IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
August 2020 Vol.:19, Issue:1

Development and Validation of Novel Stability-Indicating RP-HPLC Method for Determination of Linagliptin

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
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Submission: 24 July 2020
Accepted: 30 July 2020
Published: 30 August 2020

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: HPLC, Linagliptin, Forced degradation, Tablet dosage form

ABSTRACT

A new simple, accurate, precise and selective stability-indicating high performance liquid chromatographic (HPLC) method has been developed and validated for estimation of Linagliptin in tablet dosage form. An isocratic, reverse phase HPLC method was developed on Jasco HPLC system equipped with Grace C_{18} column (150x4.6 mm i.d.) using Methanol: Water (Triethylene amine 1 ml) in ratio 80: 20, v/v as mobile phase and detection was carried out at 294 nm. The retention time for Linagliptin was 2.70 ± 0.02 min. The drug was subjected to stress condition of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. Results were linear in the range of 5-30 µg mL^{-1}. The developed method has been successfully applied for the estimation of drug in tablet dosage form.

INTRODUCTION

Linagliptin, chemically, 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-ynyl)-3-methyl-1-[(4-methylquinazolin-2-yl) methyl] purine-2, 6-dione is an inhibitor of dipeptidylpeptidase-4 (DPP-4) enzyme which degrades the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) and used to lower the increased blood sugar level. [1]

Extensive literature review revealed that analytical methods such as spectrophotometry [2, 3] and High Performance Liquid Chromatography (HPLC) [4-18] has been reported for the estimation of Linagliptin as bulk and in tablet dosage form either as single drug or in combination with other drugs.

To best of our information, some reports were found for determination of Linagliptin in tablet dosage form by stability-indicating reverse phase high performance liquid chromatographic (RP-HPLC) method. But the reported methods have used either acetonitrile as solvent for separation which is costly. So it was necessary to develop a method which would be cost effective. The present work describes the development and validation of a simple, precise, accurate and cost effective stability indicating RP-HPLC method for determination of Linagliptin as bulk drug and in tablet dosage form in accordance with ICH guidelines. [19, 20]

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade working standard Linagliptin was obtained from Getz Pharma Research Pvt. Ltd. (Thane, India) used as such without further purification. Pharmaceutical dosage form used in this study was Tradjenta tablets (Avanscure Life Sciences Pvt. Ltd., Haryana, India) labeled to contain 5 mg of Linagliptin was procured from the local market. Triethylamine, Methanol (both AR grade) were obtained from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

HPLC system used was JASCO system equipped with Model PU 2080 Plus pump, Rheodyne sample injection port (20 µl), MD 2010 PDA detector and Borwin- PDA software (version 1.5). A chromatographic column Grace C_{18} (150 x 4.6 mm i.d. 3µm) was used. Separation

was carried out at flow rate of 1 mL min^{-1} using Methanol: Water (Triethylene amine 1 ml) in ratio 80:20, v/v and detection at 294 nm.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10 mg of drug in 10 mL methanol to get concentration of 1000 µg mL^{-1} which was diluted further to acquire final concentration 100 µg mL^{-1}.

Preparation of sample solution (Tablet Formulation Analysis)

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10 mg of Linagliptin was weighed and transferred to 100 mL volumetric flask containing about 60 mL of methanol and ultrasonicated for 15 min and volume was made up to the mark with the Methanol. The solution was filtered through Whatman paper No. 41. One mL of this solution was transferred to 10 mL calibrated volumetric flask and volume was made up to the mark with the methanol to get solution of concentration 10 µg mL^{-1} for Linagliptin. After setting the chromatographic conditions, the tablet sample solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curve. The % assay was found to be 99.61 ± 1.15 (mean ± S.D.).

