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A validated new chiral LC method for the enantiomeric separation of vildagliptin

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ABSTRACT

A simple isocratic and chiral high performance liquid chromatographic method was developed for the determination of chiral purity of S-enantiomer of vildagliptin. Method involved Chiralpak-IC (250 x 4.6 mm) column at constant room temperature using ethanol and diethylamine mixture in the ratio of 100: 0.1 (v/v) as mobile phase with a flow rate of 0.5 mL min^{-1}. The resolution (R_s) between the enantiomers was found to be greater than 4.0 and interestingly the R-enantiomer was eluted prior to the S-enantiomer in the developed method. The limit of detection (LOD) and limit of quantification (LOQ) of the R-enantiomer was 0.15 and 0.5 µg mL^{-1}, respectively, for 10 µL injection volume. The percentage recoveries of the R-enantiomer ranged from 95 to 105 in the bulk samples of vildagliptin. The test solution and mobile phase observed to be stable up to 48 h after the preparation. The method was validated as per International Conference on Harmonization (ICH) guidelines in terms of LOD, LOQ, linearity, precision, accuracy, specificity and robustness. © 2009 Trade Science Inc. - INDIA

KEYWORDS

Vildagliptin;
Enantiomeric separation;
Chiral Liquid chromatography;
Validation and Quantification.

INTRODUCTION

Vildagliptin (Galvus®, Novartis Pharmaceuticals Corporation) is a new oral anti-hyperglycemic agent (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Vildagliptin inhibits the inactivation of GLP-1[1,2] and GIP[3] by DPP-4, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas. Vildagliptin has been shown to reduce hyperglycemia in type 2 diabetes mellitus[1].

The determination of the stereo isomeric composition of pharmaceuticals is rapidly becoming one of the key issues in the development of new drugs. Among the methods currently used to achieve chiral separation of enantiomers, high resolution liquid chromatographic systems based on chiral stationary phases, CSPs (direct methods) are more rapid and are suitable for the resolution of racemic mixtures of pharmacologically active chemical entities[3-5]. Several CSPs are now available to allow the direct separation and determination of drug enantiomers and recemates. Amylose CSPs are one of these commonly employed phases used for the separation and enantiomeric purity determination. The ability of chemically modified cellulose to separate a

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variety of racemates has recently been reviewed by Okamoto[6]. Enantiomeric inclusion in chiral cavities, which might be multiple, and competitive in cellulose and amylose based CSPs seems to be responsible for the chiral discrimination[7].

Vildagliptin is produced as a single isomer and that of the R-enantiomer could be present as a chiral impurity. Enantiomers of recemic drugs often differ in the pharmacokinetic behavior or pharmacological action[8].

Till date, there is no reported validated chiral HPLC method in the literature for the enantiomeric separation of vildagliptin. Therefore, it is very imperative to develop a simple and suitable analytical method for the enantiomeric measurement of vildagliptin in bulk drug samples. Such methods could be easily adapted for stability testing, routine and in-process quality control analysis or similar studies. Our purpose was to develop a simple, sensitive, and reliable method for enantiomeric determination of vildagliptin in bulk sample which can be applied in stability studies and quality control laboratories.

This article describes liquid chromatographic method for the enantiomeric separation of vildagliptin using an amylose based chiral stationary phase, Chiralpak-IC. The developed liquid chromatographic method was validated for determination of R-enantiomer in the vildagliptin bulk samples as per International Conference of Harmonization (ICH) guidelines[9-11].

EXPERIMENTAL

Chemicals and Reagents

HPLC-grade ethanol was procured from Tedia company, Inc., Canada. Diethyl amine was procured from Fluka. Samples of S and R-enantiomers of vildagliptin confirmed by spectral characterization and SOR (specific optical rotation) were obtained from Process Research Department of Dr. Reddy's Laboratories Ltd, Hyderabad, India. The chiral column used during the study was Chiralpak-IC (250 mm X 4.6 mm, 5 μm particle size) from Daicel Chemical Industries, Japan.

