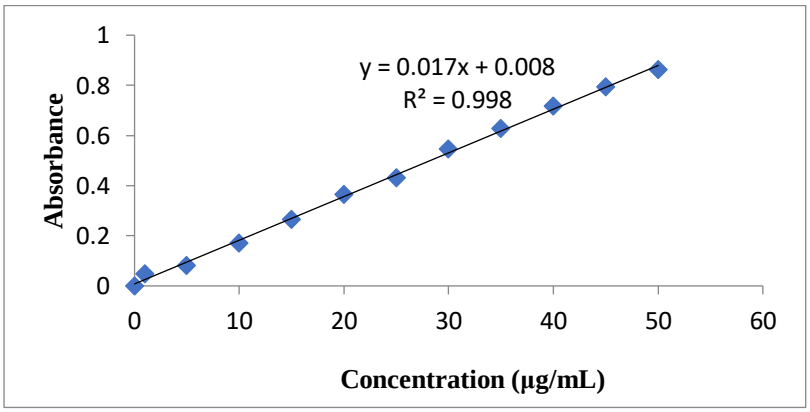


Fig. 3: Calibration curve of Linagliptin

Table 3: Results of Sensitivity

Limit of Detection	Limit of Quantification
0.0524 µg/mL	0.158 µg/mL

Table 4: Results of accuracy

Level of addition	Amount added	Amount found	%Recovery	%Mean Recovery
80%	20	20.36	101.8	
100%	25	25.14	101.06	100.5±0.45%
120%	30	30.27	100.9	

Table 5: Results of Repeatability

Concentration (µg/mL)	Absorbance	Statistical analysis
25	0.4703	
25	0.4819	
25	0.4764	Mean = 0.4769
25	0.4802	%RSD = 0.85%
25	0.4746	

Table 6: Results of Ruggedness

Concentration (µg/mL)	Analyst 1		Analyst 2	
	Absorbance	Statistical analysis	Absorbance	Statistical analysis
25	0.4703		0.4651	
25	0.4819		0.4648	
25	0.4764	Mean: 0.4769	0.4745	Mean: 0.4736
25	0.4802	%RSD: 0.85%	0.4739	%RSD: 1.58%
25	0.4746		0.4809	
25	0.4780		0.4828	

Table 7: Validation parameters of Linagliptin

Parameters	Results
Absorption maxima (nm)	243nm
Linearity range (µg/mL)	1-50
Regression Equation	y= 0.017x - 0.008
Correlation coefficient (R^2)	0.9980
Molar extinction coefficient	8.878×10^{-3}
LOD (µg/mL)	0.0524
LOQ (µg/mL)	0.158
Accuracy (%Recovery ± SD)	101.0% ± 0.45
Precision (%RSD)	LT 2%
Sandell's sensitivity (µg/cm^2/0.001 absorbance units)	5.322×10^{-3}

Precision

The precision expresses the degree of scatter between the series of results obtained from multiple sampling of homogenous sample. For the method, precision was determined by repeatability including Intra-day and Inter-day precision.

Repeatability

Repeatability is the measurement of exactness under the same conditions. By analyzing five samples of same concentration of drug, repeatability was determined and absorbances were recorded. The results of the method were given in Table 5.

Intra-day and Inter-day precision

Analysis of three different concentrations and each for three times, on the same day determines intraday precision and analysis of the same three different concentrations that carried out daily, for three consecutive days determines inter-day precision. The %RSD was found to be less than 2%.

Robustness

Robustness is the capacity of a sample to remain unchanged by small after changing the conditions. The robustness of the method was determined by altering the experimental conditions deliberately and assay was performed in the same conditions. Wavelength effect was observed at two different wavelengths like 242 and 244 which is ±1nm to actual wavelength (243nm). Assay of LIN at all the altered conditions was within 98-102%. There was no much effect on the system suitability parameters.

