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Research Article

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Method Development and Validation for Enantiomer in Sitagliptin Hydrochloride by RPHPLC

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ABSTRACT

Reversed phase chiral HPLC method for separation enantiomers of sitagliptin was developed and validated. These sitagliptin isomer were resolved on a chiralpak IC (250 mm × 4.6 mm, 5 µm) column by using liquid mobile phase of 10 mM ammonium acetate with 0.05% Di ethyl amine –acetonitrile (40:60 v/v).Both enantiomers are well resolved with the resolution of less than 3.0. Newly developed method was verified by validating according to ICH guidelines. Method was linear over the concentration range 0.0003 mg mL-1 to 0.0045 mg mL^{-1}(r^2=0.9991). The detection level and quantification level of the (s)-enantiomer were identified to be 0.0001mg mL^{-1} and 0.0003 mg mL^{-1},respectively for 20 µL injection volume. (S)-enantiomer recovery is in the ranged of 95.7 to 101.7 in API samples of sitagliptin. Both sample solution and mobile phase were stable for 48 hrs in this method. The final optimized method conditions were well established the separation of (s)-enantiomer from sitagliptin and demonstrated that the method is reproducible, accurate and robust for the quantitate estimation of enantiomeric purity of sitagliptin in API samples.

Keywords: Sitagliptin hydrochloride; (S)-Enantiomer; Reversed-phase high-performance liquid chromatography; UV detector; Validation; Active pharmaceutical ingredient

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INTRODUCTION

Sitagliptin(2R)-4-Oxo-(3-(trifluromethyl)-5,6-dihydro(1,2,4)triazolo(4,3-α)pyrazine-7(8H)-yl)-1-(2,4,5-trifluorophenyl)butane-2-amine (Figure 1) is a selective DPP-IV inhibitor for the treatment of type 2 diabetes mellitus. Type 2 diabetes is a long term metabolic disorder that is characterized by high blood sugar, insulin resistance and relative lack of insulin [1]. It enrich the glucagon-like peptides, other incretins and facilitates glucose-dependent insulin secretion. It improve the growth of insulin producing β-cells in pancreatic islets. Based on clinical findings that a diabetic individual is more likely to develop hypertension, which subsequently predisposes the patient cardiovascular diseases and many other complications [2-5]. Moreover, diabetic retinopathy will be developed with hypertension along with diabetes impairs the renal function. Hence blood pressure control is a primarily required for a hypertensive diabetic patient in order to preventive and prolong the subsequent manifestations (Figure 2).

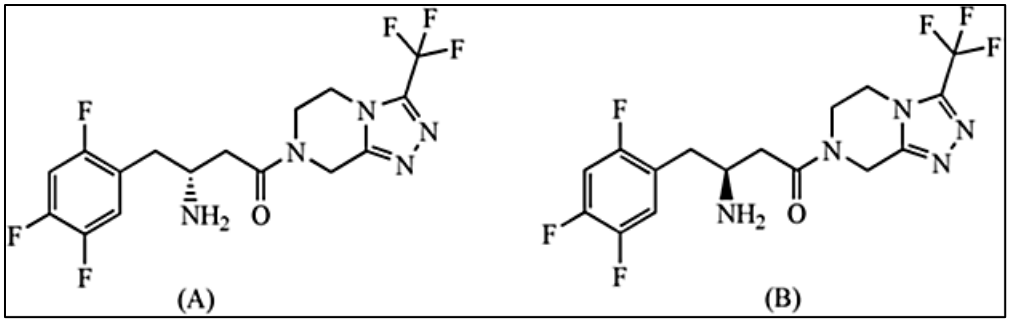

Figure 1: (A) Chemical structure of sitagliptin and (B) (S)-isomer of sitagliptin

As per literature search is suggested that number of high performance liquid chromatography methods (NPLC) have been reported for the determination of sitagliptin hydrochloride in drug product and druge substance [6,7]. In the synthetic process of sitagliptin there is possibility of carrying an undesired (s)-enantiomer of enantiopure sitagliptin, hence enantioselective analytical methods are very much required to ensure its therapeutic efficacy and safety [8-10].

Method development for the quantitate analysis of chiral compounds and assessment of enantiomeric purity is extremely challenging because the same physical and chemical properties of enantiomers make separating them very difficult. To the best of our knowledge, no RPHPLC method is available with shorter runtime as 15 min for the enantiomeric separation in sitagliptin. The pharmacopeia methods were much longer in run time. The European pharmacopeia method was 40 min for the determination of (s)-enantiomer in sitagliptin. This paper refers to a RPHPLC method for the separation of sitaglitpin enantiomers by using the modified cellulose chiral stationary phase, chiralpak IC [11,12]. The objective of this development is to fine tune the chromatographic conditions in terms of temperature, pH value of buffer solution and mobile phase composition. The developed sitaglitpin enantiomeric purity method was validated according to ICH guidelines for the quantitate determination of (s)-enantiomer in sitagliptin.