Instrumentation

Waters make an Alliance HPLC (Alliance 2695 Model, Waters Corporation, Milford, USA) equipped with in-built auto sampler and 2487 dual λ absorbance detector was used for the analysis. Photo diode array detector was also used for determining peak purity. The output signal was monitored and processed using Waters Millenium software.

The chiral columns used in method development were used Chiralcel OD (cellulose tris(3,5-di methyl phenylcarbamate) coated onto silica-gel), Chiralpak AD (amylose tris(3,5-dimethyl phenylcarmate) coated onto silica-sel), Chiralcel OJ (cellulose tris(4-methylbenzoate) coated onto silica-sel) and Chiralpak –IC (cellulose tris(3,5-dichloro phenyl- carbamate) immobilized onto silica-gel). All are Daicel make (Daicel Chemical Industries, Japan) with 5 μm particle size in (250 x 4.6) mm dimension.

Chromatographic Conditions

The chromatographic conditions were optimized using a Chiralpak IC column. The mobile phase, a mixture of ethanol and diethylamine in the ratio of 100: 0.1 mL with a flow rate of 0.5 mL min^{-1} was employed. The column temperature was maintained at 25 °C and the detection was monitored at a wavelength of 210 nm. The injection volume was 10 μL.

Preparation of Standard Solutions

Stock solution of S-vildagliptin, its R-enantiomer (2000 μg mL^{-1}) were prepared individually by dissolving an appropriate amount of the substances in the ethanol. Working solutions were prepared in ethanol.

Method Validation

As per ICH guidelines, the method was validated in terms of following parameters.

Specificity

Samples were exposed to light for an overall illumination of 1.2 million lux hours and an integrated near UV energy of 200 watt hm^2 which took 10 days in a photo stability chamber. Vildagliptin sample was exposed to thermal conditions at 60 °C for 10 days. The exposed samples were tested for peak purity using photo diode array detector.

Precision

The precision of the developed method was checked by injecting six individual preparations of 2000 μg mL^{-1} of vildagliptin spiked with 0.2 % of its, R-enantiomer. The percentage relative standard deviation

(%RSD) of peak area for the R-enantiomer was calculated. The intermediate precision of the method was also evaluated on a different lot of column, on a different instrument, by a different analyst, in different laboratories and the %RSD of peak area for six individual spiked preparations was calculated.

Limit of Detection and Limit of Quantification

Limit of detection (LOD) and limit of quantification (LOQ) for the R-enantiomer were estimated as the amounts for which signal-to-noise ratios were 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentrations. The precision of the method at LOQ was checked by analyzing six test solutions of the R-enantiomer prepared at LOQ level and %RSD of peak area was calculated.

Linearity

Linearity for the R-enantiomer was evaluated by determining six working sample solutions of R-enantiomer ranging from LOQ to 200% of the permitted maximum level of impurity (0.2%). (LOQ, 0.05, 0.10, 0.15, 0.20 and 0.40%). The peak area versus concentration of the R-enantiomer was subjected to regression analysis to calculate calibration equation and correlation coefficient.

Accuracy

Standard addition and recovery experiments were conducted to determine accuracy of the present method for the quantification of the R-enantiomer in bulk drug samples of vildagliptin. The recovery studies for the R-enantiomer were carried out in triplicate at 0.1, 0.2 and 0.3% of the vildaglitpin target analyte concentration (2000 $\mu g\ mL^{-1}$). The percentage recovery of the R-enantiomer was calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between enantiomers of vildagliptin was checked. To study the effect of flow rate on the resolution, 0.1 units of flow changed from 0.5 $mL\ min^{-1}$. The effect of column temperature on resolution was studied at 23 and 27 °C instead of 25 °C. The effect of change in percent ratio of additive was also studied.

Solution Stability

The solution stability of vildagliptin, its R-enantiomer was carried out by leaving both unspiked and spiked sample solutions at temperature 25°C on a laboratory bench for 48 h. Content of R-enantiomer was determined for every 6 h interval.

