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A VALIDATED CHIRAL HPLC METHOD FOR THE ENANTIOMERIC PURITY OF TRELAGLIPTIN

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ABSTRACT

An accurate,precise,simple and rugged Chiral HPLC method has been developed and validated for the quantitation of (S)-isomer in Trelagliptin. The enantiomeric separation of Trelagliptin was achieved Chiral pak AD-3 (250×4.6mm, 3µm) column. The ratio of Hexane,ethanol and diethyl amine (70:30:0.1)in the mobile phase were optimized to obtain the best separation. UV detection was performed at 275 nm. The method is linear over a range of LOQ – 150 % of (S)-isomer. The mean recovery of (S)-isomer was found to be in the range of 95–105%. The method is simple, rapid, accurate, selective and precise, useful in the quality control of bulk manufacturing.

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INTRODUCTION

Trelagliptin is an orally active dipeptidyl peptidase(DPP-4) inhibitors used for the treatment of type 2 diabetes. Dipeptidyl peptidase-4 (DPP-4), the serine protease is responsible for metabolism of incretin hormones glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide, plays an important role in regulating glucose homeostasis. Thus, DPP-4 is an attractive target for therapeutic intervention, and inhibitors of DPP-4 have been shown to be an effective therapy for treatment of of type 2 diabetes. Trelagliptin is potent antagonist, is a novel and effective DPP-4 inhibitor developed by Takeda California (CA, USA). It is a highly selective and longacting DPP-4 inhibitor which is effective when used in a once-weekly dosing schedule.

The compound has a chiral center and the *R* enantiomer is the active inhibitor. In pharmaceutical preparations it is very important to determine the enantiomeric purity of the drug substance. The enantiomers of racemic drugs have relatively different pharmacokinetic properties and toxicological effects. This is one of the important reasons why the regulatory authorities insist more on stringent investigation for evaluating the safety and the effectiveness of drugs containing chiral centers. Enantiomeric separations have acquired importance in all the stages of drug development and the commercialization process. The physical and chemical properties of the two enantiomers are same, so the development of methods for quantitative analysis of chiral compounds is extremely challenging and is very difficult. To best of our knowledge no HPLC method for chiral resolution of the enantiomers of trelagliptin has been reported in the literature. It was necessary to develop an efficient HPLC method for determination of the enantiomeric purity (e.e. %) of trelagliptin.

EXPERMENTAL

Materials

Samples of Trelagliptin and (S)-isomer were synthesized at Stereokem Laboratory, Hyderabad India. HPLC grade ethanol is obtained from Brompton, Ontario L6T 3Y5 (Canada), *n*-Hexane (HPLC grade) was purchased from Merck (Mumbai, India). 2-Propanol was purchased from Merck chemical (Mumbai, India). Analytical grade Diethyl amine (DEA) is purchased from Merck (India).

Equipment

The HPLC system consisted of quaternary gradient pump, auto sampler, column oven and a variable wavelength detector. The output signal was monitored and integrated using EZ-Chrom Elite Chromatography Data Software (1200 series HPLC, Agilent, USA).

Chromatographic conditions

The analysis and validation was performed on Chiralpak AD-3 column (250mm×4.6mm, 3µ) Daicel corporation. The mobile phase is a combination of Hexane, ethanol and diethyl amine in the ratio of 70:30:0.1(v/v). The flow rate was set to 1.0 mL/min, and the column was maintained at 30 °C temperature. The injection volume was 10 µL and the detector wavelength was tuned at 275 nm. The column is flushed with *n*-hexane and 2- propanol (90:10, v/v) at the end of the sequence run at a flow rate of 0.5 mL/min for 1 hour to flush out the containing DEA.

Preparation of standard solutions

Accurately weighed quantity of (R)-isomer working standard (0.1 mg/mL) is dissolved in a 100 ml volumetric flask with minimum quantity diluent and made up to mark with diluent, and the solution injected in to the system. Accurately weighed quantity of Trelagliptin sample (mg/mL) is dissolved in a 10 ml volumetric flask minimum quantity diluent and made up to mark with diluent. The solution injected in to the system.

