RESEARCH ARTICLE

Development and Validation of Analytical Method for Linagliptin Drugs in Pharmaceutical Dosage form by RP-HPLC

Chavan Avinash, Gandhimathi R

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, VELS Institute of Science, Technology and Advanced Studies (VISTAS), Chennai, Tamil Nadu, India

Received: 25th December, 2022; Revised: 10th January, 2023; Accepted: 07th February, 2023; Available Online: 25th March, 2023

ABSTRACT

The objective of the current research was to develop and validate the reversed-phase high performance liquid chromatography (RP-HPLC) method for linagliptin from formulation and bulk material. The development was performed on C18 stainless steel column using 0.1 M TEA pH 5.5 adjusted with OPA and methanol (30:70% v/v) at 0.7 mL/min flow rate. Samples were analyzed by 1024 DAD detector at 238 nm. The developed method complied with the system suitability study with acceptable asymmetric factor and number of theoretical plates. The linearity was observed between 10–50 µg/mL concentrations (R^2 = 0.999). The mean %recovery of 99.33 to 99.88% with %RSD between 0.13 to 0.24 was observed. The drug content was found within the acceptable limit in interday and intraday precision study. The method was fond robust and small changes in flow rate, mobile phase and wavelength did not hamper the accuracy and specificity of the method and all results were found within acceptable limit. Limit of detection (LoD) and limit of quantitation (LoQ) of 0.17854 and 0.54105 µg/mL, respectively, were observed. This research confirmed the development of simple, robust, sensitive, accurate and cost-effective methods that can be used to analyze Linagliptin using RP-HPLC from tablet and bulk dosage.

Keywords: Diode-array detector, Limit of Detection, Limit of Quantitation, Linagliptin, Reversed-phase high performance liquid chromatograph, Validation.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.1.35

How to cite this article: Avinash C, Gandhimathi R. Development and Validation of Analytical Method for Linagliptin Drugs in Pharmaceutical Dosage form by RP-HPLC. International Journal of Pharmaceutical Quality Assurance. 2023;14(1):203-207.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Linagliptin is an antidiabetic drug used to treat and control plasma sugar levels of patients suffering from type 2 diabetes. It is advised to take along with other antidiabetic drugs with proper regular exercise and diet. It is a potent dipeptidyl peptidase-4 (DPP-4) inhibitor and exerts pharmacological action by boosting the amounts of certain natural substances that decrease elevated blood sugar.[1,2] Following a meal, the digestive system releases incretin hormones e.g. glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), that raises their blood levels.[3] GLP-1 and GIP reduces blood sugar levels by boosting the pancreas's ability to produce and release insulin. Similarly, GLP-1 lowers blood sugar and reduces the secretion of glucagon from the pancreas, which increases the liver's ability to produce glucose and elevates blood sugar levels. The enzyme DPP-4 destroys GLP-1 and GIP, linagliptin increases their levels and metabolic activity. As a result, blood levels of GLP-1 and GIP continue to be higher, while blood glucose levels drop. Linagliptin decreases blood sugar levels by inhibiting DPP-4 and elevating GLP-1 and GIP levels.[4-6] Figure 1 depicts the Linagliptin chemical structure.

According to a review of the literature, Linagliptin has been estimated using UV and HPLC methods.[7-11] However, these techniques are a little more expensive, require more sophisticated equipment, and take longer to complete. Linagliptin still requires the development of an easy, affordable, and accurate analytical approach. The current work serves as an example of how an accurate, precise, simple, rapid, and reproducible reversed-phase high-performance liquid chromatography (RP-HPLC) method may be developed for linagliptin form marketed formulations as well as bulk materials.

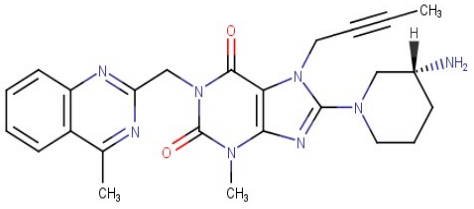

Figure 1: Linagliptin structure

**Author for Correspondence: gandhimathi.sps@velsuniv.ac.in*

MATERIALS AND METHODS

Chemical and Reagents

Swapnroop Drug and Pharmaceutical, Aurangabad, Maharashtra, India provided a working standard of pharmaceutical-grade Linagliptin as a gift sample. The marketed formulation of Linagliptin (Onderoa tablet 5 mg) was purchased from a neighbourhood pharmacy. Merck Ltd., India provided the TEA, OPA, sodium hydroxide, hydrochloric acid, hydrogen peroxide, HPLC grade acetonitrile, water, and methanol.

