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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR ASSAY OF LINAGLIPTIN IN BULK AND MARKETED DOSAGE FORM

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Linagliptin in tablet dosage form. Isocratic elution at a flow rate of 1.0 ml/min was employed on a symmetry Nucleodur C18 (150x4.6mm, 5µm in particle size) at 50°C temperature. The mobile phase consisted of Buffer: Acetonitrile: Methanol (68:85:15 (v/v/v). The UV detection wavelength was 225 nm and 5µl sample was injected. The retention time for Linagliptin was 3.61 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Linagliptin in tablet dosage form and bulk drug.

KEY WORDS: Linagliptin, RP-HPLC, UV detection, recovery, precise, 225 nm.

INTRODUCTION

Linagliptin is an oral drug that reduces blood sugar (glucose) levels in patients with type 2 diabetes.[1] Linagliptin is a class of drugs that inhibit the enzyme, dipeptidyl peptidase-4 (DPP-4). Other members of this class include sitagliptin (Januvia), and saxagliptin (Onglyza). Following a meal, in cretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP) are released from the intestine, and their levels increase in the blood.[2]

GLP-1 and GIP reduce blood glucose increasing the production and release of insulin from the pancreas.[3] 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 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. In summary, Linagliptin reduces blood glucose levels by inhibiting DPP-4 and increasing the levels of GLP-1 and GIP. Linagliptin was approved by the FDA in May 2011.[4] Type 2 diabetes is a progressive disease which may require intensification of therapy over time.

Linagliptin, 5 mg tablets are indicated as an adjunct to diet and exercise to improve glycaemia control in adult's type 2 diabetes mellitus.[5] Linagliptin is described chemically as 1piperidinyl]-7-(2-butyn-1-yl)-3, 7-dihydroC25H28N8O2 and the molecular weight is 472.54 g/mol.[6]

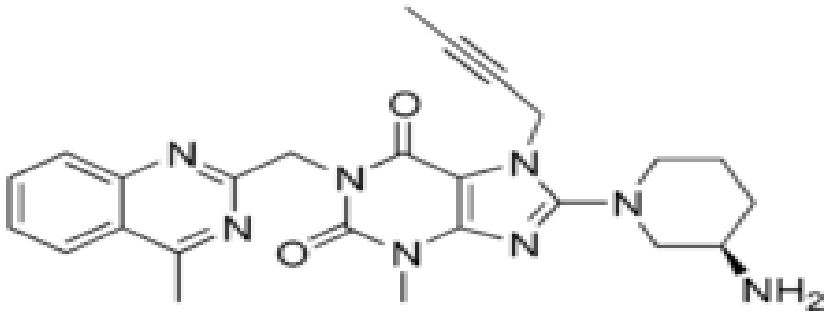


FIG: 1

MATERIAL AND METHODS

Chemicals and materials: Linagliptin was obtained as a gift sample from IPC laboratories. HPLC grade water, acetonitrile and methanol were obtained from Merck. Potassium dihydrogen phosphate and ortho phosphoric acid of HPLC grade were obtained from Merck.

Instrumentation: Quantitative HPLC was performed on agilent, with PDA detector equipped with automatic injector with injection volume 5 µl, the software installed was Ezchrome. A symmetry C18 column(150*4.6 mm,5µm,Neucleodur) was used.

Method development and optimization

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, pH and flow rate were studied. Various solvent systems were tried for the development of a suitable HPLC method for determination of linagliptin in bulk drug and

pharmaceutical dosage forms. Mobile phase tried for this purpose were buffer pH 2.5: buffer :acetonitrile:methanol (50:50), buffer pH 3:acetonitrile: methanol (60:40), buffe pH4:acetonitrile: methanol (68:32). The condition that gave best resolution and symmetry was selected. HPLC conditions are given in Table-1.