RESULTS AND DISCUSSION

Method development

To achieve separation between enantiomers of Trelagliptin, chiral stationary phases (CSPs) containing cellulose and amylose derivatives were evaluated with suitable mobile phase compositions. Various combinations of *n*-hexane: ethanol, *n*-hexane: IPA, ethanol:DEA were used as the mobile phase in our initial efforts in the normal-phase separation. These trials were made initially in the absence of DEA and then by adding DEA to the mobile phase. Attempts to separate the enantiomers on Amylose carbamate derivatized columns in normal-phase proved futile. With the use of cellulose derivatized columns the enantiomers could not be separated.

The enantiomers are separated well on Amylose carbamate derivatized CSP (Chiralpak AD-3 250mm length, 4.6 mm internal diameter and 3µm particle size) with mobile phase comprising hexane, ethanol and diethyl amine in the ratio of 70:30:0.1(v/v).Various experiments were conducted, to select the best combination of stationary and mobile phase that could give optimum resolution and selectivity for the enantiomers. No good separation was achieved on Amylose 2 and Lux Cellulose columns. Very good separation was achieved on Chiralpak AD-3 with resolution more than 2.0. Trelagliptin has NH and CO (carbonyl) functional groups and these groups could be well contributing to the interactions with the carbamate groups on CSP, which results in separation. The aromatic ring on the solute could provide additional stabilizing effect to the solute–CSP complex. The use of hexane, ethanol and diethyl amine in mobile phase provided better selectivity and resolution. A comparison of the System suitability results obtained using ethanol and 2-propanol clearly indicate ethanol is the solvent of choice (Table 1).

The addition of DEA up to 0.1% (by volume) to the mobile phase resulted in improved peak shapes, better resolution and shorter run times. The effect of ethanol concentration, DEA concentration, temperature and flow rate on resolution (*RS*), retention time (Rt) and selectivity (α) were examined and the most optimum conditions were found with mobile phase hexane, ethanol and diethyl amine in the ratio of 70:30:0.1(v/v).

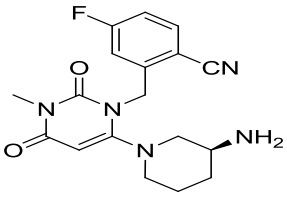


Fig. 1 Structure of (S)-Isomer:

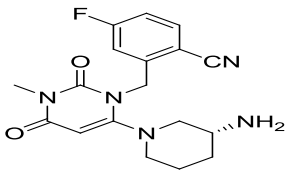


Fig. 2 Structure of (R) Isomer:

Quantification of (R) isomer

Known concentration of standard solution (0.0015 mg/ml) as used for the quantification of (S)-isomer in Trelagliptin (1.0mg/ml).Not more than 0.15% of (S)-isomer is found in Trelagliptin.

Method Validation

The LC method developed has been extensively validated for the quantification of (S)-isomer in Trelagliptin using the following parameters.

Specificity

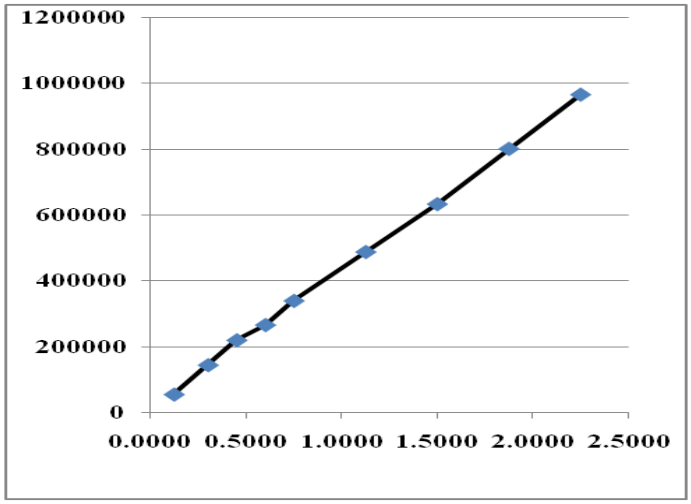
Trelagliptin and (S)-isomer are injected separately to confirm the retention times. System suitability solution was then injected. (S)-isomer and Trelagliptin are eluted at 19.3 and 21.7 minutes respectively. The resolution between the peaks as found to be 2.20.