System suitability

The system suitability was assessed by six replicate injections of the standard Linagliptin having concentration 20 µg mL^{-1}. The resolution, peak asymmetry, number of theoretical plates and height equivalent to theoretical plate (HETP) were calculated. The values obtained demonstrated the suitability of the system for the analysis of drug. The results obtained are represented in Table No. 1.

Table No. 1: System suitability parameters for proposed RP-HPLC method

Sr. No.	Parameters	Linagliptin
1	Theoretical plates	4623.28
2	HETP (cm)	0.0064
3	Resolution	3.45
4	Asymmetry factor	1.32

Forced degradation study

The stress degradation studies were performed by subjecting the bulk drug to the different stress conditions as recommended by ICH and stability was accessed. The stability studies were carried out at concentration of 100 µg µL^{-1}. The hydrolytic studies were carried out by treatment of stock solution of drug separately with 0.05N HCl and 0.05 N NaOH at room temperature for 30 min. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 20 µg µL^{-1}. The oxidative degradation was carried out in 3 % H_2O_2 at room temperature for 30 min and sample was diluted with methanol to obtain 20 µg µL^{-1} solution. Thermal stress degradation was performed by keeping drug in oven at 80ºC for period of 3 h. Photolytic degradation studies were carried out by exposure of drug to UV light for 4 d.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary target in developing this stability indicating HPLC method is to achieve the resolution between Linagliptin and its degradation products. To achieve the separation, we used a stationary phase C-18 column as stationary phase and mixture comprising of Methanol: Water (Triethylene amine 1 mL) in ratio 80: 20, v/v. The tailing factor obtained was less than two and retention time was 2.70 ± 0.02 for Linagliptin. The representative chromatogram of the standard solution is shown in Figure No. 1.

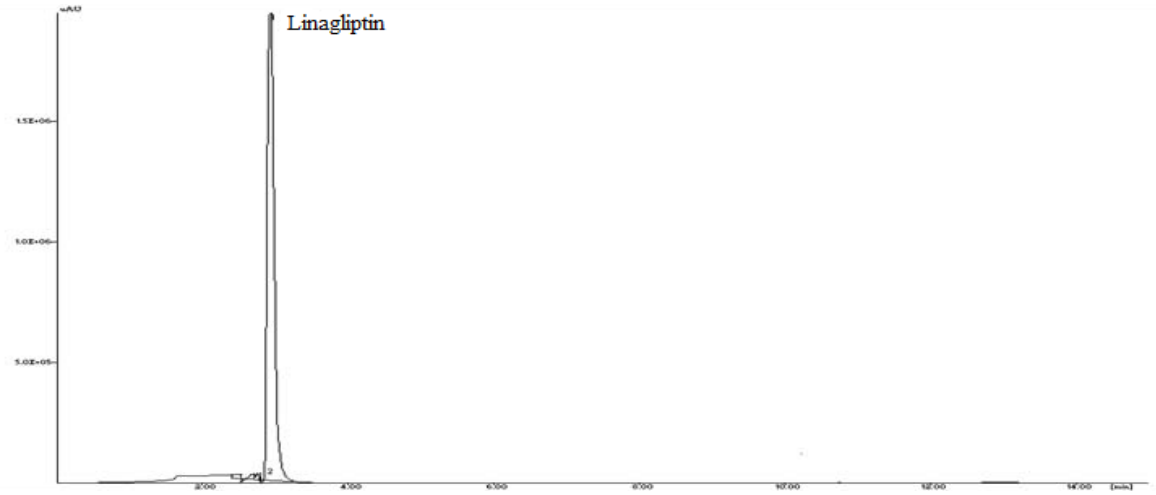

Figure No. 1: Representative chromatogram of standard drug solution (20 µg mL^{-1}, 2.70 min)

Result of forced degradation studies

The forced degradation outcomes revealed susceptibility of drug to hydrolytic, oxidative and thermal stress conditions and stability under photolytic stress conditions. Forced degradation study demonstrated that the specificity of developed method as no degradation products were eluted at retention time of drug. Figures 2-4 represents the chromatograms of acid, alkali and oxidative degradation, while Figures 5 represents the chromatogram of thermal degradation. The forced degradation studies data is summarized in Table No. 2.

