Chiral LC-PDA-ORD Method for The Separation of Linagliptin Enantiomers On Coated Polysaccharide Based Amylose Tris (3, 5-Dimethylphenylcarbamate) Stationary Phases

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Abstract: Chiral normal phase high performance liquid chromatographic (chiral-HPLC) was designed and verified for the separation of linagliptin enantiomers using coated polysaccharide chiral stationary phases. The stationary phase was amylose tris (3, 5-dimethylphenylcarbamate) (250x4.6mm, 5 µm), while the mobile phase was a mixture of 50:50:0.1% v/v. With a flow rate of 1 mL/min, orthophosphoric acid was mixed with hexane, isopropyl alcohol, and diethyl amine to achieve a pH of 5.2. The detection was seen at 225 nm. The optical rotatory dispersion (ORD) polarimeter was connected in series to the PDA outlet in order to determine the enantiomer conformation. The linagliptin retention times were found to be 5.454 and 8.772 minutes. Between 3.9 and 23.4 µg/ml, enantiomers were discovered to be linear, with a correlation coefficient of 0.9995. This method was validated in terms of linearity, LOD, LOQ, precision, accuracy, and robustness studies in accordance with ICH requirements. Novelty: The proposed analytical method for the chiral analysis of linagliptin can be used by pharmaceutical industries quality control departments.

Keywords: Linagliptin, RP-HPLC, PDA, ORD, Polysaccharide, Enantiomers.

1. INTRODUCTION

Lingulin 8-[-3-aminopiperidin-1-yl] - [(4-methylquinazolin-2-yl)-methyl] 7-butynyl-3-methyl (1) -4,5-dihydropurine-2,6-dione. Its molecular composition is C25H28N8O2. Linagliptin inhibits Dipeptidyl Peptidase-4 (DPP-4) in a competitive, reversible manner. DPP-4 inhibition slows the pace at which glucose-dependent insulinotropic polypeptide-4 (GIP-4) breaks down. When glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are present and GIP inhibits glucagon release, pancreatic beta cells produce more insulin. [1][2][3].

A thorough review of the literature indicated that there have been no publications on the chiral separation of linagliptin enantiomers reported to date. Linagliptin in bulk and its formulation were determined using RP-HPLC in the research investigation [4–7]. A RP-HPLC was created by Appalacharyulu et al. to assess the enantiomeric purity of linagliptin [8]. Linagliptin and metformin's enantiomers were simultaneously determined by Jadhav et al. [9–10] utilising conventional HPLC with a run duration analysis of 50 minutes. Simultaneous quantification [13], Determination in biological matrix [11], Stability suggesting RP-HPLC assay method [12], and other described methods [14–19].

In the currently suggested chiral separation investigation, linagliptin enantiomers are chirally separated in normal phase HPLC with a quick run time analysis of less than 10 minutes. The created approach can be successfully used for the accurate measurement of API content in linagliptin commercial samples.

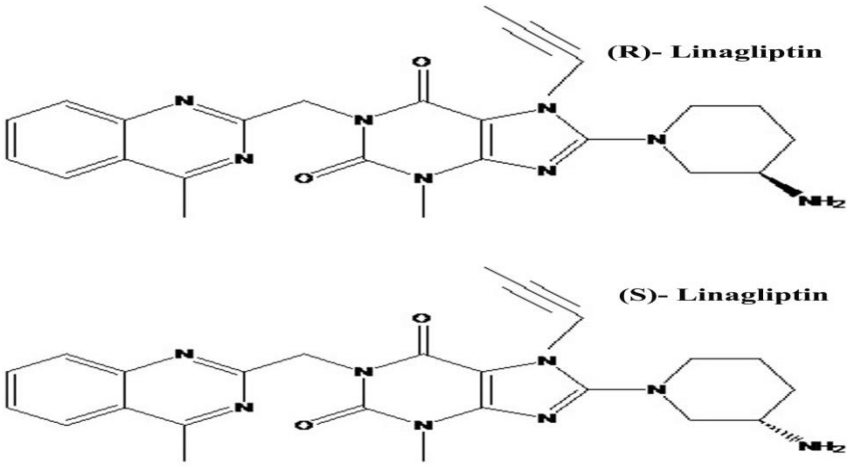

Figure 1: Structures of Linagliptin Enantiomers

2. EXPERIMENTAL

2.1. Instrumentation

Shimadzu LC-20AD LC solutions software was used to record the data from HPLC with a binary pump, SPD-M20A photo diode array detector, and HPLC data. A 20-Loop Rheodyne Injector is used. The optical rotations of the eluted analyte were investigated using a polarimeter (IBZ Messtechnik, Germany). Chiralpak-AD, Amylose tris (3, 5 dimethyl phenyl carbamate) column, Daicel, Japan, was utilised and had the following specifications: 25 cm length, 4.6mm internal diameter, and 5m particle. Shimadzu, Kyoto's analytical balance was employed.