Table 1: HPLC Condition

PARAMETER	CONDITION
Column (stationary phase)	C18 (150*4.6 mm, 5 µm, Neucleodur)
Mobile phase	Sol A:Sol B(68:32)v/v (isocratic)
Flow rate	1ml/min
Run time	10 min
Column temperature	50° c
Injection volume	5µl
Detection wavelength	225 (nm)
Drug Rt	3.61 min

Preparation of buffer (pH-2.5)

13.6gm of potassium di-hydrogen phosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. The pH of the solution was then adjusted to 2.5 with orthophosphoric acid.

Preparation of mobile phase

Solution A- Phosphate Buffer, Solution B- Acetonitrile: Methanol (85:15).The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Diluent preparation

Solution A: Solution B (1:1)v/v

Preparation of standard solution

Standard stock solution of 500 µg/ml was prepared by dissolving 25 mg of linagliptin in 50 ml of diluent. From this stock solution, 5 ml was pipette out and the volume was made up to 50 ml with the diluent to prepare working standard solution of 50 µg/ml.

Preparation of sample solution

5 mg equivalent of linagliptin tablet powder was accurately weighed and transferred into a 50 ml volumetric flask. To it, about 20 ml of diluents was added and sonicated to dissolve. The volume was made up to the mark and the solution was filtered through 0.45 µ filter under

vacuum. From this stock solution,5 ml was pipette out and the volume was made up to 10 ml with the diluents to prepare sample solution of 50 µg/ml.

Assay

Inject 5 µl of the standard and sample solution into the chromatographic system and measure the areas for the linagliptin and calculate the % assay by using the formula. The standard and sample chromatograms were shown in fig. 2.

Formula

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg.wt}}{\text{Label claim}} \times 100$$

Where

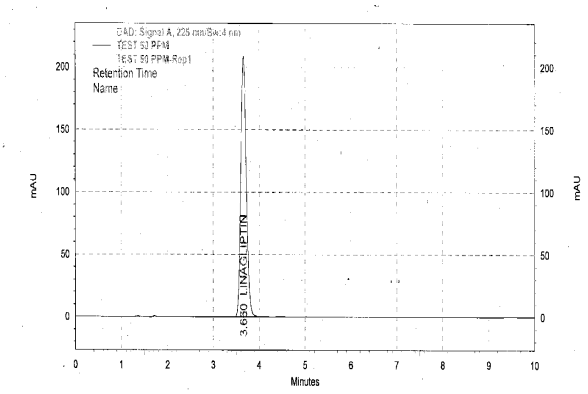
AT = average area counts of sample preparation.

AS = average area counts of standard preparation.

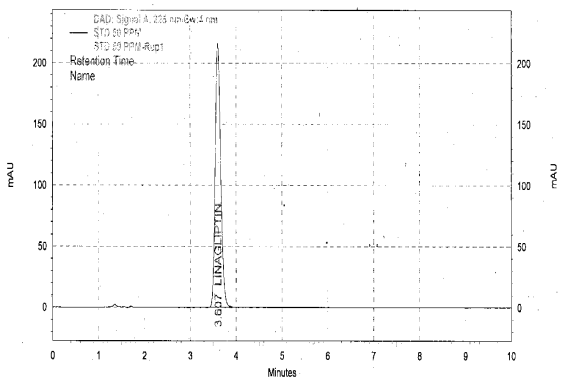
WS = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC = Label Claim of drug mg/ml**.**



Standard Chromatogram Of Linagliptin



Sample Chromatogram Of Linagliptin

Method validation

The method was validated for the following parameters such as linearity, precision, accuracy, limits of detection and quantitation, ruggedness and robustness.

