World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

DEVELOPMENT OF ANALYTICAL METHOD FOR THE DETERMINATION OF LINAGLIPTIN IN BULK DRUG AND ITS PHARMACEUTICAL FORMULATION

Dr. R. Xavier Arulappa[1*], M. Jerubin Welsingh[2], Athira S. S.[1], Ameena N. S.[1], Amina S.[1], Layana Valsan[1], Roshinee R and [2]Dr. G. R. Prasobh

[1*]Professor and HOD, Department of Pharmaceutical Chemistry, Sree Krishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram, Kerala.
[1]Department of Pharmaceutical Chemistry, Sree Krishna College of Pharmacy and Research Centre, Parassala, Thiruvanthapuram.
[2]Department of Pharmaceutical Chemistry, S.A.Raja Pharmacy College, Tirunelveli, Tamil Nadu.

***Corresponding Author: Dr. R. Xavier Arulappa**
Professor and HOD, Department of Pharmaceutical Chemistry, Sree Krishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram, Kerala.

Article Received on 19/05/2024 **Article Revised on 09/06/2024** **Article Accepted on 30/06/2024**

ABSTRACT
Analytical method development and validation is important in development of pharmaceutical preparations. The aim of the present work was to develop and validate a simple UV spectroscopic method for the determination of Linagliptin in pharmaceutical dosage form. The UV spectrum of Linagliptin in ethanol showed λ max at 290 nm. Beer's law obeyed in the concentration of 10-50mcg/ml. This method was carried out according to ICH Q2R1 guidelines by taking the parameters for linearity, accuracy, precision, ruggedness and robustness. The method was rugged and robustness with % relative standard deviation less than 2. The extraction recovery was found to be higher than 98% in all experimental conditions. Based upon the performance characteristics, the proposed method was found accurate, precise and rapid and suitable for the determination of Linagliptin for routine analysis.

KEYWORDS: Linagliptin, UV spectrophotometry, Method development, Validation.

INTRODUCTION
Linagliptin[1] is 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(Figure no.1). It's molecular formula is $C_{25}H_{28}N_8O_2$. Linagliptin is a Dipeptidyl peptidase-4 (DPP-4) inhibitor, an oral antidiabetic drug used to treat Type 2 diabetes mellitus. Ithave a moderate blood sugar lowering action. These drugs are developed as an indirectly acting secretagogues, inhibit the action of DPP-4 enzyme in the degradation of incertin hormone GLP-1 and GIP. Thus the insulin secretion is increased. The usual dosage is 5mg once daily with or without food. It is a white to yellow powder soluble in organic solvents like ethanol, DMSO, DMFA and very slightly soluble in water.[2-3]

For the estimation of linagliptin in raw materials and in pharmaceutical dosage forms, no official methods have been mentioned in U.S.P. The review of literature[4-7] provides the information that only few HPTLC and HPLC methods have been reported for the estimation of Linagliptin.

The rationale of this work is to develop a simple, accurate, rapid, precise, reproducible and cost effective spectrophotometer method for the direct quantitative estimationof the linagliptin. Here we developed a method for the determination of linagliptin in bulk drug sample and tablet dosage form and validation as per International Conference of Harmonization (ICH) Guidelines.[8]

EXPERIMENTAL MATERIALS AND METHODS
INSTRUMENTS
Spectral runs were made on a Systronics UV-spectrophotometer 2202TSVisible double beam was employed with spectral band width of 2 nm and wave length accuracy ±4 nm with automatic wavelength corrections with a pair of 10 mm quartz cell. Wenser Analytical single pan balance was used .Glassware used in each procedure were soaked overnight in a mixture of chromic acid and conc. sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven.

CHEMICAL STRUCTURE OF LINAGLIPTIN

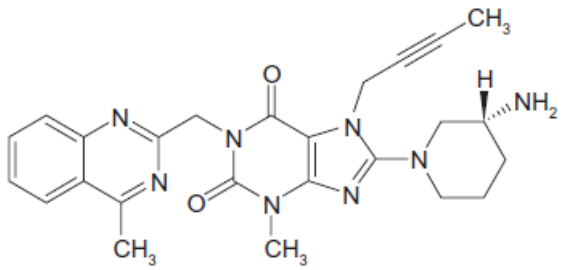

Fig.No.1.

