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RPHPLC Method for the Estimation of Saxagliptin in Pure and its Tablet Dosage Form

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Abstract

A reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of saxagliptin in tablet dosage form. Chromatographic separation was achieved on a Thermo Hypersil BDS C18 (150 mm x 4.6 mm, 5 µm) column kept at 30 °C in isocratic mode. A mixture of 0.05 M ammonium acetate buffer and methanol (47:53 v/v) was used as a mobile phase at a flow rate of 1.0 mL/min. UV detection was performed at 210 nm. The developed method was validated based on the ICH guidelines for its specificity, linearity, accuracy, precision, limit of detection and limit of quantification. Satisfactory results were obtained from the validation studies. The developed method has been applied for the determination of saxagliptin in commercial samples. The proposed method is simple, rapid, sensitive, accurate and precise and hence can be used for routine quality control of saxagliptin in tablet dosage form.

Keywords: Saxagliptin, RP-HPLC, Method development, Validation.

1. Introduction

Saxagliptin, chemically (1S,3S,5S)□2□[(2S)□2□amino□2□(3□hydroxy□ 1□adamantyl) acetyl]□2□azabicyclo[3.1.0]hexane□3□carbonitrile (Figure 1), is a new oral hypoglycaemic (anti□diabetic drug) of the novel dipeptidyl peptidase□4 inhibitor class of drugs [1]. Dipeptidyl peptidase□4 inhibitors stand for a new therapeutic approach to the treatment of type 2 diabetes that acts to stimulate glucose-dependent insulin release and reduce glucagon levels. They hinder the inactivation of incretins, predominantly glucagon-like peptide-1 and gastric inhibitory polypeptide, thereby improving glycemic control [2].

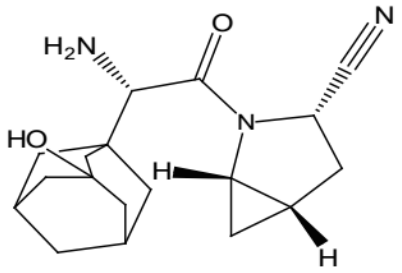

FIGURE 1: Chemical structure of saxagliptin.

Few analytical methods have been reported for the assay of saxagliptin in biological samples, bulk and pharmaceutical dosage forms alone or in combination with other medicines. Literature survey indicates that the drug can be determined by HPLC [4, 5], Liquid chromatography and tandem mass spectrometry [6, 7] and spectrophotometric methods [8, 9]. Simultaneous estimation of saxagliptin in combination with other drugs was done using spectrofluorimetric [10, 11], spectrophotometric [10, 11], high performance thin layer chromatographic [12] and HPLC [13-20] methods.

The present study describes an accurate, sensitive and precise RP-HPLC method for estimation of saxagliptin in pure and tablet formulation.

2. Experimental

2.1. Instrumentation. Waters 2695 HPLC system (Milford, Massachusetts) equipped with Thermo Hypersil BDS C18 (150 mm x 4.6 mm, 5 µm) column, Rheodyne injector with 25 µL loop, 2996 PDA detector and Empower-2 software was used. pH meter (Elico, model LI 120) and ultrasonic bath sonicator (Remi, India) were employed.

2.2. Reagents and Chemicals. Analytical grade ammonium acetate, HPLC grade milli-Q water and methanol were used. Saxagliptin was given as a gift from Novartis, Hyderabad, India. The tablets of saxagliptin were obtained from local pharmacy in India.

2.3. Preparation of Buffer Solution. To prepare the buffer solution, 3.855 g of ammonium acetate was accurately weighed and transferred into a 1000 mL volumetric flask. To get a 0.05 M ammonium acetate buffer pH 5.0, 200 mL of HPLC grade milli-Q water and 0.5 mL of glacial acetic acid were added and diluted to 1000 mL with the same solvent. The pH was adjusted with glacial acetic acid.