System Suitability

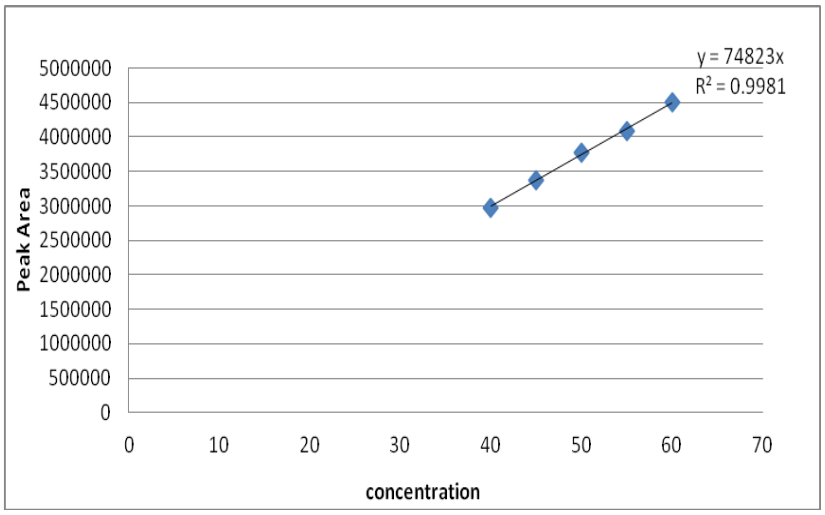
System suitability was daily performed during entire validation of this method. The results of system suitability were presented in Table 2.

Table 2: System suitability parameter

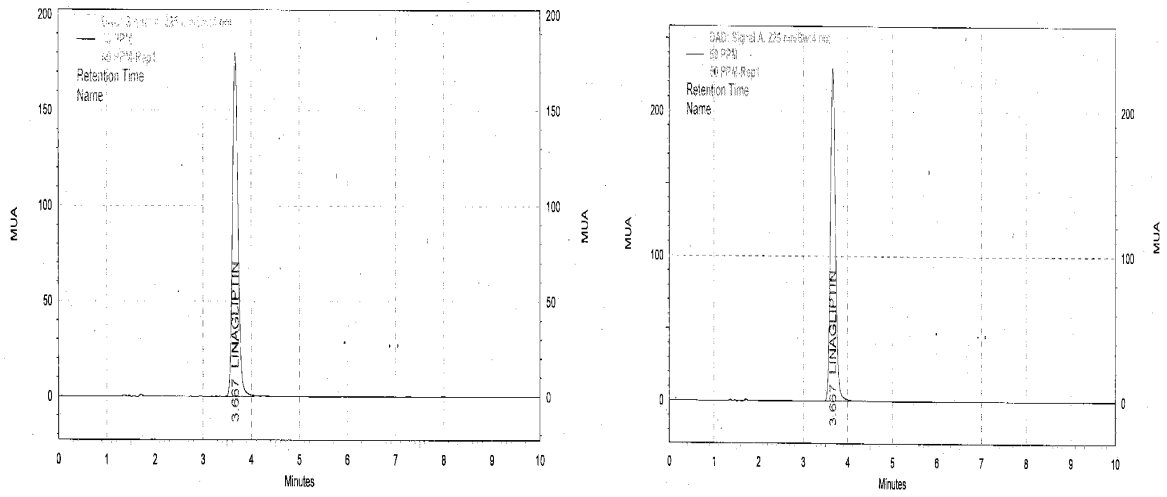
S. No	Parameter	Linagliptin
1.	Retention Time	3.60
2.	Theoretical Plates	4752
3.	Tailing Factor	1.17
4.	Area	3739792

