Validated High-Performance Liquid Chromatographic Method, for Separation of the Enantiomers of Sitagliptin Phosphate Monohydrate

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1 Abstract— A high-performance liquid chromatographic method has been developed for resolution of the enantiomers of sitagliptin phosphate monohydrate in the bulk drug. The isomers were resolved to baseline on chiralpak AD-H and Heptane: Ethanol (30: 70) with 0.1 % diethyl amine as a basic modifier. Resolution for the enantiomers was not less than 3.0. The method was extensively validated and proved to be accurate and precise. The calibration plot was indicative of an excellent linear relationship between response and concentration over the range 1 to 10 ppm for the S isomer. The limits of detection and quantification for the S isomer were 0.3 and 0.9 ppm, respectively, for an injection volume of 20 µL. Recovery of the S isomer from bulk drug samples of Sitagliptin Phosphate monohydrate was 97.12, 96.77, and 98.15% for addition of 0.5, 0.6, and 0.7%, respectively. The analytical solution was stable for 48 h. The method was found to be accurate, and suitable for quantitative determination of the S isomer in the bulk drug.

Index Terms— Column liquid chromatography, Enantiomer Separation of sitagliptin phosphate

2 Introduction

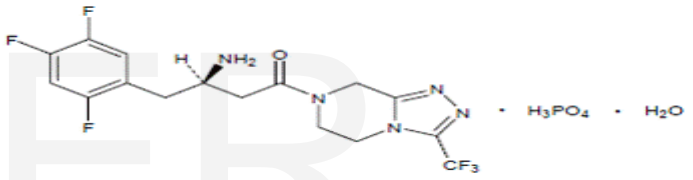
Sitagliptin phosphate monohydrate is a dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes with the chemical name 7-[(3R)-3-amino-1-oxo-4-(2,4,5trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine phosphate (1:1) monohydrate. This enzyme-inhibiting drug is used either alone or in combination with other oral antihyperglycemic agents for treatment of diabetes mellitus type 2 . Based on its unique mechanism of action, sitagliptin will provide practitioners with an additional tool in the treatment of diabetes. Review of the literature to date implies sitagliptin may be effective as monotherapy in type 2 diabetes. In addition, existing evidence supports the use of sitagliptin as adjunct therapy to sulfonylureas and metformin. Another advantage of sitagliptin use is that it appears to be free from the adverse effects of weight gain and hypoglycemia that are associated with currently available treatments [1-4]

Dipeptidyl peptidase-IV (DPP-IV) is a serine protease cleaving dipeptides from the N-terminal end of polypeptides with l-proline or l-alanine at the penultimate position and occurs as a soluble as well as membrane-bound form in many tissues and body fluids. DPP-IV either alters or abolishes many of the circulating biological peptides including glucose-dependent insulin tropic polypeptide (GIP) [5-7]

Sitagliptin phosphate monohydrate R isomer is required as a single isomer but the S isomer can be present as a impurity. Isomers of drugs often differ in pharmacokinetic behaviour or pharmacological action [8]. The term ‘isomer’ is used to describe two or more chemical compounds which can be represented by the same chemical formula. Enantiomers can be separated by use of a chiral stationary phase. In the literature there is no any evidence for the High-Performance Liquid Chromatographic Method, for Separation of the Enantiomers of Sitagliptin Phosphate Monohydrate

3 Experimental

4 Chemical and Reagents



The chemical structure of sitagliptin phosphate monohydrate is given in Fig. 1. Heptane was purchased from Ranbaxy Fine Chemicals (India); ethanol and diethyl amine is purchased from Fisher Scientific (India).

Figure 1
The chemical structure of Sitagliptin Phosphate Monohydrate

5 Chromatography

Method development and validation were performed with Agilent 1200 series. The system was controlled by EZ Chrom computer software. Compounds were separated on a 250 mm × 4.6 mm i.d., 5-µm particle, Chiralpak AD-H column. The mobile phase was a 30:70 mixture of heptane and ethanol containing 0.1 % diethyl amine as modifier. The mobile phase flow rate was 1.0 mL min^{-1}. The column was maintained at 40 °C temperature and the detector wavelength at 268 nm. The injection volume was 20 µL.

6 Standard Solutions

Solutions of Sitagliptin phosphate monohydrate and its S isomer were prepared by dissolving weighed quantities in the methanol. The final concentrations of the solutions were approximately 10 ppm for S isomer of Sitagliptin and 2000 ppm for Sitagliptin phosphate monohydrate.

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7 Method Development and Optimization

The objective of this work was separation and accurate quantification of the S isomer of Sitagliptin Phosphate monohydrate. A mixed solution containing approximately 10 ppm S isomer and 2000 ppm Sitagliptin phosphate monohydrate in the mobile phase was used for method development. To develop a rugged and appropriate HPLC method for separation of the enantiomers of sitagliptin, different mobile phases, stationary phases were evaluated. In all trials with other columns poor retention times and unsatisfactory resolution were obtained for the enantiomers of the sitagliptin.

A variety of experiments were conducted to select the best chiral stationary and mobile phases enabling optimum resolution and selectivity for the two isomers. No resolution was achieved on cyclodextrin, protein and cellulose based chiral columns by use of different mobile phases. When mobile phase containing heptane and ethanol in the ratio of 30: 70 containg 0.1 % diethyl amine as a modifier on a 250 mm × 4.6 mm, 5-µm particle, amylase based Chiralpak AD-H column. Addition of diethyl amine enhanced chromatographic efficiency and resolution of the isomers, resulting in very good resolution. In the optimized method, the results are indicative of suitability for the purpose intended.