RESULTS AND DISCUSSION

Method Development and Optimization

The objective of our work is to develop a suitable and rugged HPLC method for the separation of vildagliptin enantiomers enabling the accurate quantification of R-isomer. The preliminary trials carried out in reverse phase chiral columns were not fruitful in the separation of these isomers. Subsequently, various chiral columns namely Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak IC of Daicel were employed in normal phase mode and polar organic mode. All the columns chosen for the study are of 250 nm length and 4.6 mm internal diameter. Various experiments were conducted to select the ideal stationary and mobile phases that would give the optimum resolution and selectivity for two enantiomers. While no separation was achieved on Chiralcel OD-H and Chiralcel OJ-H, chiral recognition noticed on Chiralpak AD column was not efficient. Poor resolution was observed on Chiralpak AD-H while using n-Heptane: ethanol (90:10 v/v) as mobile phase and no significant improvement observed in resolution by adding the additives like trifluoroacetic acid and diethyl amine. Very good separation was achieved on Chiralpak IC column using polar organic mode consisting of ethanol and diethyl amine (100: 0.1, v/v) mobile phase. The resolution between vildagliptin enantiomers was found to be 4.0.

The chiral selector contained in Chiralpak IC is cellulose tris (3,5-dichlorophenylcarbamate) immobilized on silica gel. Unlike coated columns, this immobilized column has universal solvent compatibility[12]. Polar organic solvents, typically ethanol, methanol and acetonitrile are widely used as mobile phases for chiral separation on the polysaccharide-derived CSPs. The use of solvents of this kind has several advantages such as the simplicity in mobile phase preparation, fast separations, compatibility with LC-MS (Liquid chromatography-mass spectrometry) and the possibility to enhance the sample solubility in the mobile phase. A basic additive

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(most commonly diethylamine) in the mobile phase is in general beneficial for the separation of chiral compounds of basic nature.

The separation of vildagliptin enantiomers on Chiralpak IC may be ascribed to the interaction between the solute and the polar carbamate group on the CSP. The carbamate group on the CSP can interact with solute through hydrogen bonding using the C=O and NH groups, which are present in both CSP and vildagliptin. In addition, the dipole-dipole interactions are possible between the C=O group on the CSP and C=O group on the vildagliptin. Further, the nature of the substituents on the phenyl ring of the CSP affects the polarity of phenyl groups. In this case, we believe that chloro substitution on phenyl ring of the CSP favored the chiral recognition between vildagliptin enantiomers.

The structure and configurations of R and S-isomers of vildagliptin are displayed in Figure 1. The chromatogram of the mixture of R and S-isomers and the chromatogram of S-isomer spiked with R-enantiomer are displayed in Figure 2(a) and Figure 2(b), respectively.

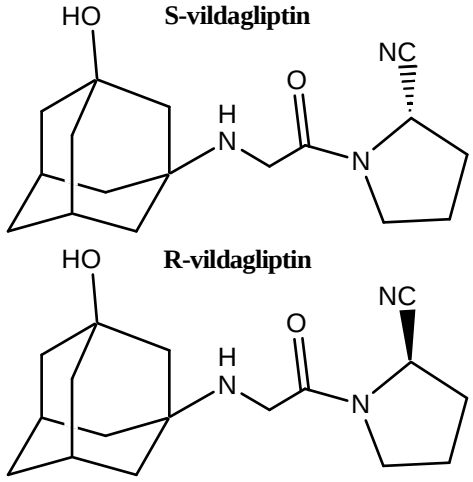

Figure 1 : Chemical structure of S and R-vildagliptin.

Validation Results of the Method

Specificity

It was observed that rigorous stress of the vildagliptin sample did not cause any significant degradation and change in the content of vildagliptin in a 10 days study period. Peak purity was obtained for vildagliptin and the R-enantiomer by using a photo diode array detector and no interference was observed in stress samples. Hence the developed method was found to be selective.