LOCATION OFλ max

The working standard solution was scanned in UV range 200-400nm in 1cm quartz cell against solvent blank. The UV spectra of the drug show the spectrum wavelength selected for the estimation of drug was 290nm as λ max. At 290nm Linagliptin shows maximum absorbance (fig. no. 2).

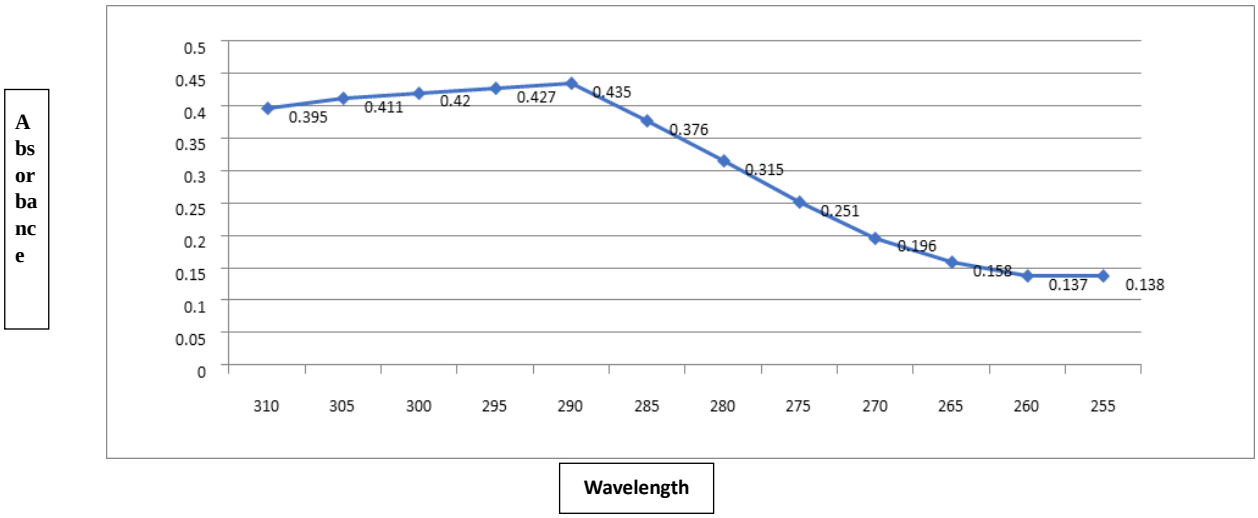

Fig No:2.

MATERIAL

Linagliptin was kindly gifted from Sahana Pharmaceuticals, Nagercoil, Tamil Nadu, India. The commercially available tablet were obtained from market. Ethanol AR grade was used as solvent obtained from Shiv scientific industries and deionized water was used.

PREPARATION OF STANDARD SOLUTION

25mg of pure Linagliptin was weighed and transferred into 25ml standard flask. To this, add 10 ml ethanol to dissolve the drug and then makeup to 25ml with deionized water [Standard stock solution – I (1 mg/ml)]. 5ml of standard stock solution was pipetted into 50ml volumetric flask and it was diluted to 50ml with diluent [Solution II (100µg/ml)].

SELECTION OF WAVELENGTH FOR ANALYSIS OF LINAGLIPTIN

Accurately measured, 2ml of standard stock II solution was transferred into 10ml volumetric flask and diluted to 10ml of given concentration (20µg/ml). And it was used for initial spectral scan in UV range of 255-310nm to detect maximum wavelength and further dilutions for linearity were prepared from the stock solution.

METHODVALIDATION

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, sensitivity, and specificity according to ICH Q2(R1) Guideline and USP guidelines.

LINEARITY AND RANGE

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration of an analyte in the sample. The range of an analytical procedure to the interval between the upper and lower concentration of an analytein the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. The linearity of the analytical method was demonstrated over the concentration range investigated by triplicate analysis (n=3) at a concentration range of 10-50µg/ml. The absorbance obtained at respective concentration was recorded and the graph is plotted as concentration (µg/ml) versus absorbance. The linear regression equation and the coefficient correlation were obtained from the UV probe software. Fig.No.3.