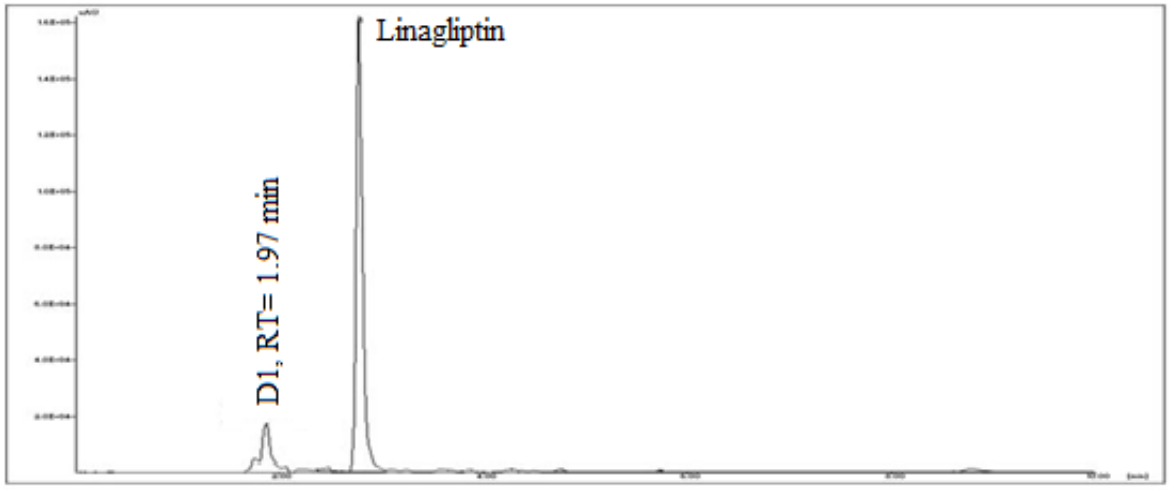

Figure No. 2: Chromatogram obtained after acid degradation with degradation product (D1, RT 1.97 min)

Figure No. 3: Representative chromatogram obtained after alkali degradation with degradation product at (D2, RT 2.49 min)