Linearity and range

The linearity of the method was studied over a concentration covering a range of LOQ to 150 %. Each solution was injected in triplicate and the mean responses recorded were plotted against concentration. The correlation coefficient for (S)-isomer was found to be 0.99, which indicated good linearity. The calibration equation for(S)-isomer was found to be y =106814x-686.7

Linearity of (S)-isomer

S.No	Linearity Level	Concentration (µg/mL)	Average Area
1	LOQ	0.1220	55454
2	20%	0.2998	144567
3	30%	0.4497	220145
4	40%	0.5996	266290
5	50%	0.7495	339835
6	75%	1.1242	488057
7	100%	1.4990	633275
8	125%	1.8737	801015
9	150%	2.2485	965546
Slope			419330
Y-Intercept			17163
CC			0.99



Accuracy

The accuracy of the method was evaluated by spiking the known amount of unwanted isomer ((S)-isomer) to the drug subsatance from LOQ, 50%, 100% and 150% levels. The recoveries were calculated from the slope and the intercept obtained for the calibration curve. The recoveries ranged from 97.0% to 101%.

Table 1: Accuracy data for (S) – Trelagliptin.

(R)-isomer spiked level (%)	% Recovery
LOQ (n=3)	97.7
50 (n=3)	100.2
100 (n=3)	99.97
150 (n=3)	98.9

Precision (repeatability and reproducibility)

The repeatability of the method was evaluated by calculating the % RSD of the unwanted isomer in spiked samples for six replicate injections and the reproducibility was expressed in terms of % RSD in area obtained for analysis performed, six times. The precision studies for (S) - Trelagliptin were performed at the limit of quantification (LOQ) and at 150% of analyte concentration. The results were precise for estimation of the unwanted isomer (Table 2).

Table 2:

Ruggedness (Differeint day and different analyst) (R) -isomer (n=6)	**%RSD 0.72**
Precision (%RSD)	
LOQ (n=6)	1.42
150 % (n=6)	0.97

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

The limits of detection and quantitation were estimated by obtaining the detector signal to noise ratio. LOD and LOQ are determined by performing serial dilution of the S-isomer and by calculating the signal to noise ratio at that known concentration levels of solution. The limits of detection and quantitation were found to be 0.0001 µg/mL and 0.002 µg/mL respectively. A typical peak signal to noise ratios is between 2–3 at LOD level and 9–10 at LOQ level.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, i.e. change in flow rate by 0.1 mL/min,change in the ratio of mobile phase (2% absolute) and change in temperature (5°C) .The method was demonstrated to be robust over an acceptable working range of its HPLC operational parameters and results are shown in Table 3.

Table 3.

Flow rate(mL/min)	Effect of change in flow rate		
	Compound	**RT**	**Resolution**
0.9	(S)-isomer	20.1	0.0
	Trelagliptin	22.7	2.5
1.0	(S)-isomer	19.3	0.0
	Trelagliptin	21.7	2.3
1.1	(S)-isomer	18.4	0.0
	Trelagliptin	20.3	2.0

Composition(v/v/v/v) Hexane:Ethanol:DEA	Effect of change in Mobile phase composition		
	Compound	**RT**	**Resolution**
68:32:0.1	(S)-isomer	15.5	0.0
	Trelagliptin	17.3	2.01
70:30:0.1	(S)-isomer	19.3	0.0
	Trelagliptin	21.7	2.3
72:28:0.1	(S)-isomer	21.9	0.0
	Trelagliptin	22.8	2.4