2.4. Chromatographic Condition. Saxagliptin was eluted in Thermo Hypersil BDS C18 (150 mm x 4.6 mm, 5µm) column using a mobile phase consisted of a mixture of ammonium acetate buffer, pH 5.0 and methanol in the ratio of 47:53 v/v at 30 ^{o}C. Detection was monitored at 210 nm. The injection volume was 25 µL and the total runtime was 8 min.

2.5. Preparation of Standard Stock Solution. Standard stock solution of saxagliptin was prepared by dissolving 100 mg of drug in 100 mL of methanol to get 1000 µg/mL.

2.6. Preparation of Sample Solution. Twenty tablets were accurately weighed, powdered and a portion of the powder equivalent to 10 mg/mL of saxagliptin was transferred into a 50 mL volumetric flask. It was dissolved in methanol and filtered through a 0.2 µm membrane filter. The filtered sample solution was diluted and used for the analysis.

After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded into the 25 µL fixed-sample loop of the injection port. The sample solutions were injected six times and the chromatograms were recorded.

2.7. Method Validation. The developed method was validated according to the ICH guidelines [21] for its specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness.

3. Results and Discussion

3.1. Method Development and Optimization: The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the analysis of saxagliptin in pure and in pharmaceutical dosage form. A variety of chromatographic parameters such as detection wavelength, pH of mobile phase, concentration of buffer solution, effect of composition of mobile phase, column temperature, flow rate and injection volume were examined and optimized. The wavelength was fixed at 210 nm based on the optimum response observed at the specified conditions.

The optimized mobile phase to achieve good resolution and symmetric peak shape for the drug was composed of ammonium acetate bu□er (50 mM ammonium acetate, adjusted to pH 5.0 with glacial acetic acid) and methanol in the ratio of 47:53 v/v. Likewise, the best signal was observed at a column temperature of 30 °C, an injection volume of 25 µL and a flow rate of 1 mL/min reducing the total runtime to 8 minutes on a Thermo Hypersil BDS C18 (150 mm x 4.6 mm, 5 µm) column.

System suitability studies were performed by injecting saxagliptin standard solutions in six replicates and system suitability parameters such as USP plate number - 3529, tailing factor – 1.12 and retention time – 3.02 minutes were estimated, which revealed acceptable results. The results of system suitability study and validation parameters are summarized in Table 1.

3.2. Method Validation

3.2.1. Specificity: The specificity of the proposed method was evaluated by injecting solutions of standard, sample and blank separately. The absence of interfering peaks of additives in a pharmaceutical formulation at the retention time of saxagliptin demonstrated the specificity of the method. Chromatograms of a standard, sample and blank solutions are shown in Figures 2, 3 and 4, respectively.