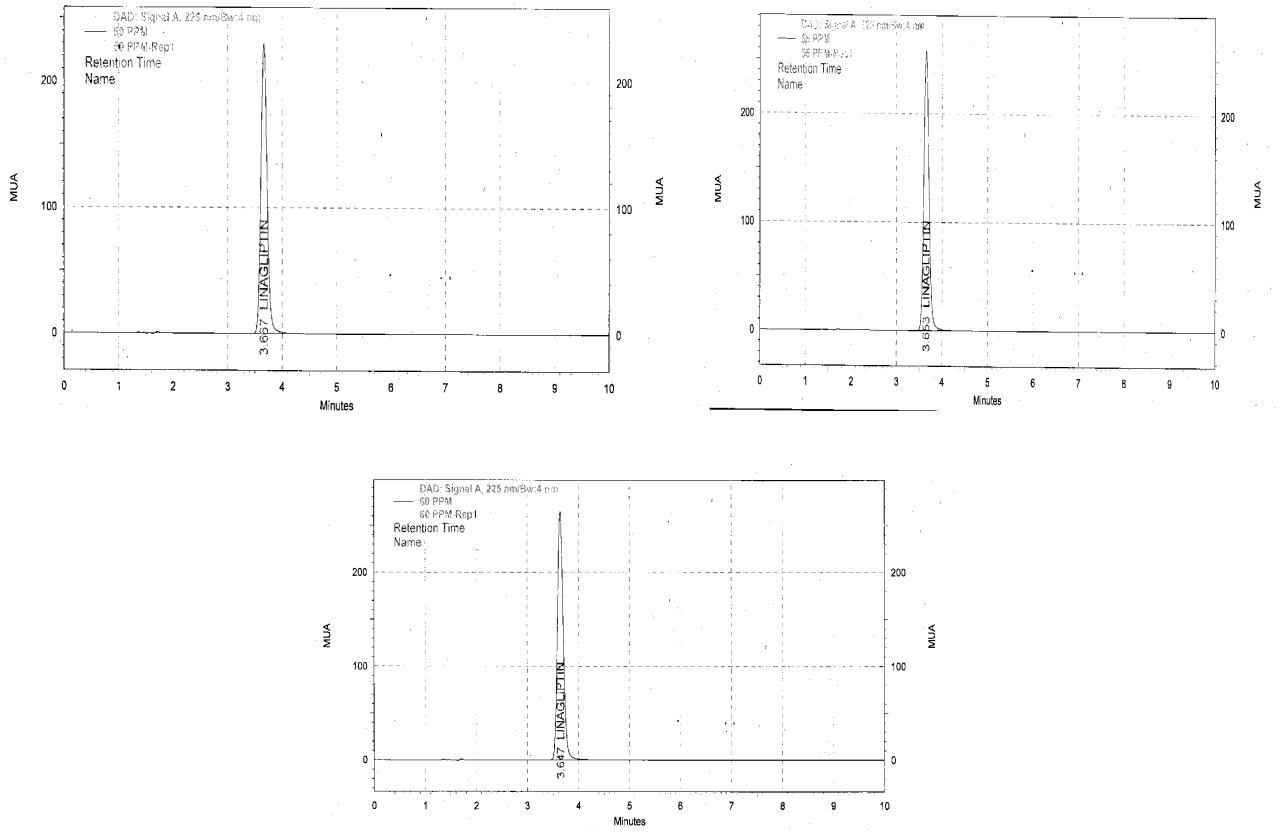
Range of linearity

Aliquots of standard linagliptin stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of linagliptin were in the range of 40-60 µg/ml. Each of these drug solutions (5µL) was injected into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 225 nm and a Calibration graph was obtained by plotting peak area versus concentration of linagliptin.