Instrumentation and Chromatographic Condition

Agilent's LC 1100 series autosampler system with a reciprocating plunger pump, Diode array detector (DAD), with auto injection system was used to conduct the chromatographic study. All calibrated volumetric glassware (Borosil) was used in this validation process. The modular HPLC system included the agilent quaternary gradient HPLC pump, a solvent degasser, an autoinjector, and a DAD detector. The data generated before, during, and after chromatographic analysis were recorded and evaluated using a data ace chromatography data system. On an Eclipse XDE C-18 agilent column (250 mm 4.6 mm *i. d*., and 5 m particle size), the chromatographic separation was performed using a mixture of methanol: 0.1% TEA in the ratio 70:30 v/v (pH adjusted to 2.7 with OPA) as the mobile phase at a flow rate of 0.7 mL/min. At 238 nm wavelength, the samples were analyzed. Before use, the mobile phase was sonicated on an ultrasonic bath and filtered using a 0.45 micron filter.

Preparation of Standard and Stock Solution

Linagliptin (10 mg) was carefully transferred into a 10 mL glass volumetric flask and 1000 µg/mL stock solution was prepared with mobile phase. The volumetric flask containing stock solution was covered with aluminum foil to protect it from the light. The samples were diluted properly with mobile phase to give 10 to 50 µg/mL concentrations

Selection of Wavenumber for Linagliptin

After baseline correction, the UV spectrophotometer scanned with 10 µg/mL working standard solution between 400 to 200 nm against methanol as a blank. The UV-analyst software displayed a maximum wavelength of 238 nm.

System Suitability Study

Following the optimization of the chromatographic conditions and parameters, an identical standard solution of linagliptin (10 µg/mL) was injected to HPLC system and RT, theoretical plates, peak area and other variables were monitored. The obtained findings were compared to the acceptance criteria specified in the ICH recommendations Q2R1.[12]

Validation of the Developed Method[12]

Linearity and Range

Aliquots of the standard Linagliptin solutions from stock were added to volumetric flask (10 mL) and volume was adjusted using mobile phase to get 10 to 50 µg/mL linagliptin concentrations. The peak areas and retention times of each of these drug solutions (20 µL) that were injected into the column under the specified conditions were noted. Using a DAD detector set to 238 nm for sample analysis, a calibration curve was constructed.

Accuracy

The concentrations used were 80, 100, and 120% to analyze the recovery studies using the standard method. The procedure involved combining 0.8, 1.0, and 1.2 mL of standard solution with 0.2 mL of tab solution having10 µg/mL concentration. The % accuracy was determined by using the following formula:

$$\% \text{ Accuracy} = \frac{\text{Mean measured concentration}}{\text{Nominal Concentration}} \text{ x } 100$$

Precision

Following each injection of linagliptin, chromatograms were collected at 10, 30 and 50 µg/mL concentrations that covered the whole range. The interday and intraday precision, was calculated on two consecutive days. After injecting the three standards at 10, 30, and 50 µg/mL, the mean peak area was calculated from the chromatograms using the integration method. In each instance, the percent RSD was computed, and then conformity with the stipulated requirements was determined by examining the results.

LoD and LoQ

These parameters for the Linagliptin were determined as per ICH guidelines using the following formula

$$LoD=3.3\times\sigma/S$$

$$LoQ=10\times\sigma/S$$

Where S is the slope and σ is SD. By measuring the standard deviation of the responses that were obtained for each of the standard concentrations of linagliptin during the linearity investigation, LoD and LoQ were estimated.

Robustness

The defined chromatographic parameters were changed to evaluate the robustness parameter, including flow rate, the detection wavelength, and the mobile phase concentration. The effect on %RSD was observed.

Assay

Linagliptin (10 mg) was carefully transferred into a 10 mL glass volumetric flask and 1000 µg/mL stock solution was prepared with mobile phase. The volumetric flask containing stock solution was covered with aluminium foil to protect it from the light. The samples were diluted properly with mobile phase to give 10–50 µg/mL concentrations. To calculate the assay, the standard and sample solutions were injected into the HPLC system.