EXPERIMENTAL SECTION

Chemicals

Sitagliptin and (s)-Isomer of sitagliptinwere provided by synthetic laboratory of Dr. Reddy's laboratories Ltd., (Visakhapatnam, India). Acetonitrile was procured from Merck (Mumbai, India); Ammonium acetate was purchased from Merck (Mumbai, India); Diethyl amine was purchased from rankem.

Instrumentation and Chromatographic Conditions

HPLC system with make Agilent Infinity 1260 series and weights measurements are taken by using analytical balance with make Sartorius and model MSA 225S-100-DA. The mobile phase was filtered by using 0.45 µm PTFE filters and sonicated to degas. The chromatographic data is processed through empower-3 software. The chromatographic conditions were adjusted using a chiral stationary phase, Chiralpak-IC (250 mm × 4.6 mm, 5 µm). The isocratic mobile phase was a homogeneous mixture of 10 mM ammonium acetate with 0.05% DEA and acetonitrile (40:60 v/v), which was pumped through the column at flow rate of 1.0 mL min^{-1}. The column oven temperature was maintained at 25°C and the eluent was monitored at wave length 266 nm. The injection volume is 20 µL.

Sample Preparation

Sitagliptin test solution (2.0 mg mL^{-1}) and (s)-enantiomer (2.0 mgmL^{-1}) were prepared by dissolving the appropriate amount of the sample in the mobile phase. The test concentration of sitagliptin was fixed to be 2 mg mL^{-1}. Sitagliptin test solutions was spiked with low level of (s)-enantiomer were prepared by adding quantitate amounts of other unwanted enantiomer stock solution with pipette in to the quantitate amount of sitagliptin stock solution, and makeup to the volume with the mobile phase and sonicated the solution to mixed well.

RESULTS AND DISCUSSION

To develop a new robust and rugged RPHPLC method to resolve both enantiomers, different stationary phases and mobile phases were employed. Priliminary column screening involved with Chiralcel OD-3(250 mm × 4.6 mm, 3 µm); Chiral Pak ADH (250 mm × 4.6 mm, 5 µm); Chiral Pak IC (250 mm × 4.6 mm, 5 µm) with mobile phase of n-Hexane: IPA: DEA (400:600:0.5;v/v/v). No separation is achieved. Also employed the column of Chiral Pak IC (250 mm × 4.6 mm, 5 µm), which is immobilized column for normal phase and reversephase. This column is monitored for different mobile phases such as with 10 mM ammonium acetate adjusted pH 8.0 with DEA and methanol (50:50; v/v); 10 mM ammonium acetate pH adjusted with 8.0 with DEA and Acetonitrile (40:60; v/v); then finally with 10 mM ammonium acetate with 0.05% DEA and acetonitrile (40:60, v/v).Continued this buffer, which provided best resolution and selectivity between two enantiomers.

Method Validation

The described method is validated in terms of Limit of detection (LOD), Limit of quantification (LOQ), Linearity, recovery, stability, precision and accuracy according to the international conference on harmonization (ICH) guidelines.

Method Precision

Method reproducibility is determined by evaluating the repeatability of retention times and the component areas of each enantiomer. For this study sitagliptin (2 mg mL^{-1}) spiked with (s)-enantiomer (0.003 mg mL^{-1}) was analyzed six replicates. The R.S.D values are less than 0.5% for the areas of sitagliptin and (s)-enantiomer, less than 2.0% for the retention times. The values are as tabulated below. All these analytical data values indicated that the method is precise.

Detection Limit and Quantification Limit

The detection limit (LOD) is a minimum concentration of analyte which can be clearly distinguished from the baseline and assessed by signal to noise ratio method. The quantification limit (LOQ) is minimum concentrations of the analyte where analyte can be quantified with appropriate precision and accuracy, is assessed by signal to noise ratio. LOD and LOQ values were assessed by injecting the diluted (s)-enantiomer solution. The precision of the developed method for (s)-enantiomer was tested by injecting the six test solutions of the (s)-isomer prepared at LOQ level calculating the percentage relative standard deviation of the area. The LOD and LOQ concentrations were estimated to be 0.0001 mg mL^{-1} and 0.0003 mg mL^{-1} for the (s)-enantiomer respectively. The method precision for the (S)-enantiomer at LOQ was below 5.0% R.S.D. Hence this method has adequate sensitivity for the detection and estimation of the (S)-enantiomer in sitagliptin.