Calibration Curve of Linagliptin





Linearity Chromatogram of Linagliptin

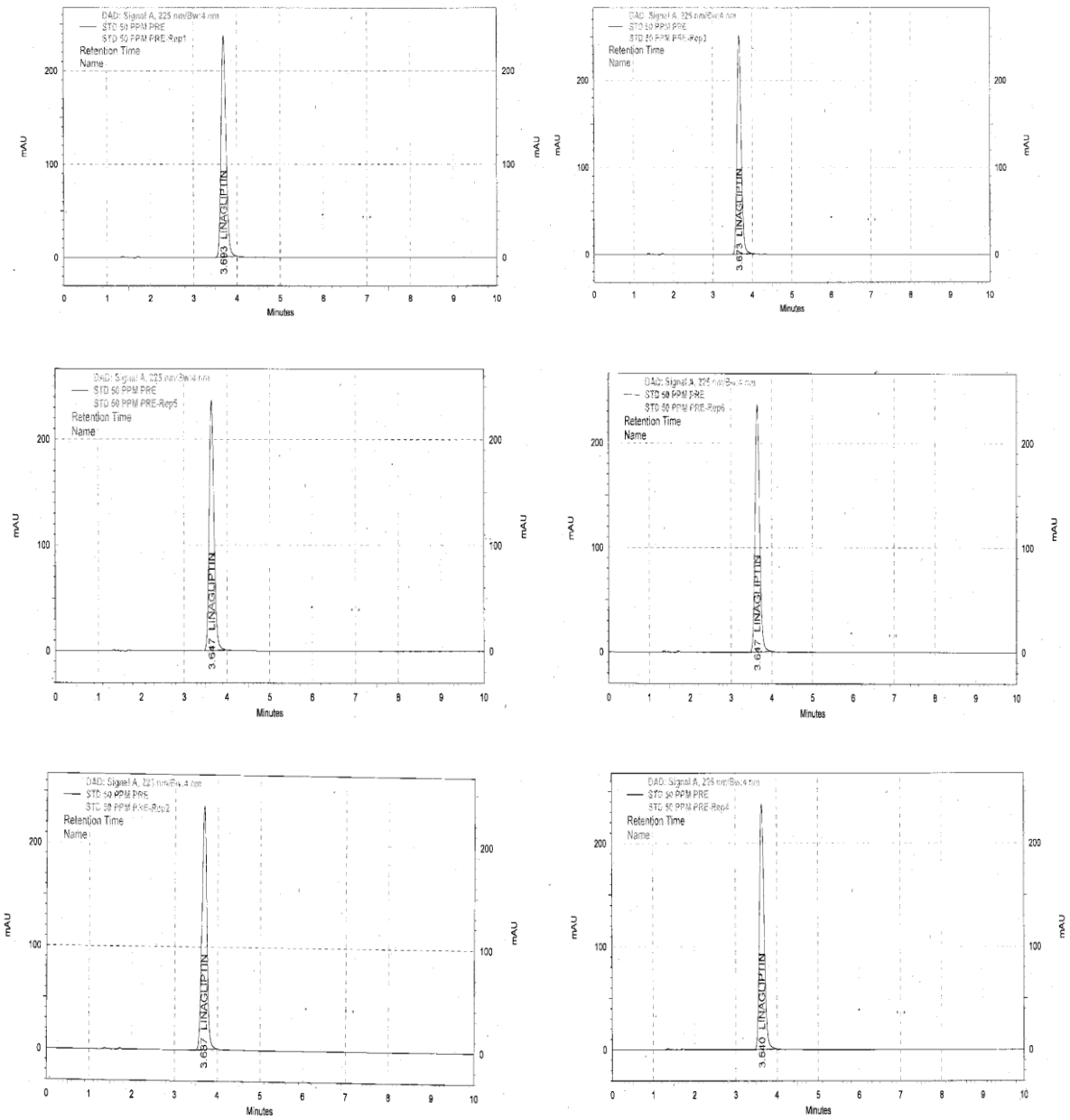
Table: 3 Linearity Results of Linagliptin

S. No.	Concentration	Area
1	40µL	2970894
2	45 µL	3365179
3	50 µL	3778827
4	55 µL	4088021
5	60 µL	4499002
Correlation Coefficient		0.99800

Precision: Repeatability of the method checked by injecting replicate injections of 50 ppm of the solution for six times on the same day as intraday precision study of LINAGLIPTIN and the RSD was found to be 0.43 for intraday and 0.66 for interday.

Table: 4 Precision Result of Linagliptin

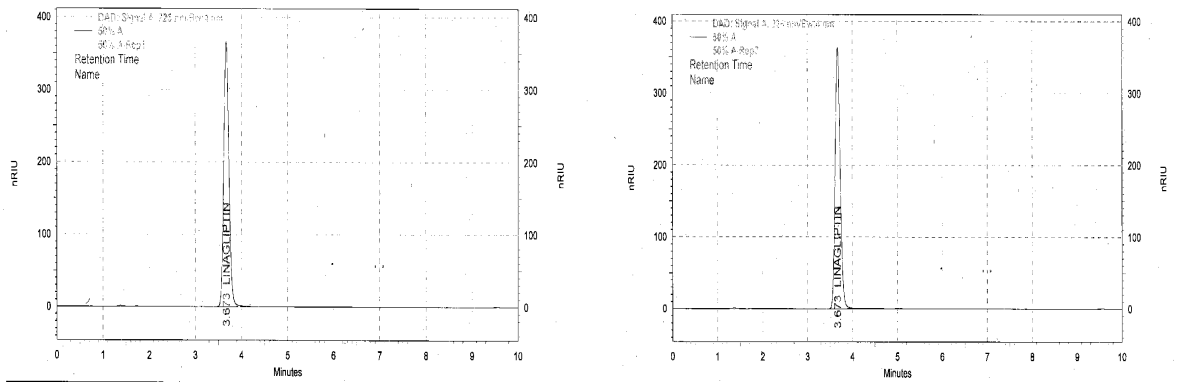
Injection	Concentration (PPM)	Intraday Precision	Interday Precision
1	50	3736224	3706555
2	50	3693735	3706084
3	50	3695237	3700278
4	50	3687721	3739792
5	50	3698385	3764330
6	50	3696398	3706555
	RSD	0.43	0.66



