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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF NEW ANTIDIABETIC AGENT LINAGLIPTIN IN BULK AND IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A novel isocratic reverse phase liquid chromatography method for determination of Linagliptin was developed and validated after optimization of various chromatographic conditions. A Khromosil C18, 5µm column having 150×4.6 mm i.d., with mobile phase containing 0.02 M potassium dihydrogen phosphate : acetonitrile (70:30, v/v, pH 5.0 adjusted with 1% OPA solution) was used. The flow rate was 1.2 mL $min^{-1}$and effluents were monitored at 226 nm. The retention time of Linagliptin was 4.2min. The linearity for Linagliptin was in the range of 0-75µg mL^{-1} with coefficient of correlation 0.999. The proposed method was validated with respect to linearity, accuracy, precision and robustness.

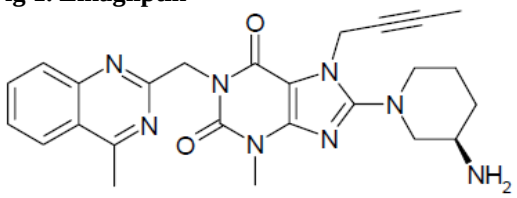
Keywords: Linagliptin, RP- HPLC, Tradgenta Tablets, Validation.

INTRODUCTION

Linagliptin is described chemically as 1H-Purine-2,6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butyn-1-yl)-3,7-dihydro-3-methyl-1-[(4-methyl-2-quinazolinyl) methyl]-

The empirical formula is $C_{25}H_{28}N_8O_2$. The structural formula is shown in fig (1).

Fig 1. Linagliptin



Linagliptin is a white to yellowish, not or only slightly hygroscopic solid substance. It is very slightly soluble in water (0.9 mg mL^{-1}). Linagliptin is soluble in methano (ca. 60 mg mL^{-1}), sparingly soluble in ethanol (ca. 10 mg mL^{-1}), very slightly soluble in isopropanol (<1 mg mL^{-1}), and very slightly soluble in acetone (ca. 1 mg mL^{-1}) [1-3].

Linagliptin is an oral drug that reduces blood sugar (glucose) levels in patients with type 2 diabetes. Linagliptin is a member of a class of drugs that inhibit the enzyme, dipeptidyl peptidase-4 (DPP-4). Following a meal, incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released from the intestine, and their levels increase in the blood. GLP-1 and GIP reduce blood glucose by increasing the production and release of insulin from the pancreas. GLP-1 also reduces blood glucose by reducing the secretion by the pancreas of the hormone, glucagon, a hormone that increases the production of glucose by the liver and raises the blood level of glucose. The net effect of increased release of GLP-1 and GIP is to reduce blood glucose levels. Linagliptin inhibits the

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enzyme, DPP-4, that destroys GLP-1 and GIP and thereby increases the levels and activity of both hormones. As a result, levels of GLP-1 and GIP in the blood remain higher, and blood glucose levels fall. Linagliptin reduces blood glucose levels by inhibiting DPP-4 and increasing the levels of GLP-1 and GIP [4-8].

MATERIALS AND METHODS

Chemicals and Reagents

HPLC grade Acetonitrile from Merck specialties Pvt Ltd, Mumbai. Chemicals and Whatman GFC filter were used in the study. Analytically pure Linagliptin was procured as gratis sample from reputed laboratory. Water HPLC grade was obtained from Rankem laboratories.

Tablet formulation (Tradjenta 5mg) manufactured by Eli Lilly and Company containing labeled amount of 5 mg of Linagliptin film coated tablets was purchased from local market.

Equipments

The instrument was a Water Alliance 2695 separation module, having water 2996 photodiode array detector in isocratic mode. The system was connected with the help of Empower2 software in a computer system for data collection and processing. The analytical column used is Khromosil C18.

Chromatographic condition

The mobile phase consists of a mixture of 0.02N Potassium dihydrogen phosphate (pH adjusted to 5 with 1% o-phosphoric acid) (70 volumes) and Acetonitrile (30 volumes) was filtered through 0.45 µm nylon membrane filter before use. The injection volume was 20µL with a flow rate 1.2 mL min^{-1} and detection wavelength 226 nm having ambient condition and run time 15 min.