Temperature change in (°C)	Column Temperature variation		
	Compound	**RT**	**Resolution**
25	(S)-isomer	20.8	0.0
	Trelagliptin	23.4	2.5
30	(S)-isomer	19.3	0.0
	Trelagliptin	21.7	2.3
35	(S)-isomer	17.7	0.0
	Trelagliptin	19.46	2.0

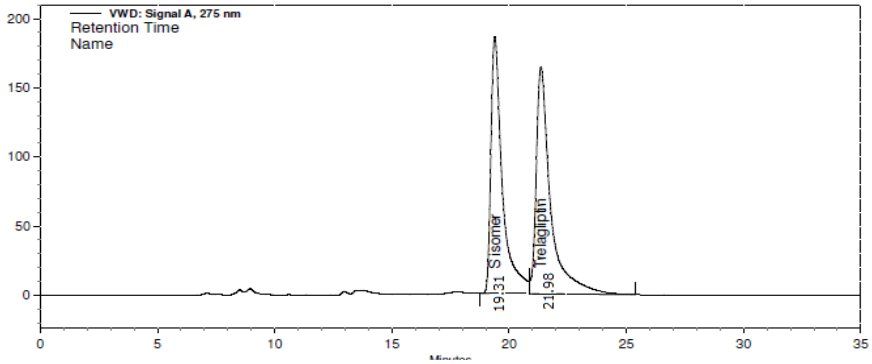


Fig a: Typical Chromatogram of enantiomeric separation of racemic trelagliptin.

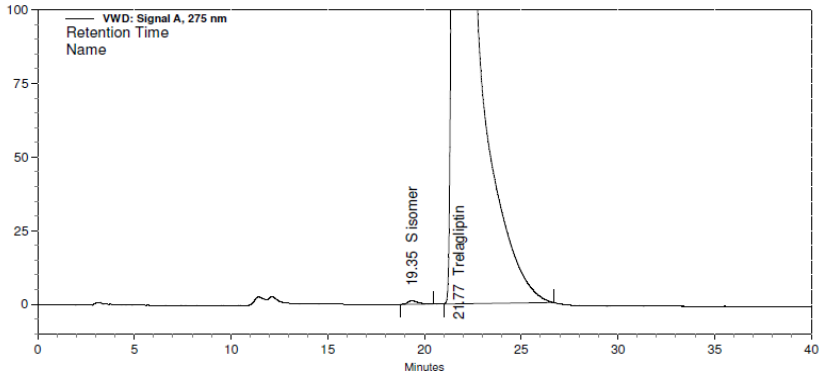


Fig b: Typical Chromatogram of System suitability solution.

Batch analysis

The (S)-isomer content in three batch samples of Trelagliptin as determined and found to be less than 0.15% m/m. Other related substances were evaluated by reverse phase HPLC method, known and unknown impurities are less than 0.1% and total impurities less than 1.0% (excluding (S)-isomer content by chiral stationary phase HPLC).The chromatogram showing racemic sample and System suitability solution with (S)-isomer (0.15%) are shown in Fig a & b.

Stability in solution

Standard solutions of (*S*) - Trelagliptin and (*R*) - Trelagliptin were prepared in the mobile phase at analyte concentration. Each standard solution was analyzed immediately after preparation (Fig. 4a and b) and divided into two parts. One part was stored at 2–8 ˚C in a refrigerator and the other at bench top in tightly capped volumetric flasks. The stored solutions of each isomer were reanalyzed after 24 h. No change in either the chemical or enantiomeric purity was observed. The area obtained for each isomer after 24 hours did not show any significant change compared with the area of initial analysis. This indicates that both isomers were stable in the mobile phase for at least 24 hours when stored either at 2–8 ˚C or at bench top.

CONCLUSION

A chiral HPLC method for the separation of Trelagliptin enantiomers was developed and validated. The chiral separation was achieved in cellulose carbamate derivatized column (Lux Cellulose-3, Phenomenex). This method is simple, accurate and has provided good linearity, precision and reproducibility. The results of analysis obtained with this HPLC method and a validated CE method are comparable. The practical applicability of this method was tested by analyzing various batches of the bulk drug and formulations of Trelagliptin.

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