Ruggedness

Ruggedness is a measurement of reproducibility of the sample at different conditions like laboratories, analysts, instruments, reagents etc. By using two different analysts, the sample was analyzed and absorbances were recorded. The results were given in Table 6.

RESULTS AND DISCUSSION

Spectrum scan was performed to the standard solution over a range of 200-400nm to decide the detection wavelength. The maximum absorbance (λ_{max}) of LIN was found to be 243nm. Excepients in the tablet solution did not interfere with the absorbance of standard solution of LIN at 243nm. Hence, quantitative analysis and validation were performed at this wavelength. The Beer-Lambert's law was obeyed by this drug in the linearity range of 1-50µg/mL, Regression equation was found to be y = 0.017x-0.008 with slope and intercept as 0.017 and 0.008 respectively. The regression coefficient (R^2) was found to be 0.9980. Relative standard deviations from the measurements were found to be always less than 2%. By observing %RSD values of intraday and inter-day precision which is less than 2%, the developed method was found to be precise. The method was also found to be accurate where recoveries were ranging from 98 – 102%. LOD & LOQ were found to be 0.0524µg/mL and 0.158µg/mL respectively. It indicates that the method is sensitive. Robustness and ruggedness were performed by analyzing the sample at different wavelengths and by different analysts respectively. As the % RSD of robustness and ruggedness were found to be less than 2%, the method was found to be robust and rugged. Molar Extinction Coefficient and Sandell's Sensitivity were calculated. Validated parameters were given in Table 7.

CONCLUSION

The method development in UV spectrophotometer for the determination of Linagliptin has the advantage of being simple, economical and applicable to various concentration ranges with high precision and accuracy. This method was validated as per the ICH guidelines. The validation results were found to be satisfactory. Hence, this method can be successfully applied to analyze solid dosage forms.

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REFERENCES

1. Four phase III trials confirm benefits of B's Oral, Once-daily type 2 Diabetes therapy, Genetic Engineering & Biotechnology news, 2010.

2. Home, Philip D, *Diabetes care*, 2013, 36 (4): 3361-3367.
3. Adooq Bioscience LLC, Product data sheet on Linagliptin, 2012.
4. Tripathi K.D, Essential of Medical Pharmacology, 2013: 515-516
5. Chandra K Sekhar, A new UV method for the determination of linagliptin in bulk and pharmaceutical dosage forms, *International Journal of Universal Pharmacy and Biosciences*, 2014: 1-6.
6. Sujan Bank, Palash Karmakar and Md. Anowar Hossan Maih Development and validation of a UV-spectrophotometric method for determination of vildagliptin and linagliptin in bulk and pharmaceutical dosage form. *Bangladesh Pharmaceutical Journal*, 2015; 18(2): 163-168.
7. Lakshmi B, Reddy, A Novel RP-HPLC method for the quantification of linagliptinin formulations. *Journal of Atoms and Molecules*, 2012: 155-164.
8. Lakshman Rajubudugu. A validated RP-HPLC method for the determination of linagliptin, *American Journal of Phamatech*, 2013: 436-469.
9. Janardhan Swamy. Analytical Method Development and Method Validation for the simultaneous estimation of Metformin Hydrochloride and Linagliptinin Bulk and tablet Dosage form by RP-HPLC method, *International Journal of Pharmacy*, 2013: 594-600.
10. Ramzai. El-Bagaryl, Ehab F. Elkadyl, Bassam M. Ayoub Spectrophotometric Methods for the Determination of Linagliptin and Metformin Hydrochloride and Simultaneous Determination of Linagliptin and Metformin Hydrochloride using High Performance Liquid Chromatography, *International Journal of Biomedical Sciences*, 2013: 41-51.
11. Michael E., Schartz S., Krull, "Analytical Method Development and Validation", 2004: 25-46.
12. ICH Harmonized Tripartite Guideline, CH Q2B, Validation of analytical procedures: methodology, 1997.