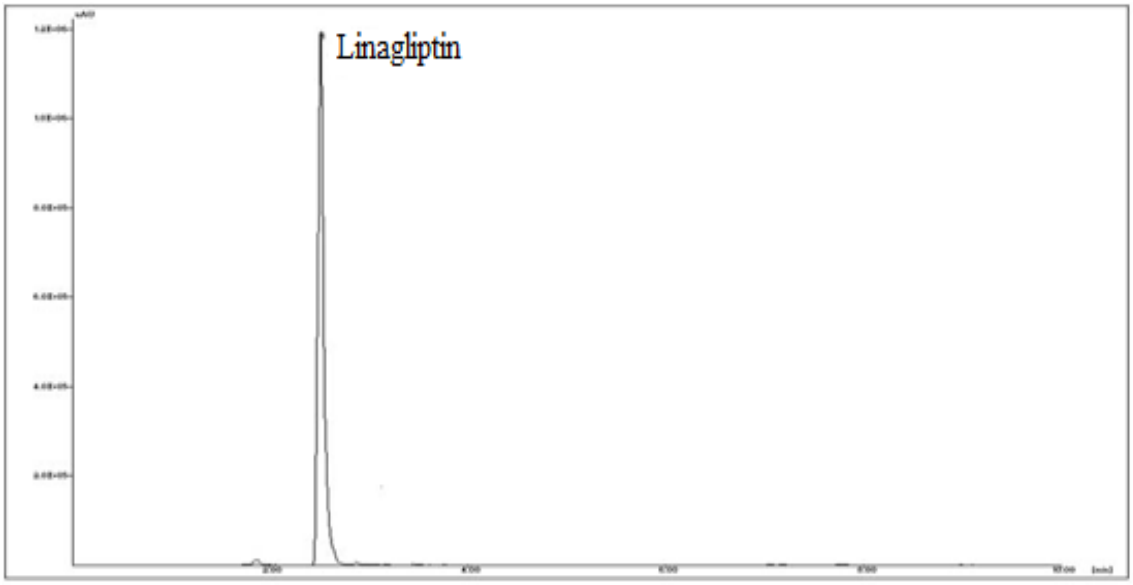

Figure No. 4: Representative oxidative degradation chromatogram of Linagliptin

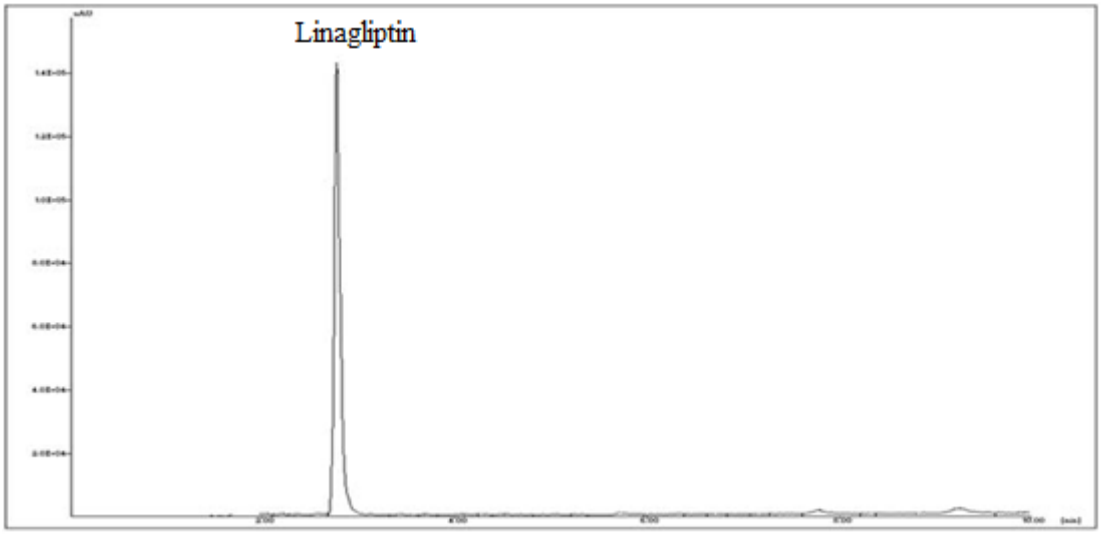

Figure No. 5: Representative chromatogram after dry heat degradation

Table No. 2: Summary of stress degradation studies

Stress conditions/ duration	**% Recovered**	**% Degradation**
Acidic / 0.05 N HCl/ Kept at RT for 30 min	85.23	14.77
Alkaline /0.05 N NaOH/ Kept at RT for 30 min	81.23	18.77
Oxidative /3 % H_2O_2 / Kept at RT for 30 min	90.13	09.87
Dry heat/ 80ºC/ 3 h	88.27	11.73
Photolysis: UV light 200 watt h square $meter^{-1}$ 4 d	99.12	stable

Method Validation

The method was validated for linearity, accuracy and intra-day and inter-day precision, specificity and robustness, in accordance with ICH guidelines.

Linearity

The linearity of the responses of the drug was verified at six concentration levels, ranging from 5-30 µg mL^{-1}. The calibration graph was obtained by plotting peak area versus the concentration and data was treated by least-squares linear regression analysis. The equation of the calibration curve found to be y = 56194x + 41806 with coefficient of correlation 0.996. The calibration curve obtained is represented in Figure No. 6.