2.2. Material & Reagents

A 99.8% weight-to-weight pure linagliptin sample was obtained from a local manufacturing facility in Hyderabad. Hexane, isopropyl alcohol, and diethyl amine solvents of HPLC quality were utilised. We bought 4 mg Linagliptin tablets under the trade name Tradjenta.

2.3. Chromatographic Parameters

Chiralpak-AD, an amylose tris (3,5-dimethylphenylcarbamate) column with a specified length of 25 cm, an internal diameter of 4.6 mm, and a particle size of 5 m, was used to perform the chiral separation. Isocratic elution at room temperature with a 20-L sample injection volume and a 1-mL/min flow rate. Attending tests, the optimum mobile phase contained 0.1% Diethyl amine, 50% Isopropyl alcohol, and 50% Hexane. At 225 nm, the analysis was carried out. The analysis took 30 minutes to complete.

2.4. Preparation of Mobile Phase

Hexane, isopropyl alcohol, and diethyl amine were combined to create the mobile phase in a 50:50:0.1 ratio. After being sonicated for 35 minutes, the mixture was via a filter with a 0.45 millimetre pore size.

2.5. Preparation of Diluent

Both the standard and sample solutions were made using the mobile phase as a diluent.

2.6. Preparation of Individual Standard Solution

A separate 100 mL volumetric flask was used to hold each 10 mg dose of linagliptin and it’s S-isomer. The samples were dissolved in an adequate amount of diluent and diluted to the required level to achieve 100 µg/mL.

2.7. Preparation of Working Standard Solution

A 100 mL calibrated flask was filled with 10 mg each of linagliptin and its entantiomer-S. The analytes were dissolved using an adequate amount of diluent, and the diluent was added up to the mark to obtain 100 µg/mL.

2.8. Preparation of Working Sample Solution

In order to achieve a concentration of 1 mg/mL, ten milligrams of linagliptin were weighed, transferred to a volumetric flask with a volume of 10 mL, and then sufficiently diluted with diluent to dissolve by sonication.

2.9. System Suitability

Prior to validation, the system appropriateness was estimated. To assess the system's suitability, the standard solution was examined six replicate times. The relative standard deviation expressed as a percentage should be less than 2%. The other system appropriateness factors were the symmetry of peak, tailing factor, and theoretical plates indicating column efficiency.

3. RESULTS AND DISCUSSION

3.1. Method Development

The methodology was designed using two separate columns, each measuring 25 cm x 4.6 mm and 5 mm. In order to improve the analytical approach for the chiral separation of Linagliptin enantiomers, numerous mobile phase and flow rate experiments were conducted. Following tests with 100:0.1 ethanol and DEA at a flow rate of 0.7 mL/min and 100:0.1 isopropyl alcohol (IPA) and diethyl amine (DEA) at a flow rate of 0.7 mL/min revealed no enantiomers on the cellulose tris (3, 5-dimethylphenylcarbamate) column. The enantiomeric resolution was substantial even though the trial was run on a Cellulose tris (3, 5-dimethylphenylcarbamate column with mobile phase Hexane, Ethanol, and DEA in the ratio 50:50:0.1 at a flow rate of 0.7mL/min.

The following trails were conducted on the Amylose tris (3, 5-dimethylphenylcarbamate column, and they revealed the enantiomeric peaks with resolution greater than two. I IPA and DEA at a flow rate of 0.7 mL/min in a 100:0.1 ratio ii) 100:0.1 ethanol and DEA at a flow rate of 0.7 mL/min. iii) A 1 mL/min flow of 50:50:0.1 ethanol, methanol, and DEA. iv) IPA, Methanol, and DEA at a flow rate of 1 mL/min at a ratio of 50:50:0.1. A column of amylose tris (3, 5-dimethylphenylcarbamate) of 25 cm by 4.6 mm and 5 m was used for the optimized approach, along with a mobile phase made up of hexane, IPA, and DEA mixed 50:50:0.1 at a flow rate of 1 mL/min. The theoretical plates observed for enantiomers were larger than 2000, and the retention periods for R and S-Linagliptin were 5.4 and 8.7 min, respectively. Both the R and S enantiomers' tailing factors were under 1.5.