RESULT AND DISCUSSION

Method Development and System Suitability

The analytical method development and its validation is a regulatory requirement that ensures that the developed method can efficiently quantify the drug from dosage form of bulk pharmaceuticals.[13] Using the RP-HPLC method, the

Table 1: HPLC condition

Sr. no	*Parameter*	*Conditions*
1	HPLC	Agilent (1100)
2	Software	Chemstation
3	Column	Id 4.6 × 250 mm length
4	Particle size	5.0 µm
5	Stationary phase	C18 (Agilent)
6	Mobile phase	Methanol: 0.1% TEA in the ratio 70:30 v/v (pH adjusted to 2.7 with OPA)
7	Wavelength	238 nm
8	Flow rate	0.7 mL/min
9	Temperature	33°C
10	Volume	20 µL

Table 2: System suitability study results

Sr. no	*Parameters*	*Linagliptin*	*Acceptance criteria*
1	Asymmetric factor	0.68	NMT 2.00
2	Number of Theoretical plates	4880	NLT 2000
3	Retention time	3.28	……

Table 3: Linearity results of Linagliptin

Sr. No.	*Conc (µg/mL)*	*Area*
1	10	979.32770
2	20	2291.39575
3	30	3779.34082
4	40	5066.08545
5	50	6411.53760
Correlation coefficient (R^2)		0.9996
Regression equation		Y=136.44x-388.27

analytical method was developed for linagliptin to estimate the concentration from bulk and marketed tablets. Various parameters were tested (mobile phase, flow rate, wavelength etc) to optimise the simple and robust analytical method. The optimised conditions for the mobile phase was found to be Methanol: 0.1%TEA in the ratio 70:30 v/v (pH adjusted to 2.7 with OPA), a column size of 250 mm 4.6 mm, a particle size packing of 5 µm, and a flow rate of 0.7 mL/min. The optimized HPLC conditions are presented in Table 1. The various system suitability parameters tested are summarised in Table 2 as follows. The peak was observed at a retention time of 3.28 for standard of linagliptin (Figure 2) and same retention time for sample of linagliptin (Figure 3) which is acceptable and indicates the applicability of the developed method.

Validation of the Developed Method

Range and Linearity

Excellent linearity was found between 10–50 µg/mL concentration for developed and validated method of Linagliptin. Y=136.44x-388.27 can give regression equation. Over a wide range of concentrations, the calibration curve revealed a linear relationship between concentrations and peak

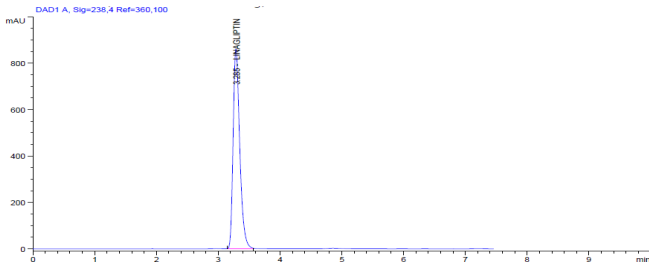

Figure 2: Linagliptin standard chromatogram

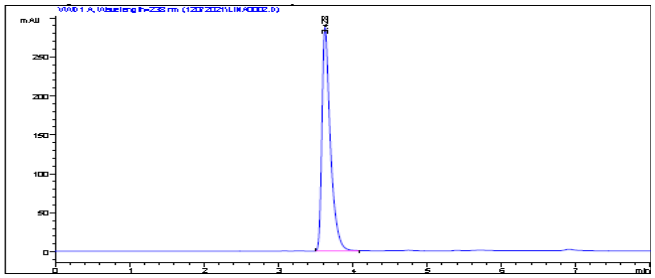

Figure 3: Sample Chromatogram of Linagliptin

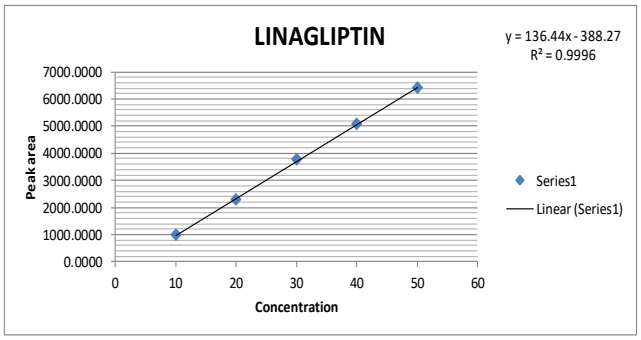

Figure 4: Calibration curve of Linagliptin

Table 4: Accuracy level in HPLC

Level of Recovery (%)	*%RSD*	*Standard Deviation*	*Mean %Recovery*
80	0.24	0.24	99.88
100	0.17	0.17	99.33
120	0.13	0.13	99.77