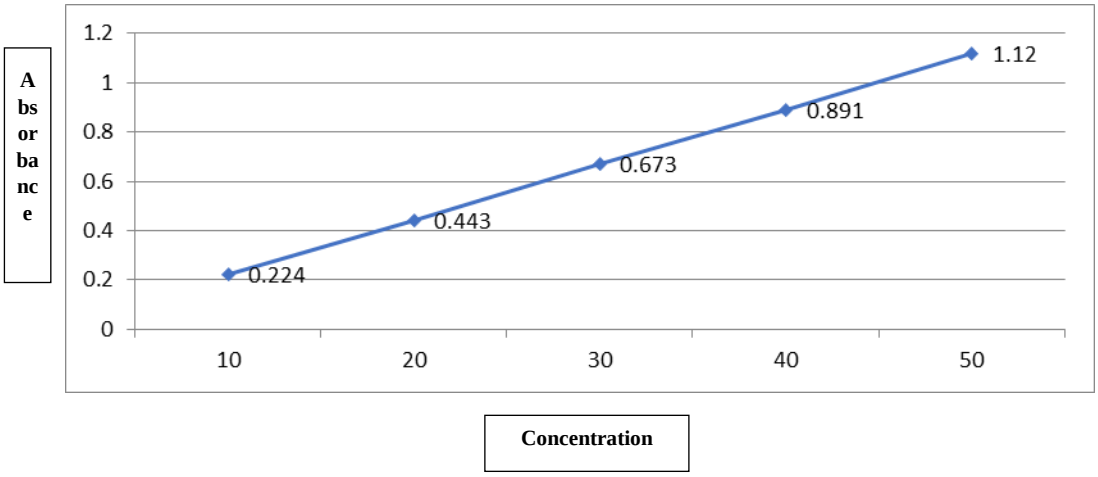

Fig No.: 3

ACCURACY[9-12]

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is termed as trueness. The accuracy of proposed method was determined on the basis of recovery study. Recovery study was carried out by spiking standard working solution to sample solution (formulation). The final concentration of Linagliptin was determined at each level of the amount, three determinations were performed. The percentage recovery was calculated as mean ± standard deviation.

PRECISION

The precision of an analytical method is the degree of reproducibility among individual test results when the procedure was applied repeatedly to multiple sampling of homogenous sample. The precision of an analytical method is usually expressed as standard deviation.

LIMIT OF DETECTION (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value.

The limit of detection (LOD) was determined bypreparing solution of different concentration from 10-20 mcg/ml

LOD=3.3 x SD/S

Where SD – Standard deviation;S –slope

LIMIT OF QUANTIFICATION (LOQ)

The detection limit is the lowest amount of analyte in a sample which can be detected but not quantitates. The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve.

LOD = 10 SD/S

Where SD = standard deviation; S = slope

SENSITIVITY

The sensitivity of the method was determined by calculating the different parameter like molar absorptivity and Sandell's sensitivity.

ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity remains unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the proposed method the solution 20µg/ml of standard Linagliptinsolution was prepared and analysed by a change in wavelength. The wavelength was selected λ max ± 1 (i.e.) 289-291nm respectively for the standard linagliptin solution.

RUGGEDNESS

The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days.

To establish ruggedness of the proposed method, the solution of 20 µg/ml of standard linagliptinsolution was prepared and analysed with the change in the different analyst.

RESULTS AND DISCUSSION

The proposed method for determination of linagliptin showed molar $mole^{-1}$ cm^{-1}. From the calibration curve it was found that it shows linearity in the range 10-50mcg/ml with regression coefficient 1.875. Linear regression of absorbance on concentration gave the equation Y = with a correlation coefficient (r) of. The detection wavelength showing λ max (maximum wavelength) at 290nm.

ACCURACY

The percentage recovery and % RSD were calculated.The mean percentage recovery and % RSD where found to be within limits and it is less than 2, which explains the present research paper is accurate in method development of linagliptin. The mean, standard deviation and percentage relative standard deviation

(%RSD) were calculated. The results were shown in table.