3.2. Method Validation

3.2.1. Specificity

To determine each analyte's unique retention time, chromatographic analysis was performed on linagliptin and its enantiomer-S at a concentration of 100 g/mL. Prepared and examined was the working standard, which contained spiking analytes of linagliptin and enantiomer-S at a concentration of 100 g/mL. By comparing the chromatograms of the standard solution and blank, the specificity of the method was determined, and No diluent interference was found to have occurred during the analytes' retention duration in the standard solution. [Fig-2]. Figure 3 shows the Optical Rotator Dispersion (ORD) chromatograph with a Polari meter detector to validate the enantiomers.

3.3. System Suitability

Prior to validation, the system appropriateness was estimated. To assess the system's suitability, the standard solution was examined six replicate times. The system's appropriateness for analysis was demonstrated by the

%RSD for the R and S linagliptin enantiomers being larger than 2000, the theoretical plates column efficiency being less than 2%, and the tailing factor being less than 1.5.

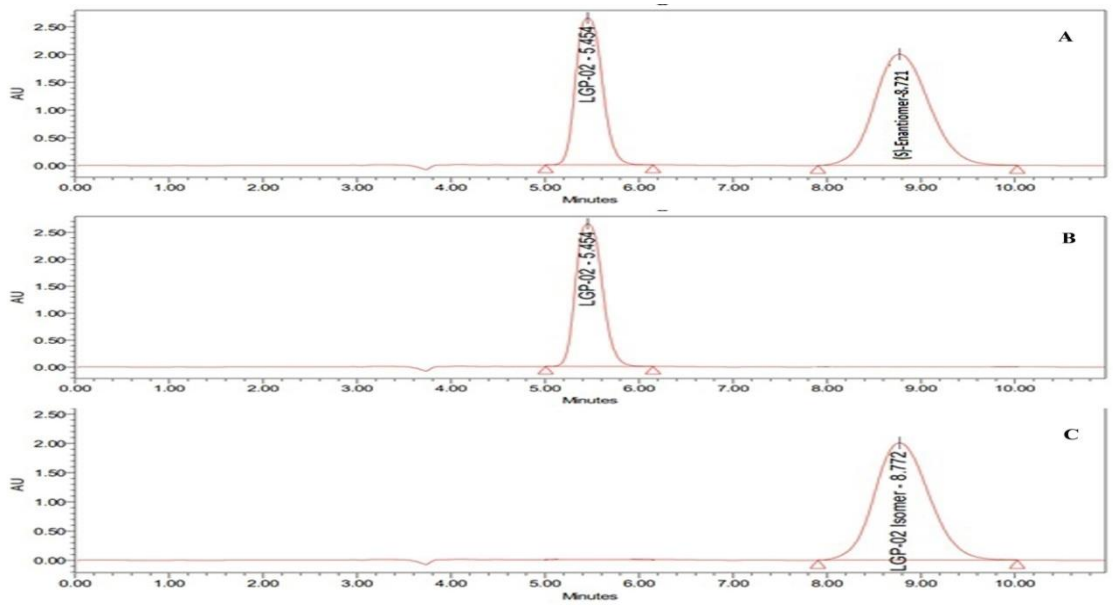

Fig 2: A) Spiked chromatograms of (R) and (S) Linagliptin B) Chromatogram of (R)-Linagliptin C) Chromatogram of (S)-Linagliptin