sizes. The linearity results of Linagliptin are summarised in Table 3 and the calibration curve presented in Figure 4.

Accuracy

According to ICH Q2R1 guideline, the accuracy study was performed at 80, 100, and 120% levels to analyse the recovery of the Linagliptin using developed analytical method.[12] Table 4 contains the observed findings of the accuracy study. The mean %recovery of 99.33 to 99.88 was observed with %RSD between 0.13 to 0.24. All the obtained results were within the range of acceptable limits.

Precision

The precision study was performed as per ICH guideline. As a part of intermediate precision, the system was evaluated on a variety of days i.e interday and intraday precision. The three

Table 5: Interday and Intraday precision for HPLC

Conc (µg/mL)	Interday Precision		Intraday Precision	
	Mean ± SD	%Amt Found	Mean ± SD	%Amt Found
10	978.26 ± 7.86	100.18	985.55 ± 5.68	100.71
30	3770.96 ± 1.84	101.64	3748.43 ± 9.62	101.09
50	6416.01 ± 11.11	99.77	6417.74 ± 3.71	99.79

standard solutions were used. The peak area was integrated before being analyzed statistically to establish the mean peak area, standard deviation, and percentage of quantity. The results had shown to fall within the parameters that were set. The tabulated findings from the precision study are presented in Table 5. In the interday precision study, the % of the amount was found in the range of 99.77 to 101.64% while Tables 5 and 6 provide the tabulated results from the precision study. The percentage of the amount was found to be between 99 and 101% in the precision study.

LoD and LoQ

The LoD for Linagliptin was determined to be 0.17854 µg/mL, while the LoQ was determined to be 0.54105 µg/mL, as indicated in Table 6. The lower LoD and LoQ results showed excellent, sensitive method towards detection as well as the quantification of Linagliptin.

Robustness

The results obtained from the robustness study are presented in Table 7. It was shown through this investigation that minor variations in flow rate, wavelength, and mobile phase had no effect on the method's accuracy and specificity, which is acceptable by the standards. These observations clearly indicated that the developed method was robust.

Analysis of Marketed Formulation

The marketed formulation (Onderoa tablet 5 mg) was analyzed using developed and validated analytical method. The linagliptin estimation from the tablet was performed and it was found to be 100.91%. The details of the marketed formulation analysis is presented in Table 8. The standard chromatogram of linagliptin and its formulations are presented in Figures 5 and 6, respectively.

Table 6: LoD and LoQ results for Linagliptin

Sr. No	Parameter	Measured value
1	LoD	0.17854
2	LoQ	0.54105

Table 7: Results of Linagliptin Robustness study

Parameter	*Modification*	*Level*	*± SD*	*%RSD*
Flow rate	± 0.2 mL/min	0.6	2.96	0.03
		0.8	22.68	0.35
Wavelength	± 2 nm	277	30.26	0.41
		279	31.11	0.43
Mobile phase (Methanol: OPA)	24:76	24:76	2.57	0.04
		26:74	9.07	0.12

Table 8: Results of marketed formulation analysis

Formulation	Dosage	Sample Conc.	Amt. of sample estimated	%Estimation of drug in tablet
Onderoa	5 mg	30 ppm	30.27	100.91