Linearity of (S)-Enantiomer

Detector response line was measured by preparing six linearity solutions of the (S)-enantiomerranges from the concentration of 0.0003 mg mL^{-1} (LOQ) to 0.0045 mg mL^{-1}(i.e., LOQ, 25%, 50%, 75%, 100%, 125% and 150%) in the mobile phase. The percent relative standard deviation of the slope and Y-intercept of the calibration curve was calculated .Good linearity of the (S)-enantiomer was estimated over six levels. The linear regression equation $y = 0.0967x + 279$.Correlation coefficient $r^2 = 0.9991$.The R.S.D of the slope and % Y-intercept of the calibration curve were 10.1 and 1.8.

Quantification of the (S)-Enantiomer in Samples (Recovery)

The standard (s)-enantiomer addition and recovery experiments were conducted for the sitagliptin samples(2 mg mL^{-1}) spiked with (S)-enantiomer in API samples in triplicate at 50% level (0.0015 mg mL^{-1}),100% level (0.0030 mg mL^{-1}) and 150% level (0.0045 mg mL^{-1}) (Table 1). Recovery was calculated and achieved from range of 95.7% to 101.7%.

Table 1: Evaluation of validation parameter

S.No	Validation parameter	Criteria	Result	Limit
1	Method precision	Resolution	6.7	NLT 2.0
		Tailing factor	1.2	NMT1.5
		%RSD for S-isomer area	0.51	NMT2.0
2	System precision	%RSD for S-isomer area	0.32	NMT2.0
3	LOQ Precision and Accuracy	Resolution	6.9	NLT2.0
		Tailing factor	1.2	NMT2.0
		%RSD of S-isomer area	4.8	NMT10
		Recovery at LOQ (%)	93.4	85 to 115
4	Linearity and recovery	Resolution	6.8	NLT2.0
		Tailing factor	1.2	NMT1.5
		Recovery at 50%	101.7	85 to 115
		Recovery at 100%	95.7	85 to 115
		Recovery at 150%	96.1	85 to 115
		Correlation	0.9991	NLT0.999
		%Y-intercept	1.8	± 5.0

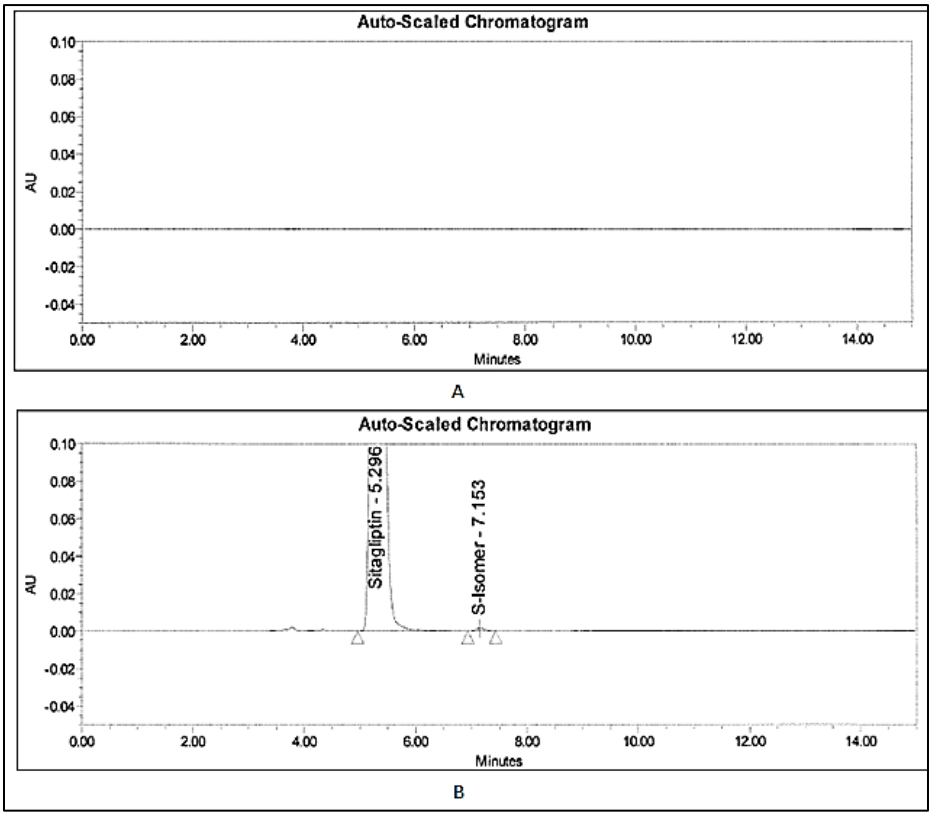

Figure 2: A) Blank, B) System suitability solution

CONCLUSION

This newly developed enantiomeric selective Sitagliptin reverse phase high performance liquid chromatography (RP-HPLC) method was simple, rapid and accurate, which is capable to separate the enantiomer impurity of sitagliptin. Method is accurate, precise, robust, selective and sensitive. The validated method can be used for the chiral purity as well as the quantitative determination of the chiral impurity in the bulk samples.

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