PRECISION
Repeatability of the method was studied by precision experiment. The %RSD of linagliptin was found to be 1.098.

APPLICATION OF THE PROPOSED METHOD
The proposed method was successfully developed and validated for the estimation of linagliptinin pharmaceutical formulations. The proposed method was compared with the reference method.

STANDARD METHOD
20 tablets were discarded from the strips and each tablet was weighed accurately. The average weight was determined and was found to be 180 mg they are crushed into the powder form. Then, weigh accurately 350 mg of tablet powder sample (equivalent to 10 mg API) into a clean and dry 10 ml volumetric flask. Diluted with 10 ml of methanol and the flask was kept in ultrasonic bath for shaking. Filter the solution through Whattmann filter paper. After filtration, an aliquot of 0.4ml of the solution was transferred into a clean 10ml volumetric flask. The volume was made upto the mark with methanol. Absorbance of this solution was recorded at 297 nm.

The proposed method obeyed Beer's law in the concentration range 2-10 µg/ml with correlation coefficient value of 0.9998. The accuracy of method was accessed by recovery studies and found to be within the range, 98 to 102 %.

STUDY OF FORMULATIONS
Accurately measured standard stock solution was diluted upto 10ml with diluent to get the concentration range 10 to 50µg/ml. The absorbance of each of the solution was measured at 290nm against blank (diluent), a calibration curve was found to be linear.

QUANTIFICATION OF FORMULATIONS
20 tablets of linagliptin were taken for the analysis. The average weight of tablet was calculated and the tablet was powdered in a glass mortar. Tablet powder equivalent to 25 mg was accurately weighed was dissolved in diluent. It was filtered and the residue was washed with diluent and then the volume was made up to 25 ml with diluent (solution-1) 5ml of solution-I was pipetted into 50ml volumetric flask and the volume was made up to 50ml with diluent(solution-II). From this 2ml was pipetted into 20ml volumetric flask followed by the addition of ethanol to produce final drug concentration of 20µg/ml. The absorbance of solution was measured at 290nm against blank. The same procedure was repeated five times. In similar manner standard absorbance was measured with pure drug in same final concentration that of assay method. The readings were recorded in the table no:1

VALIDATION OF SPECTROPHOTOMETRIC ACCURACY PROPOSED UV- METHOD
Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method.

STANDARD SOLUTION
Standard solution was prepared as per described in standard dilution.

SAMPLE SOLUTION
In order to justify the reliability and suitability of the proposed method of the recovery studies were carried out. The recovery experiment was performed on the tablet of linagliptin. The powder equivalent to 25mg was weighed accurately and dissolved in diluent. It was filtered and the residue was washed with diluent an aliquot of 5ml of standard solution (1mg/ml) of pure sample of linagliptin was added to the flask. It was shaken well and the volume was made up to 25 ml with diluent and the procedure for the assay of the tablet was followed. The experiment was repeated 5 times. The results were shown in table no:2. The percentage of recovery was calculated by using the formula,

$$\textbf{\% Recovery}=\frac{A}{B+C}\times 100$$

Where,
A=Total drug estimated (mg)
B=Weight (mg) of drug contributed by tabletPowder
C=Amount of pure drug added (mg)

Table 1: Result of Analysis of Tablet Linagliptin

SL NO.	Brand Name	Avg. Wt. of tablet (mg)	Wt. of std drug (mg)	Std. abs	Wt. of tablet powder (mg)	Test abs	Content drug in tablet (mg)	Avg. content (mg)
1	SANLING-5	176	25	0.443	881 882 883 884 885	0.438 0.439 0.440 0.441 0.442	4.934 4.938 4.948 4.952 4.956	4.945

± SD=0.0093

Table 2: Result of Recovery Study

Sl. No.	Brand Name	Wt. of std. drug (mg)	Std. abs	Avg. wt. of tablet powder (mg)	Wt. of tablet powder (mg)	Amt. of pure drug added	Absorbance of recovery sample	% of recovery
1	SANLING-5	25	0.443	176	881	5	0.475	102
2					882	5	0.480	
3					883	5	0.485	
4					884	5	0.490	
5					885	5	0.495	

Table 3: Assay of Reference method.