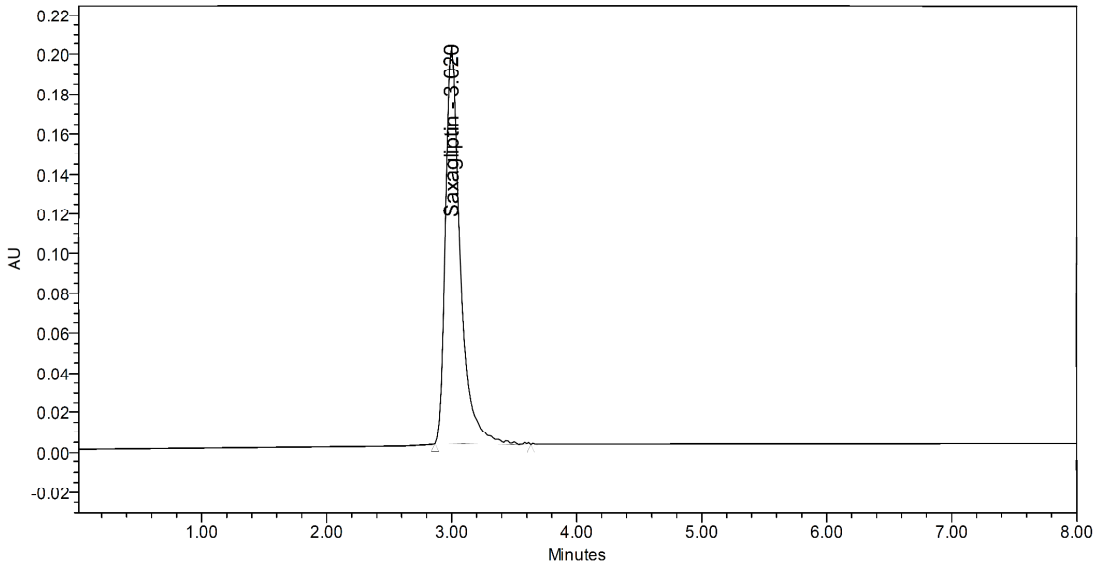

FIGURE 2: A typical chromatogram of saxagliptin standard.

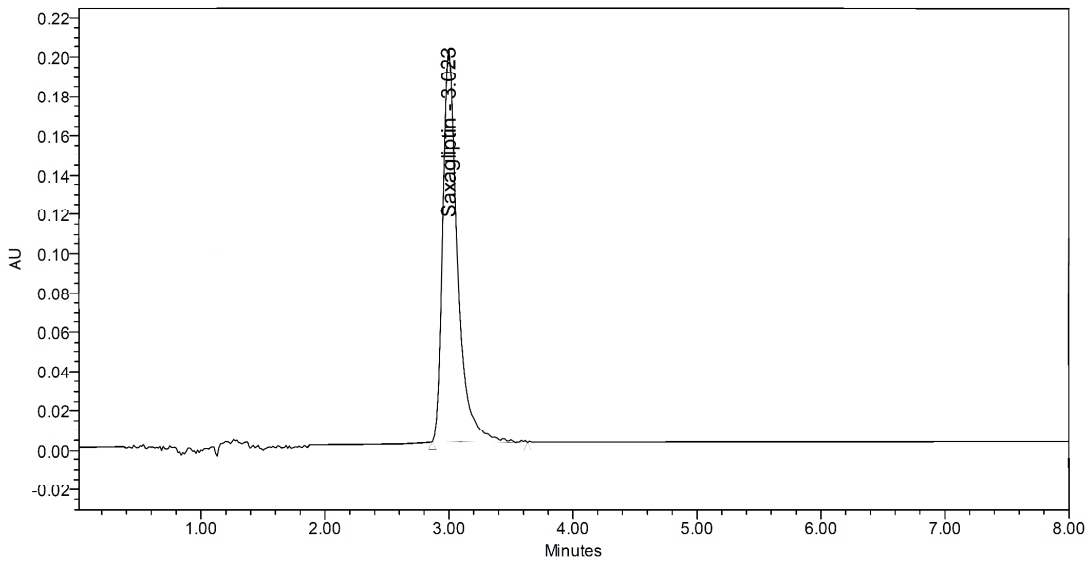

FIGURE 3: A typical chromatogram of saxagliptin sample solution.

