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Original Article

Chiral separation of sitagliptin phosphate enantiomer by HPLC using amylose based chiral stationary phase

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ABSTRACT

Background: Although a number of methods are available for evaluating sitagliptin phosphate (SGP), a common method for separation if its potential enantiomer and its possible impurities with good efficiency remains unavailable. With the objective of developing a method for rapid separation with shorter runtimes, a simple, precise, accurate stability indicating normal phase High-performance liquid chromatographic (NRP-HPLC) coupled with a photodiode array detector method was developed for the quantitative determination of (S)-enantiomer in API substance and as well as drug product.

Methods: The proposed novel method uses the mixture of n-heptane-ethanol-diethylamine (DEA) 35:65:0.1 (v/v/v) as a mobile phase. The enantiomer of sitagliptin phosphate was baseline resolved on a Chiralpak AD-H (250 mm×4.6 mm, 5 μm) column. The flow rate of the mobile phase is 1.0 mL/min and the detector wavelength monitored at 265 nm. The developed method was extensively validated.

Result: In these conditions, linearity over the concentration range 400-2250 ng/ml for (S)-enantiomer was obtained. The limit of detection and quantification were 150 and 400 ng/ml, respectively. The intra and inter-day precision was less than 1.5%. The recovery of (S)-enantiomer was within 107% in bulk drug.

Conclusion: The proposed method was found to be suitable, precise, and accurate for the quantitative determination of (S)-enantiomer in bulk drugs as well as in pharmaceutical formulations.

1. Introduction

The physiological environment within a living organism is mostly chiral. Therefore, chiral discrimination has been an issue in the development and use of pharmaceutical drugs. Enantiomers of racemic drugs often differ in pharmacokinetic behavior or pharmacological action.[1] In recent years, research has been intensified to understand the aspects of the molecular mechanism for stereoselective biological activities of the chiral molecules. The development of analytical methods for the assessment of enantiomeric purity is challenging due to the fact that enantiomers possess virtually identical

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properties.[2] In the pharmaceutical industry, much emphasis is put on chiral analysis. The reason is the potentially different behavior of the enantiomers of a chiral drug molecule after administration. To cause a therapeutic effect, an administered molecule has to interact with a target receptor. For chiral drug molecules only one enantiomer (the eutomer) will fit properly into this receptor, resulting in the desired therapeutic effect. The other enantiomer (the distomer) can either not interact or can interact less intense with the receptor, which generally causes a lower effect. Occasionally the distomer interacts with other receptors, causing side or even toxic effects. As a consequence, the enantiomers of drug candidates must be subjected to supplementary investigations during development processes: the eutomer has to be distinguished from the distomer during identification and impurity determinations of the drug substance. For drug products, it should be confirmed that the eutomer is present in the required dose while the distomer level should be analyzed as impurity, as prescribed in the guidelines imposed by the International Conference on Harmonisation (ICH), more precisely in guideline Q6A (decision tree number 5).[3,4]

According to the regulatory authorities, an enantioselective HPLC method should be able to separate the optically active drug substance from the enantiomeric impurity and other potential organic impurities. Potential organic impurities include chiral and/or achiral starting materials, intermediates and by-products from the drug substance manufacturing process. Enantiomers are strictly similar in structure to the active product ingredient (API). So, a chemo- and enantioselective HPLC purity appears a critical step in the development of high-quality manufacturing processes and quality-control methods. Sitagliptin Phosphate is chemically 7-[(3R)-3-amino-1-oxo-4-(2,4,5 trifluorophenyl) butyl]-5,6,7,8-tetrahydo-3-(trifluoromethyl)-1,2,4-Triazolo [4,3-a] pyrazine phosphate (1:1) monohydrate (Fig. 1),an oral antidiabetic agent that blocks dipeptidylpeptidase-4 (DPP-4) activity. Currently it is available in the market under the brand name of Januvia. Januvia is an orally-active inhibitor of the dipeptidylpeptidase-4 (DPP-4) enzyme. The DPP-4 enzyme inactivates incretin hormones, which are involved in the physiologic regulation of glucose homeostasis. By inhibiting DPP-4, Januvia increases and prolongs active incretin levels. This in turn increases insulin release and decreases glucagon levels in the circulation in a glucose-dependent manner. Januvia is specifically indicated for the improvement of glycemic control in patients with type II diabetes mellitus as monotherapy or combination therapy with metformin or a peroxisome proliferator activated receptor gamma (PPAR) agonist (e.g., thiazolidinediones) when the single agent does not provide adequate glycemic control. Several HPLC methods are reported for determination of sitagliptin phosphate in tablet dosage and combination with other drugs in pharmaceutical formulation, and plasma.[5–10] So far to our present knowledge no chiral HPLC methods were reported in the literature for the enantiomeric separation of sitagliptin and accurate quantification of its potential (S)-enantiomer. The aim of the present article describes the quantitative determination of S-enantiomer of sitagliptin phosphate in bulk drug samples by using normal phase chromatography.

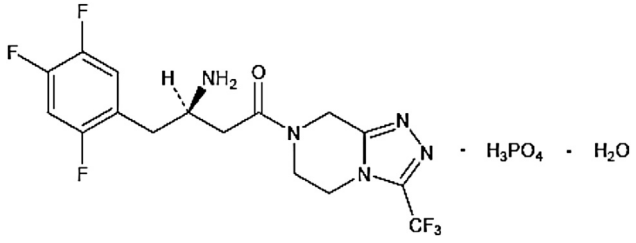

Fig. 1 – Chemical structure of sitagliptin phosphate monohydrate.

2. Experimental

2.1. Chemicals

Sitagliptin and its enantiomer were obtained by the Process Research Department of Hetero Drugs Limited, Hyderabad, India. Commercially available tablets containing 32.13 mg of sitagliptin phosphate monohydrate were purchased at a local drugstore. HPLC grade n-Heptane, ethanol was purchased Merck (Germany) were used to prepare the mobile phase, diethylamine from Rankem (India) of reagent grade quality.

2.2. Equipment

Agilent 1100 series (Germany) HPLC system equipped with degasser auto sampler, auto injector, thermostatic compartment, and photodiode array detector was utilized for method development and validation. The output signal was monitored and processed using Agilent Chemstation software.

2.3. Sample preparation

Stock solution of (S)-enantiomer (0.03 mg/mL) and sitagliptin phosphate (0.03 mg/mL) were prepared by dissolving the appropriate amount of the substances in methanol. The analyte concentration of sitagliptin phosphate was fixed as 2.0 mg/mL in mobile phase.

2.4. Chromatographic conditions

The chromatographic conditions were optimized using a amylose based chiral stationary phase Chiralpak AD-H (250 mm × 4.6 mm, 5 μm, Daicel make) which was safeguarded with a 1 cm long guard column. The mobile phase was n-heptane:ethanol:diethylamine (35:65:0.1, v/v/v). The flow rate was set at 1.0 ml/min. The column was maintained at 25 °C and the detection was carried out at a wavelength of 265 nm. The injection volume was 20 μL. Methanol was used as diluent. Cellulose based chiral stationary phases Chiralcel OD-H and Chiralcel OJ-H (Daicel make) were also employed during method development. All calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.