Sl. No.	Weight of tablet powder	%label claim	Mean	S.D	%RSD
1	360.4	98.87	0.1562	0.0017	1.098
2	360.5	97.71			
3	360.6	100.7			

Table 4: Optical Characteristics, Data, Precision and Accuracy of the proposed method for Linagliptin.

Parameter	Meter
λ Max	290nm
Beer's law limits (µg/ml)	10-50 µg/ml
Molar Absorptivity (Lit $mole^{-1}$ cm^{-1})	1.71×10^{4} $mole^{-1}cm^{-3}$
Sandell's sensitivity (µgkg^{2}/0.001 abs unit)	0.0379
Regression equation (y=a+bc)	0.1872
Slope (b)	0.1548
Correlation coefficient	0.8462
% Relative Standard Deviation	1.098%

*Average of five determinations.

CONCLUSION

The proposed UV-Visible Spectroscopy is a simple, low-cost method can be easily be applied to linagliptin control sample analysis in bulk and pharmaceutical formulations. It has a more comprehensive dynamic range for the study with excellent accuracy and precision value. The proposed method does not require any laborious clean up procedure before analysis and simple methodology for its estimation. Therefore, it can easily accommodate in the laboratories of research, and pharmaceutical industries for the quantification of Linagliptin in pure and pharmaceutical dosage forms.

ACKNOWLEDGEMENT

A moment of remembrance to the eternal repose of the soul of our founder Chairman, **Dr. K MONIKANTAN NAIR.** My heartfelt thanks to our Managing Trustee **Smt.P.GEETHA MONIKANTAN** and our Chairman **Dr.GOUTHAMKRISHNA M**. for providing laboratory facilities and for instructive suggestions.

The authors would like to express gratitude to **Dr. M.G. ETHAYARAJAN M.B.B.S. M. Diab., M.D., Ph.D., (cardio) Managing director and Mr. T. RAMESH, B.Pharm., General Manager, Sahana Pharmaceuticals**, Nagercoil, Kanyakumari, Tamil Nadu for providing gift sample of Linagliptin.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCE

1. FDA Approved Drug Products: Linagliptin oral tablets. (http://www.accessdata.fda.gov/drugsatfda_docs/label/2022/201280s025lbl.pdf)
2. Tripathi KD. Essentials of Medical Pharmacology, New Delhi: Jaypee Brothers Medical Publishers, 2018; 8: 297-298.
3. CIMS Medica, CIMS Medica India Private Limited, 2024; 46: 164-165.
4. ReshmaDhakate, Development and Validation of UV Spectroscopy for the determination of Linagliptin in bulk and marketed dosage form, International Journal of Pharmaceutical sciences, 2024; 2(2): 584-589.
5. Anchal Shukla, simultaneous estimation of Dapagliflozin and Linagliptin using reverse phase – HPLC, 2024; 2(2): 111-118.
6. J.N. Suresh Kumar, Quantitative analysis of Linagliptin in its pharmaceutical dosage form by using UV Spectroscopy and RP-HPLC techniques, World Journal of Pharmaceutical research, 2022; 11(11): 597-610.
7. Anvesha V., Validated stability Indicating and Assay method development of Linagliptin in Formulation

by RP-HPLC using Quality by design, The Electronic Journal of Chemistry, 2022; 12(2): 48-61.
8. Kenneth A Connors. Textbook of Pharmaceutical Analysis, Whiley Publisher, 1999; 3: 585-591.
9. Donald J Pietrzyk, Clyde and W Frank. Analytical Chemistry, Academic press Inc, 1974; 2: 51-53.
10. G. V. Mante, A. T. Hemke and M. J. Umekar, International Journal of Chen Tech Research, 2018; 11(1): 242-248.
11. Thyagarajan Deepan, Magharla Dasaratha Dhanaraju; Current Issues in Pharmacy and Medical sciences, 2018; 31(1): 39-43.
12. Mitali V. Verma, Chirag J. Patel, M.M Patel; International Journal of Applied Pharmaceutics, 2017; 9(5): 33-41.