Standard preparation

Stock solutions were prepared by accurately weighing 10 mg of Linagliptin and transferring to 10 ml volumetric flasks containing 3 ml of methanol. The flasks were sonicated for 10 min to dissolve the solids. Volumes were made up to the mark with methanol, which gave 1000 µg Ml^{-1}. Aliquots from the stock solutions were appropriately diluted with mobile phase to obtain working standards of 50 µg mL^{-1} of drug. Typical standard chromatogram of Linagliptin is shown in Fig (2) [9-12].

RESULT AND DISCUSSION

Estimation of Linagliptin in tablet dosage form

The HPLC procedure was optimized with a view to develop precise and stable assay method. Linagliptin was run in different mobile phase composition and different pH ranges (5 to 7) of mobile phase with different C18 columns Agilant Xdb (100 mm x 4.6 mm i.d.,5 µm), hypersil BDS (150 mm x 4.6 mm i.d., 5µm) at ambient temperature (25º and 30º C).The flow rate was also varied from 0.5 mL to 1.2 mL min^{-1}. The mobile phase consists of and a mixture of 0.02N potassium dihydrogen phosphate (pH adjusted to 5) (70 volumes) and acetonitrile (30volumes) was filtered through 0.45 µm nylon membrane filter before use. The column used is Khromosil C18, 5µm column having 150×4.6 mm i.d.

Twenty tablets were weighed and crushed to fine powder. The powder equivalent to 25mg of Linagliptin was taken in a 25 mL volumetric flask and made up with methanol. The resultant mixture was filtered through 0.45 µm nylon filter. From this filtrate 5mL of solution was pipette out into 100 ml standard flask and made up with mobile phase. The sample solution was chromatographed similar to standard solution and concentrations of Linagliptin in tablet samples were calculated using regression equation. Typical sample chromatogram of Linagliptin is shown in Fig (3).

Method Validation

The described method has been validated for the assay of Linagliptin using following parameters.

Accuracy

The accuracy of the method was determined by recovery experiments. Placebo was spiked with known quantities of standard drugs at levels of 50 to150% of label claim. The recovery studies were carried out 3 times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table (1).

System suitability studies

The system suitability test was carried out on freshly prepared stock solution of Linagliptin to check various parameters such as column efficiency, tailing factor and number of theoretical and presented in Table (2).The values obtained were demonstrated the suitability of the system for the analysis of the drug. System suitability parameter may fall within 3% standard deviation range during routine performance of the method.

LOD and LOQ

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Linagliptin was found to be 0.9 µg mL^{-1}.The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 2.95µg mL^{-1} for Linagliptin respectively.

Linearity and Range

Linearity was studied by preparing standard solution at five different concentration levels. The linearity

range was found to be 0-75 µg mL^{-1}. 20µL of each solution was injected into chromatograph. Peak areas were recorded for all the chromatogram. Calibration curve was constructed by plotting peak areas (Y axis) against the amount of drug in µg mL^{-1} (X axis). Peak area of linearity range and the parameters were calculated and presented in Table 3 The linearity curve of Linagliptin was shown in Fig(4).

Specificity

Specificity of the method was determined by injecting the diluted placebo. There was no interference of placebo with the principle peak, hence the developed analytical method was specific for Linagliptin in tablet dosage form.

Precision

System precision

The system precision of the method was established by six replicate injections of the standard solution containing Linagliptin. The percentage RSD were calculated and presented in Table (4). From the data obtained, the developed RP-HPLC method was found to be precise.

Method precision

The method precision of the method was established by carrying out the analysis of Linagliptin in dosage form (n=6) using the proposed method. The low value of the relative standard deviation showed that the method was precise The results obtained were presented in Table (5).

Robustness

Robustness of the method was determined by making slight change in the chromatographic condition. It was observed that there were no marked changes in the chromatograms, which. The results of robustness were presented in Table (6).

Table 1. Results of accuracy studies

S. No	%Accuracy	Peak area	Amount Added (mg mL^{-1})	Amount Found (mg mL^{-1})	%Recovery	Avg %Recovery
1	50%	1051124	0.025	0.025133	100.5304	101.0179
		1058124	0.025	0.0253	101.1999	
		1059417	0.025	0.025331	101.3235	
2	100%	2082382	0.05	0.04971	99.58038	98.9629
		2073687	0.05	0.049582	99.16458	
		2052342	0.05	0.049072	98.14385	
3	150%	3144006	0.075	0.075174	100.2318	98.9439
		3078953	0.075	0.073618	98.15788	
		3087873	0.075	0.073832	98.44225	