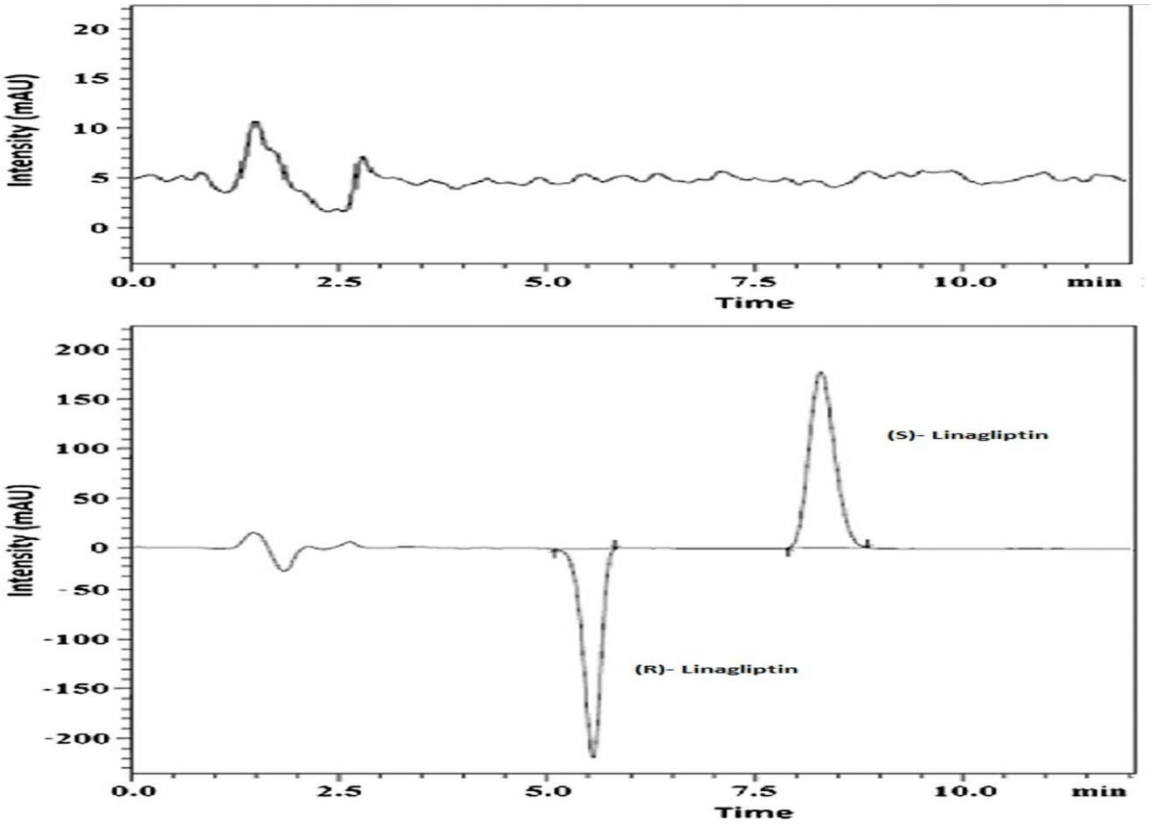

Fig 3: ORD Chromatograph of Linagliptin Enantiomers

3.4. Linearity

Different percentage aliquots of the enantiomers (R) and (S) Linagliptin were produced and examined, ranging from 25 to 150% of working standard specifications (Table-1). To prove the method's linearity, the calibration curve between peak area and concentration was created (Fig-4). The curve's correlation coefficient, which was 0.999, indicated that the procedure was linearly sound.

Table 1: Linearity data for (R) & (S) linagliptin enantiomers.

Level	(R)-Linagliptin		(S)-Linagliptin	
	Conc.µg/mL	Peak area	Conc. (µg /mL)	Peak area
25%	3.9	3134.9	3.9	5391
50%	7.8	6106.2	7.8	10774
75%%	11.7	9473.2	11.7	16154
100%	15.6	12632	15.6	21567
130%	20.3	16421.3	20.3	28350
150%	23.4	18560	23.4	32150
	y =801.2x+ 11.13		y = 1382.x + 6.914	
	R^2 = 0.9995		R^2 = 0.9997	

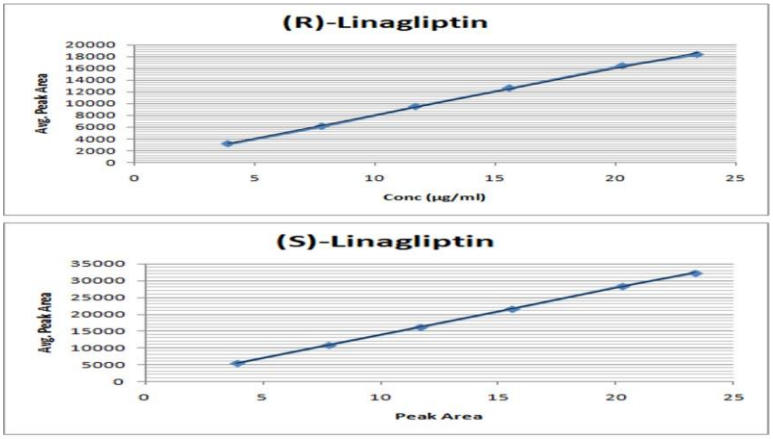

Figure 4: Calibration curve of linagliptin

3.5. Precision

At the intermediate and system levels, the precision was determined. For the precision research, the working standard at 100% level with six determinations was used. The findings obtained demonstrated the procedure precision with the% RSD at below 2. In Table 2, the system precision results are provided. The results of the intermediate precision are shown in Table 3.