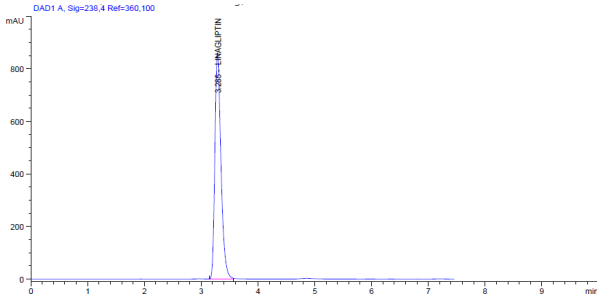

Figure 5: Standard Chromatogram of Linagliptin

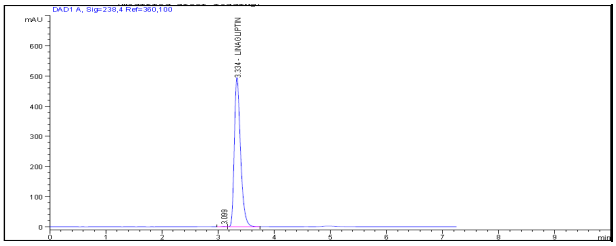

Figure 6: Chromatogram of formulation

Table 9: Summary of the developed and optimized parameter

S. No	*Parameter*	*RP-HPLC method*
1	Linearity	10–50 µg/mL
2	Correlation coefficient	0.999
3	Method precision %RSD	0.80
4	Accuracy	99.33–99.88%
5	Range	Lower 80% Higher 120%
6	LoD	0.17854 µg/mL
7	LoQ	0.54105 µg/mL

The summary of the developed and validated analytical method is summarised in Table 9.

CONCLUSION

The current study supports the development and validation of the linagliptin method using the simple, sensitive, accurate, robust, and affordable RP-HPLC. A high percentage of the recovery study demonstrates that the developed method was free of excipients utilized in pharmaceutical formulation, and the recovery study was statistically validated. Therefore, the aforementioned techniques may be used in quality control laboratories to determine how much linagliptin is present in both bulk and commercial formulations.

REFERENCES

1. Badugu LR. A Validated RP-HPLC method for the determination of Linagliptin. American Journal of PharmTech Research. 2012;2(4):463-470.

2. McGill JB. Linagliptin for type 2 diabetes mellitus: a review of the pivotal clinical trials. Therapeutic advances in endocrinology and metabolism. 2012 ;3(4):113-24.
3. Nauck MA, Meier JJ. Incretin hormones: Their role in health and disease. Diabetes, Obesity and Metabolism. 2018; 20:5-21.
4. Deeks ED. Linagliptin. Drugs. 2012;72(13):1793-824.
5. Graefe-Mody U, Retlich S, Friedrich C. Clinical pharmacokinetics and pharmacodynamics of linagliptin. Clinical pharmacokinetics. 2012;51(7):411-427.
6. Lewin A, DeFronzo RA, Patel S, Liu D, Kaste R, Woerle HJ, Broedl UC. Initial combination of empagliflozin and linagliptin in subjects with type 2 diabetes. Diabetes care. 2015;38(3):394-402.
7. Ganorkar AV, Askarkar SS, Gupta KR, Umekar MJ. Validated stability indicating and assay method development of linagliptin in formulation by RP-HPLC using quality by design. Orbital: The Electronic Journal of Chemistry. 2020:48-61.
8. Madhusudhan P, Reddy MR, Devanna N. RPHPLC method development and validation for simultaneous determination of linagliptin and empagliflozine in tablet dosage form. International advanced research journal in science, engineering and technology. 2015;2(2):95-99.
9. Vankalapati KR, Alegete P, Boodida S. Stability-indicating ultra-performance liquid chromatography method development and validation for simultaneous estimation of metformin, linagliptin, and empagliflozin in bulk and pharmaceutical dosage form. Biomedical Chromatography. 2021;35(4):e5019.
10. Banik S, Karmakar P, Miah MA. Development and validation of a UV-spectrophotometric method for determination of vildagliptin and linagliptin in bulk and pharmaceutical dosage forms. Bangladesh Pharmaceutical Journal. 2015;18(2):163-168.
11. Rajbangshi JC, Alam MM, Hossain MS, Islam MS, Rouf AS. Development and validation of a RP-HPLC method for quantitative analysis of Linagliptin in bulk and dosage forms. Dhaka University Journal of Pharmaceutical Sciences. 2018;17(2):175-182.
12. Validation of Analytical Procedures: Text And Methodology Q2(R1). https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf
13. Kolimi P, Shankar VK, Shettar A, Rangappa S, Repka MA, Murthy SN. Development and Validation of HPLC Method for Efinaconazole: Application to Human Nail Permeation Studies. AAPS Pharm Sci Tech. 2022;23(1):1-7.