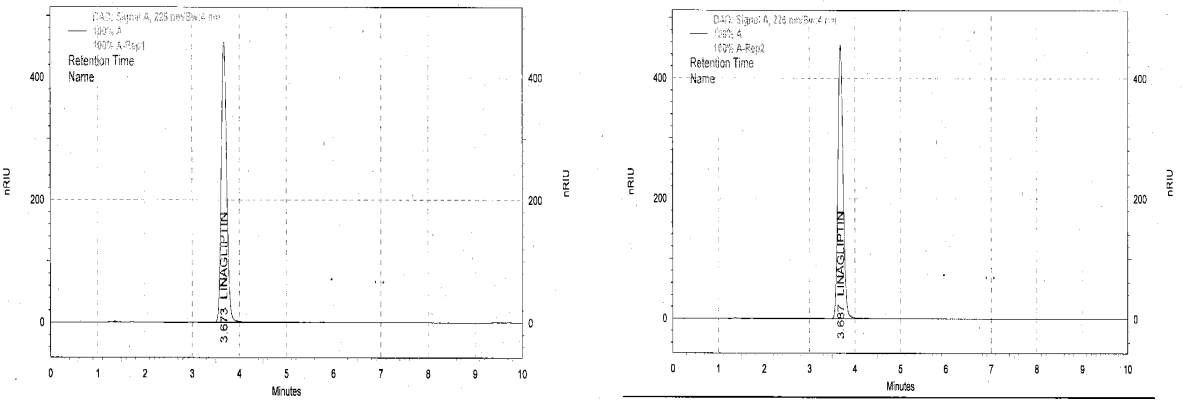
Precision Chromatogram

ACCURACY

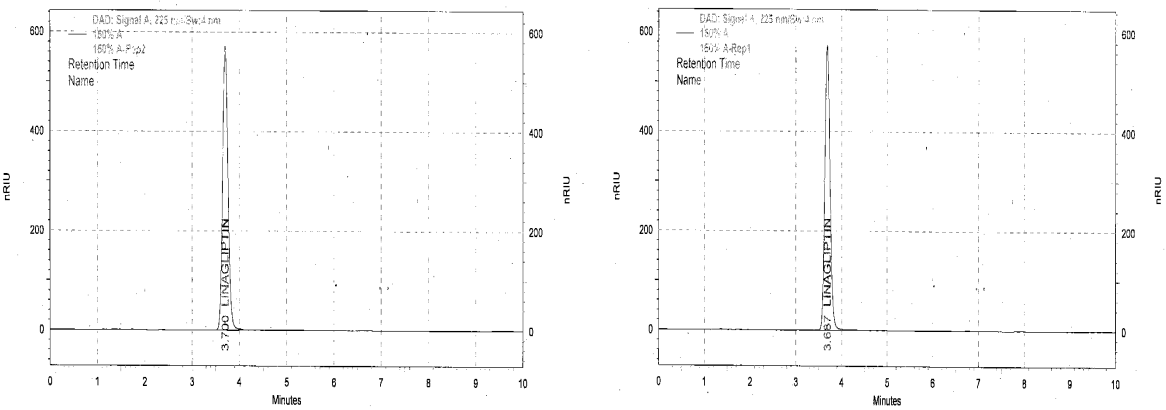
Recovery studies of the drug were carried out for determining accuracy parameter. Accuracy is the closeness of results obtained by a method to the true value. It is the measure of exactness of the method. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out in 50% 100% and 150% levels.