8 Method Validation [9]

8.1 System Suitability

The performance of the method was determined by injecting a mixed solution of 10 ppm S isomer and 2000 ppm Sitagliptin phosphate monohydrate. Because the peaks of these isomers are a critical pair in the chromatogram, quantification criteria were that resolution of the two isomers should not be less than 3.0 and the tailing factor no more than 1.5, which ensure baseline separation of two isomers and symmetrical peak shape.

8.2 Linearity

The linearity of an analytical procedure is its ability to furnish responses which are directly proportional to analyte concentration. Linearity for the S isomer was determined in the range 1ppm to 10 ppm. The regression equation was obtained by plotting peak area against concentration.

8.3 LOD and LOQ

The limit of detection, defined as smallest amount of analyte that can be clearly detected above the baseline, was estimated as the amount for which the signal-to-noise ratio was 3. The limit of quantitation, defined as lowest concentration of analyte that can be quantified with acceptable precision and accuracy, was estimated as the amount for which signal-to-noise ratio was 10 . Solutions of the S isomer were prepared in the concentration range 0.5 to 5 ppm and LOD and LOQ were also calculated by use of the equations LOD = $3.3 \times S_{YX}/S$ and LOQ = $10 \times S_{YX}/S$, where S_{YX} is residual variance from the regression and s is the slope of the calibration plot.

8.4 Accuracy and Recovery

Standard addition and recovery experiments were conducted to determine the accuracy of the method for quantification of the *S* isomer in bulk drug samples. The study was performed in triplicate for amounts of the isomer equivalent to 0.5, 0.6, and 0.7% of the total Sitagliptin phosphate monohydrate concentration. Recovery of the *S* isomer was 97.12, 96.77, and 98.15%, respectively.

8.5 Stability in Solution

The stability, at room temperature, of solutions of Sitagliptin phosphate monohydrate and its *S* isomer in the methanol was evaluated by injecting the solutions at intervals of 1, 2, 12, 24, and 48 h. The overall relative standard deviation of the peak area was calculated for all the injections. The results showed the solutions were stable for at least 48 h.

9 Results and Discussion

Typical retention times, t_R, for Sitagliptin phosphate monohydrate and its *S* isomer were approximately 13.36 and 11.02 respectively and resolution between them, R_S, was 3.73. Separation of the isomers of Sitagliptin phosphate monohydrate on the Chiralpak AD-H column is shown in Fig. 2. The USP tailing factors for the isomers were 1.01 and 1.20, respectively. In the study of repeatability the relative standard deviations of the retention times of isomers were 0.57% for Sitagliptin phosphate monohydrate and 0.71% for the *S* isomer. In the study of intermediate precision, results showed that RSD was of the same order of magnitude as in the repeatability study.

The estimated limits of detection and quantification for the *S* isomer were 0.3 and 0.9 ppm, respectively, when signal to noise ratios of 3 and 10 were used as criteria. Method precision for the *S* isomer at the limit of quantification was better than 2.71% RSD. Good linearity was observed for the *S* isomer over the concentration range 1ppm to 10 ppm, coefficient $r = 0.99$. Standard addition experiments were conducted in triplicate to determine recovery of the *S* isomer from bulk samples at levels of 0.5, 0.6, and 0.7% of total analyte concentration. At these levels recovery was 97.12, 96.77, and 98.15%, respectively and RSD was 3.27%. No significant changes in the concentrations of Sitagliptin phosphate monohydrate and its *S* isomer were observed in methanol during testing of stability in solution. Solutions of Sitagliptin phosphate monohydrate and its *S* isomer were therefore stable for at least 48 h.

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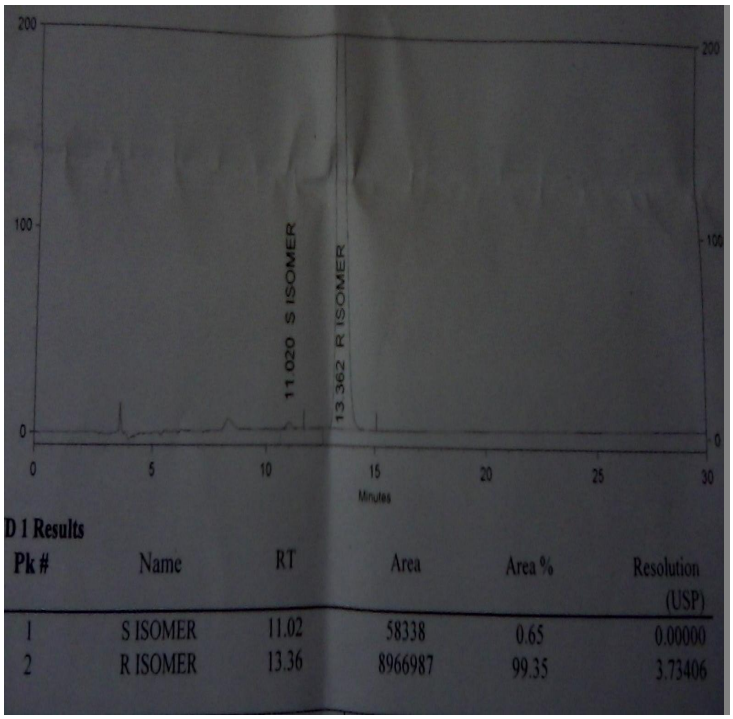

Fig. 2 Typical chromatogram obtained from Sitagliptin phosphate monohydrate bulk sample spiked with 0.50% of the *S* isomer, showing the resolution achieved. Peaks: 1 = Sitagliptin phosphate monohydrate, 2 = *S* isomer

10 Conclusion

A simple, specific, linear, precise, and accurate enantiomeric separation method has been developed and validated for analysis of the *S* isomer of Sitagliptin phosphate monohydrate in the bulk drug. Resolution was not less than 3.0, which is better than that of methods for the isomers.

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