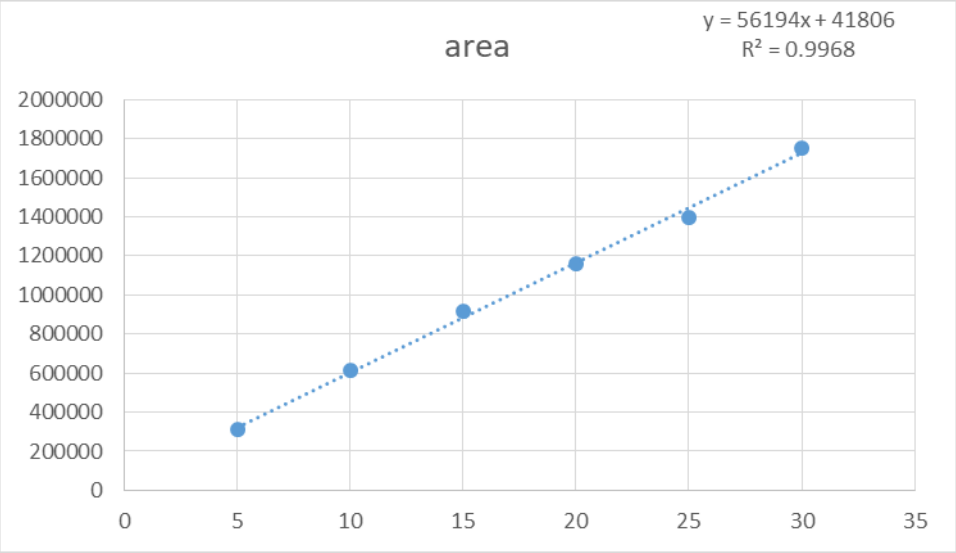

Figure No. 6: Calibration curve for Linagliptin

Precision

One set of three different concentrations of standard solutions of Linagliptin was prepared. All the solutions were analyzed thrice, in order to record any intraday variations in the results. Intra-day variation, as RSD (%), was found to be in the range of 0.53 to 1.37. For Inter day variations study three different concentrations of standard solutions in linearity range were analyzed on three consecutive days. Interday variation, as RSD (%) was found to be in the range of 0.57 to 1.38.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 0.83µg mL^{-1} and 2.52 µg mL^{-1} respectively.

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80 %, 100 % and 120 %. Basic concentration of sample chosen was 10 µg mL^{-1} from tablet solution. The drug concentrations were calculated from linearity equation. The results obtained are shown in Table No. 3.

Table No. 3: Recovery studies

Drug	Amount taken (µg mL^{-1})	Amount added (µg mL^{-1})	Total amount found (µg mL^{-1})	% Recovery*	% R. S. D.[a]
Linagliptin	10	08	17.95	99.76	1.03
	10	10	20.13	100.68	1.26
	10	12	21.95	99.79	0.58

*Average of three determinations, R.S.D. is relative standard deviation.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be ≥991, indicating the no interference of any other peak of degradation product, impurity or matrix.

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which flow rate of the mobile phase and wavelength at which the drugs were recorded was altered and the effect on the area of drug was noted. It was observed that there were no marked changes in the chromatograms, which demonstrated that the HPLC method developed is robust.

CONCLUSION

Stability indicating RP-HPLC method without interference from excipients or degradation products has been developed and validated for determination of Linagliptin as bulk drug and in tablet dosage form. The developed method can be used for assessing the stability of Linagliptin in bulk drug and in pharmaceutical dosage form. The RP-HPLC method is developed by using easily available and cheaper solvents hence can be considered as economic.

ACKNOWLEDGEMENTS

The authors express their gratitude to Getz Pharma Research Pvt. Ltd. (Thane, India) for the gift sample of pure Linagliptin. Thanks are also extended to Dr. Ashwini Madgulkar, Principal, AISSMS College of Pharmacy for providing research facilities to carry out the research work.

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