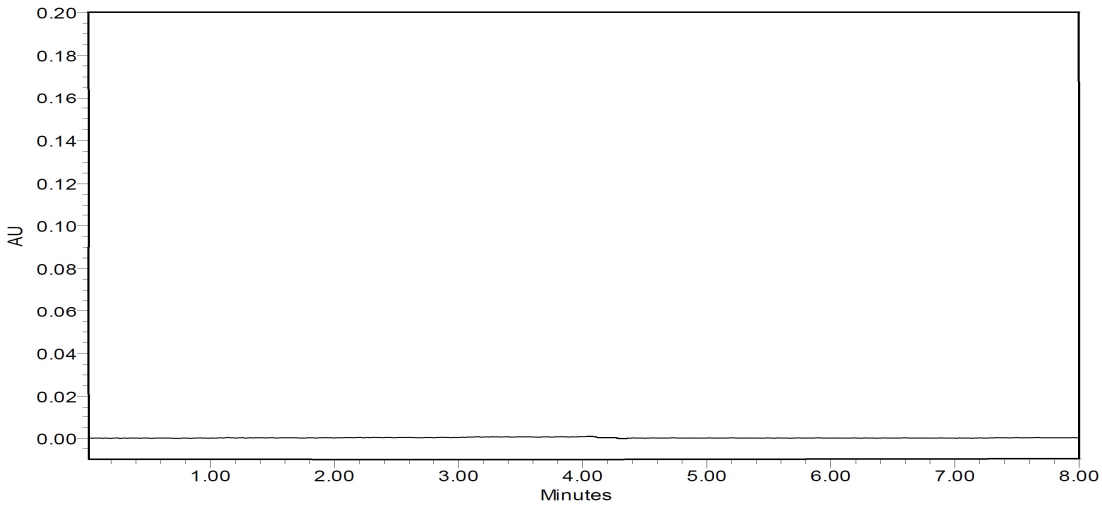

FIGURE 4: A typical chromatogram obtained from blank.

3.2.2. Linearity: Working standard solutions containing saxagliptin in various concentrations in the range of 10-150 µg/mL were prepared with the mobile phase and injected into the HPLC system. Calibration curve was constructed by plotting mean peak area against the corresponding drug concentrations. The detector response was found to be linear in the concentration range of 10-150 µg/mL, which was proved by high correlation coefficient (r^2 = 0.999).

3.2.3. Accuracy: The accuracy of the developed method was evaluated using recovery study by the standard addition technique. Known amounts of standard drug were added to the pre-analyzed sample

solutions and analyzed. Percent recoveries were in the range of 99.84% -100.52%, which show the excipients in pharmaceutical formulation do not interfere with the determination of saxagliptin.

3.2.4. Precision: Precision of the proposed method was evaluated by determining intra- and inter-day precisions as percent relative standard deviation (% RSD) on the peak areas. The intra- and inter-day precisions were estimated by analyzing the prepared samples on the same and three consecutive days, respectively. The % RSD values of the intra- and inter-day precisions were 0.01 and 0.02, and the findings indicated that the method is precise.

3.2.5. Sensitivity: The LOD and LOQ for saxagliptin were calculated based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. The LOD and LOQ values obtained were 0.045 and 0.137 µg/mL, respectively.

TABLE 1. Validation parameters and system suitability study.

Parameters		**Values**
Concentration range, µg/mL		10-150
Correlation coefficient (r^2)		0.999
LOD, µg/mL		0.045
LOQ, µg/mL		0.137
% Recovery*		99.84 - 100.52
Precision	Intra-day (n=6)	0.01
(% RSD)	Inter-day (n=18)	0.02
USP theoretical plates*		3529
USP tailing factor*		1.12
Retention time, min		3.02

* Average of six determinations.

3.2.6. Robustness: To verify the robustness of the developed method, the effect of small changes in flow rate of the mobile phase on the results was investigated. The influence of flow rate were evaluated by changing from 1.0 to 0.8 and 1.2 mL/min. At all varied conditions, the % RSD for the assay values (n=6) were below the acceptance limit of 2%. In addition, the values of tailing factor for the saxagliptin peak were < 1.5 indicating the method robustness. The results of analysis of variance demonstrated that the peak areas were not significantly ($p > 0.5$) affected by changing the variable. Therefore, the assay values of saxagliptin were not influenced by these small variations of flow rate (± 0.1 mL/min).

3.3. Application of the Method: Analysis of Commercial Samples

The developed HPLC method has been successfully applied to quantify saxagliptin in tablets. Mean content of 99.99% of the label claim was obtained, which was in good agreement with the label claim for the formulation.

The proposed method is more sensitive, precise and faster than the reported analytical methods in the literature. The developed method indicated lower limit of detection and quantification [5, 10] and analytical run time [5]. The shorter run time leads to the low volume of mobile phase consumption, which makes the method cost effective.

4. Conclusion

A sensitive, precise and accurate RP-HPLC method was developed for the estimation of saxagliptin in its dosage form. The method is simple with excellent sensitivity. As there is no official method, the proposed method can be used for routine analysis of the saxagliptin in pharmaceutical preparation.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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