The mean %recovery is well within the acceptance limit, hence the method is accurate

Table 2. System Suitability Studies

S. no	peak area	Retention Time	Theoretical plates	Tailing factor
1	2081801	4.216	4039	1.11
2	2101070	4.319	3932	1.15
3	2094054	4.38	3941	1.11
4	2099531	4.217	4100	1.1
5	2093628	4.3	3977	1.13
AVG	2094017	4.2864	3997.8	1.12
SD	7575.163	0.070323	70.94153	0.02
%RSD	0.361753	1.640603	1.774514	1.785714

Table 3. Result of Linearity

S.No	Pipetted from stock (mL)	Volume of flask (mL)	Concentration µg mL^{-1}	% of linearity level	Peak area
1	0	0	0	0	0
2	0.125	10	12.5	25	65209
3	0.25	10	25	50	1205065
4	0.5	10	50	100	2298165
5	0.625	10	62.5	125	2913681
6	0.75	10	75	150	3569378

Table 4. System precision results

S.No	Peak A ea
1	2081801
2	2101070
3	2094054
4	2099531
5	2093628
6	2076856
AVG	2091157
Stdev	9746.212
%RSD	0.466068

Table 5. Method precision result

S.No	Peak Area	% Assay of Dosage Form
1	2124657	101.5004
2	2130499	101.7795
3	2103228	100.4767
4	2123178	101.4297
5	2109707	100.7862
6	2103990	100.5131
AVG	2115877	101.0809
SD	11692.41	0.558567
%RSD	0.552604	0.552694

Table 6. Method Robustness of Linagliptin in Dosage Forms

Condition	Change	Retention time (Min)	% RSD
Temperature	+5º C	4.58	0.15
	-5ºC	4.65	0.17
Flow rate	+0.2mL min^{-1}	3.52	0.12
	-0.2mL min^{-1}	5.94	0.47