Table 2: Precision data

Injection no.	Peak Area	
	(R)-Linagliptin	(S)-Linagliptin
1	12146	20645
2	12220	20731
3	12150	20611
4	12321	20821
5	12241	20840
6	12350	20855
Mean	12238	20750.5
S.D	84.8221669	104.758293
%RSD	0.69310481	0.50484708

Table 3: Results for intermediate precision.

Injection No.	**Analyst-I /Day -I /Instrument I /Column-I**		**Analyst-II /Day-II / Instrument-II/ Column-II**	
	(R)-Linagliptin	**(S)-Linagliptin**	**(R)-Linagliptin**	**(S)-Linagliptin**
1	12156	20687	12189	20604
2	12235	20721	12267	20725
3	12189	20641	12321	20614
4	12325	20701	12214	20874
5	12278	20827	12358	20714
6	12265	20921	12145	20654
Mean	12241.33	20749.67	12249	20697.5
SD	61.58788	104.1742	81.2773	99.78727
%RSD	0.503114	0.502052	0.663542	0.482122

3.6. Accuracy

By spiking (R) - and (S)-enantiomers to the fixed formulation concentration of 20 g/L, the method's accuracy was evaluated. Three degrees of working concentration—50%, 100%, and 150%—were used to test the accuracy. Nine determinations were made in all, with duplicate samples being prepared for each accuracy level. Table-4 presents the findings of the % recovery at various levels.

Table 4: Summary of Percent recovery and Percent RSD.

Level	Spiked conc. (µg/mL)	Measured conc. (µg/mL)	% Recovery	Average	% RSD
Percentage recoveries for (R)-Linagliptin					
50.0%	7.8	7.75	99.35	99.14	0.19
	7.8	7.72	98.97		
	7.8	7.73	99.10		
100.0 %	15.6	15.12	96.92	97.97	0.95
	15.6	15.32	98.20		
	15.6	15.41	98.78		
150.0 %	23.4	23.15	98.93	98.86	0.75
	23.4	23.30	99.57		
	23.4	22.95	99.35		
Percentage recoveries for (S)-Linagliptin					
50.0%	7.8	7.71	98.84	99.01	0.53
	7.8	7.77	99.61		
	7.8	7.69	98.58		
100.0 %	15.6	15.35	98.39	100.44	1.80
	15.6	15.78	101.15		
	15.6	15.88	101.79		
150.0 %	23.4	23.15	98.93	99.94	0.90
	23.4	23.45	100.21		
	23.4	23.56	100.68		

3.7. Limit of Detection (LOD) and Limit of Quantification(LOQ)

The slope of the calibration curve and the deviation of the Y-standard intercept were used to define the limits of detection and quantification. When compared to noise, the signal for LOD should be three times higher than that for LOQ, which should be ten times higher. For (R)-Linagliptin, the LOD and LOQ were determined to be 0.62 g/mL and 1.9 g/mL, respectively. The levels of (S)-Linagliptin were discovered to be 0.38 g/mL and 1.16 g/mL, respectively.

3.8. Robustness

Minor alterations were purposely made to show the method's robustness. During the robustness examination, deliberate changes were made to the mobile phase, flow rate, and injection volume. the robustness of the technique is indicated by the %RSD being less than 2%.

Table 5: Robustness Study

Deliberate Changes	%RSD
Actual Chromatographic conditions	2.00
Change-1,(Hex: IPA: DEA): 48 : 52 : 0.1	4.21
Change -2,(Hex: IPA: DEA): 52 : 48 : 0.1	1.67
Change -3, Flow Rate: 1.2 mL/min	3.07
Change -4, Flow Rate: 0.8 mL/min	4.53
Change -5, Injection volume : 5.0 µL	3.92
Change -6, Injection volume : 15.0 µL	2.11

CONCLUSION

Enantiomers of linagliptin were successfully separated and identified using a straightforward normal phase HPLC combined with photo diode detector and polarimetric detector. The formulation and the active pharmaceutical ingredient underwent an assay using the analytical method. According to ICH requirements, the method's precision, accuracy, robustness, and other factors were validated. The chiral analysis of Linagliptin can be done using the analytical approach in the quality control division of the pharmaceutical industry.

ACKNOWLEDGEMENT

The authors would like to thank Viganan's University for their continuous encouragement and encouragement of this research study.

Conflict of Interest: The Authors are declared that there is no conflict of interest for the current manuscript.

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