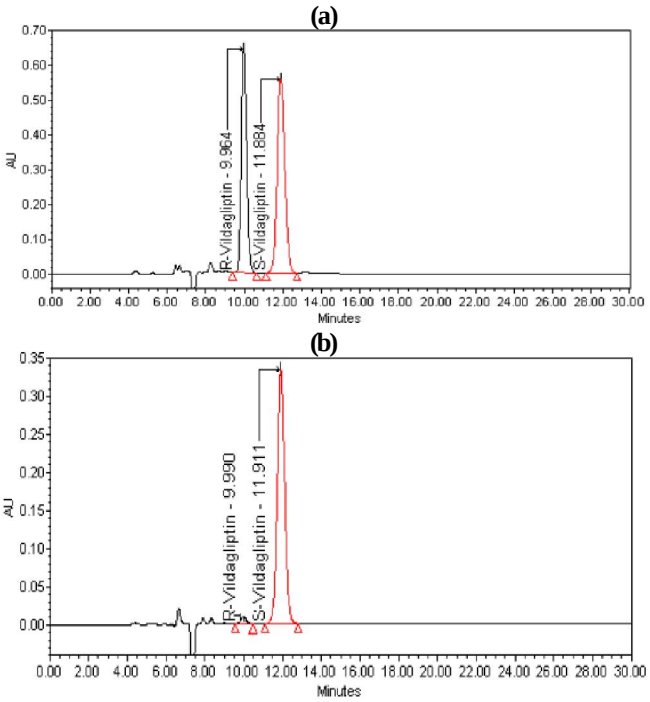

Figure 2 : (a) LC chromatogram of R and S-vildagliptin, (b). LC chromatogram of S-vildagliptin spiked with 1 % R-vildagliptin.

Precision

The %RSD for the area of R-enantiomer under precision study was found to be within 2.5 confirming the good precision of the method and for the intermediate precision study it was found to be within 3.0 confirming the ruggedness of the method.

Limit of detection and quantification

The LOD and LOQ concentrations were estimated to be 0.15 and 0.5 µg mL^{-1} for R-enantiomer, when signal-to-noise ratios of 3 and 10 were used as the criteria. The method precision for R-enantiomer at limit of quantification was less than 4.5 % RSD. The results are listed in TABLE 1.

TABLE 1 : Precision at the concentration of 0.5 µg mL^{-1}

Injection no.	R-vildagliptin area
01.	14880
02.	14444
03.	15855
04.	15311
05.	16270
06.	15393
Mean	15358
R.S.D. (%)	4.27

Linearity

The calibration curve was drawn by plotting the peak area of R-vildagliptin versus its corresponding concentration. The correlation coefficient was observed as 0.999. The results are listed in TABLE 2. Linearity was checked for the R - enantiomer over the same concentration ranges for two consecutive days. The percentage of RSD of the slope and Y-intercept of the calibration curves for the R-enantiomer were 3.6 and 4.8 respectively.

Accuracy

The percentage recovery of the R-enantiomer in bulk drug sample of vildagliptin ranged from 95 to 105.

Robustness

In all the deliberate varied chromatographic conditions carried out (flow rate, column temperature and mobile phase composition) the resolution between the enantiomers of vildagliptin was greater than 3.0 illustrating the robustness of the method.

Solution Stability

TABLE 2 : Linearity of R-isomer

S.No	Concentration of R-isomer ($\mu g\ mL^{-1}$)	R-vildagliptin area
01.	0.5	15393
02.	1.0	28177
03.	2.5	47683
04.	3.5	69240
05.	5.0	91449
06.	10.0	181846
	Correlation coefficient	0.999

No significant changes were observed in the R-enantiomer content of the vildagliptin sample during solution stability experiments when performed using the developed method. The solution stability experiment data confirms that sample solutions used during the study were stable up to 48 h.

CONCLUSION

A new chiral HPLC method was developed for the separation of two enantiomers of vildagliptin. Chiralpak IC column has shown excellent selectivity for vildagliptin enantiomers and the developed method is quite simple, sensitive, reproducible and can be used for determination of enantiomeric purity of vildagliptin. This method was found to be robust in the estimation of R- isomer.

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