Fig 2. Standard chromatogram of Linagliptin

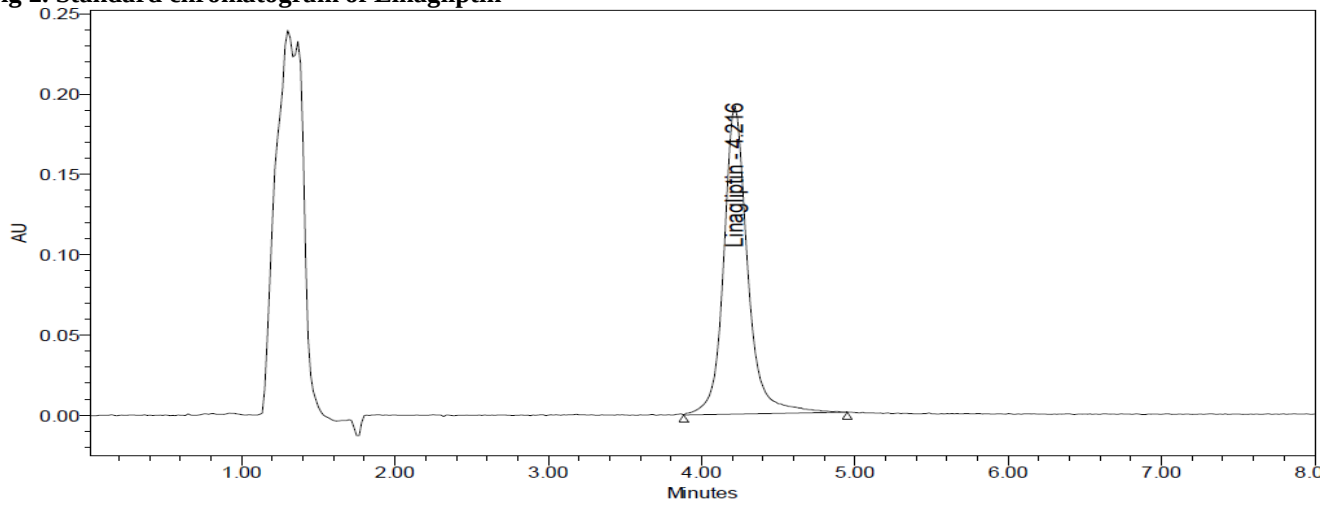


Fig 3. chromatogram of Linagliptin sample

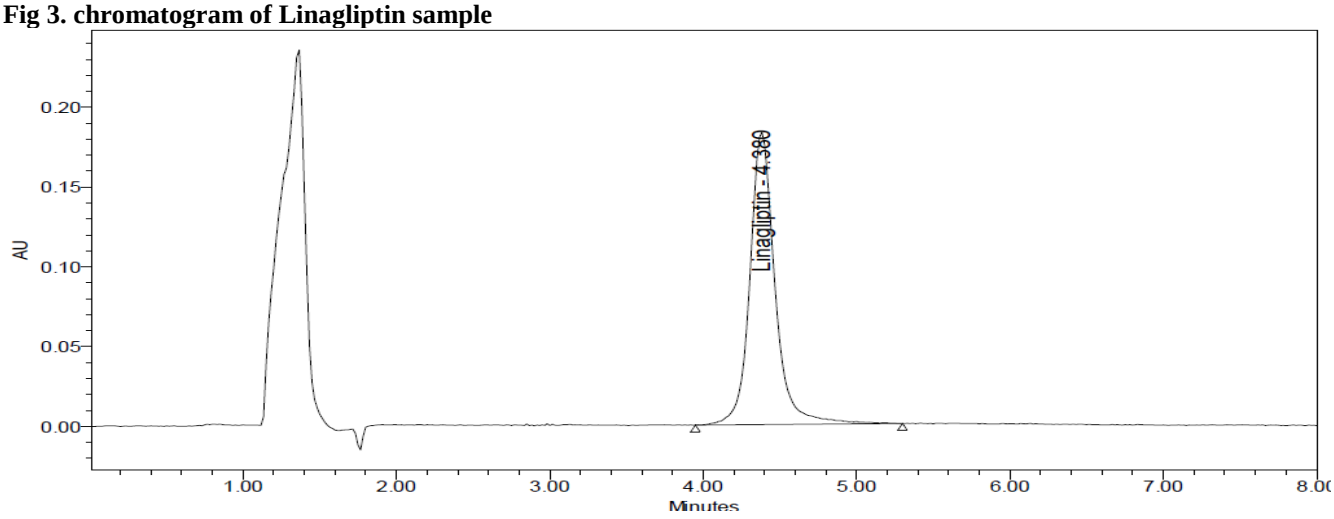
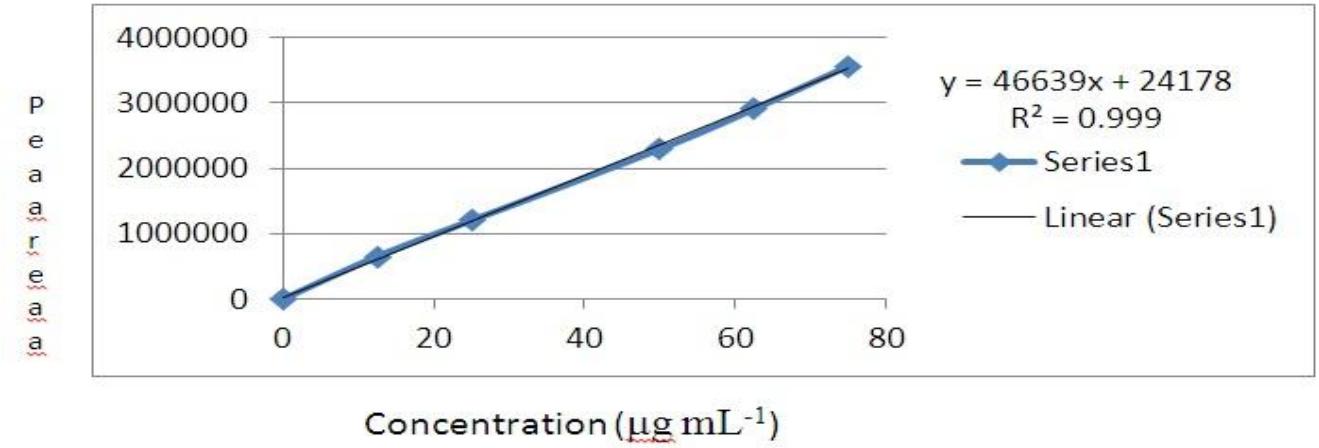


Fig 4. **The linearity curve of Linagliptin concentration vs. peak area**



CONCLUSION

The proposed RP-HPLC method for the estimation of Linagliptin in tablet dosage forms is accurate, precise, linear, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw material, formulation and dissolution studies.

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