Accuracy chromatograms for 50% solution



Accuracy chromatograms for 100% solution



Accuracy chromatograms for 150% solution

Table: 5 Accuracy Results of Linagliptin

% CONCENTRATION	AREA	AMOUNT ADDED (mcg)	AMOUNT FOUND (mcg)	% RECOVERY	MEAN RECOVERY
50%	5596272	25	24.77	99.0830	
100%	7481609	50	49.45	98.8984	99.9305
150%	9886337	75	76.36	101.8103	

LOD AND LOQ

The LOD and LOQ were determined for linagliptin, based on the standard deviation (SD) of the response and slope (S) of the regression line as per ICH guideline according to the formulae given below

$$LOD = 3.3 \times \frac{SD}{S}$$

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

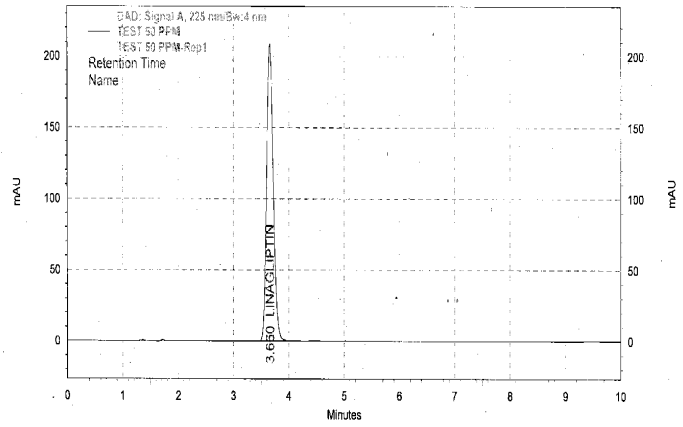
Table: 6 LOD And LOQ Results of Linagliptin

PARAMETER	MEASURED
LOD	0.70 ppm
LOQ	2.13 ppm

FORMULATION ANALYSIS

Table: 7 Assay Result of Linagliptin

S.NO	Tablet	Tablet	Sample conc	Sample estimated	% of Drug Estimated in Tablet
1	TRADJENTA	5 mg	50 ppm	49.95	99.90



CHROMATOGRAM OF FORMULATION

RESULT AND DISCUSSION

To optimize the mobile phase, various proportions of buffers with methanol were tested. Mobile phase composition was changed and the method development was started by symmetry C18 (4.6 x 150 mm, 5 mm, Neucleodur) column and with a flow rate of 1.0 ml/min, and detection wavelength of 225 nm. Injection volume was 5 µL, and run time was for 5 min. The mobile phase consists of phosphate buffer (pH 2.5) and Acetonitrile,

methanol. The retention time of linagliptin was found to be 3.61 minutes. The assay result was found to be 99.90%. Quantitative linearity was observed over the concentration range of 40-60 µg/ml. the correlation co efficient was found to be 0.998. The numbers of theoretical plates obtained were 4752, which indicates the efficiency of the column. The limit of detection and limit of quantitation were found to be 0.70 and 2.13 µg/ml, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate.

CONCLUSION

A simple and rapid RP-HPLC method was developed for the estimation of Linagliptin in API and pharmaceutical
Dosage forms.

Method was developed on Symmetry C18 (4.6 x 150 mm, 5µm, Make:Neucleodur).The mobile phase was phosphate buffer (pH 2.5): Acetonitrile :Methanol (68:85:15) % ratio with a flow rate of 1.0 ml/min. The chromatograms were recorded at 225 nm wavelength. The retention time for Linagliptin was found to be 3.61.

The developed method was validated in terms of accuracy, precision, linearity and robustness and results were validated statistically.

Therefore it was concluded that the proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical and can be used for the estimation of Linagliptin in API as well as in pharmaceutical dosage forms

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