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Research Article

RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SEXAGLIPTIN AND PIOGLITAZONE IN TABLETS

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ABSTRACT
A simple, selective, accurate high Performance Liquid Chromatographic (HPLC) method was developed and validated for the analysis of Sexagliptin and Pioglitazone. Chromatographic separation achieved isocratically on a C18 column [Use Inertsil C18, 5m , 150 mm x 4.6 mm] utilizing a mobile phase of acetonitrile/phosphate buffer (60:40, v/v, pH 7.0) at a flow rate of 0.8 ml/min with UV detection at 260nm.Aceclofenac was used as an internal standard. The retention time of Sexagliptin, pioglitazone and aceclofenac was 2.48, 4.45 and 6.34 min respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation.This study aimed at developing and validating an HPLC method, being simple, accurate and selective, and the proposed method can be used for the estimation of these drugs in combined dosage forms
Keywords: Sexagliptin, Pioglitazone, RP-HPLC, Validation.

INTRODUCTION

Saxagliptin (figure 1) is chemically (1S, 3S, 5S)-2-[(2S)-2-Amino-2- (3hydroxytricyclo [3.3.1.13, 7] dec-1-yl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile previously identified as BMS-477118 (figure 1), is a new oral hypoglycemic agent (anti-diabetic drug) of the new peptidyl peptidase - 4 (DPP-4) inhibitor class of drugs [1-8]. Saxagliptin recently approved for the treatment of type-2 diabetes mellitus [9-17]. Literature survey reveals that the drug can be estimated only by LC-MS/MS [18], Spectrophotometric method [19] and no HPLC method have been reported. The present study describes a simple, sensitive, accurate and precise HPLC method for the estimation of Saxagliptin in bulk and pharmaceutical dosage forms.

Several HPLC methods have been reported for determining pioglitazone hydrochloride (figure 1) in tablets [20-23]. The quantitative determination of pioglitazone in human serum by direct-injection HPLC mass spectrometry and its application to a bioequivalence study has also been reported[24]. Yamashita determined pioglitazone and its metabolites in human serum and urine[25] and Zhang and Lakings reported an assay method for Pioglitazone alone in dog plasma[26]. Potentiometric sensors were fabricated for the determination of pioglitazone in some pharmaceutical formulations .

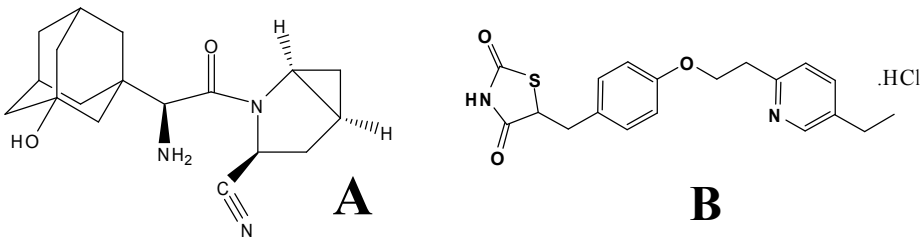

Figure 1. Chemical structures of (A) Sexagliptin (B) Pioglitazone

EXPERIMENTAL

Materials and Reagents

HPLC grade Sodium dihydrogen phosphate (NaH2PO4) disodium hydrogen phosphate Na2HPO4), acetonitrile-procured from Merck, India. High pure water was prepared by using Millipore Milli Q plus purification system.

Chromatographic Conditions

A High Performance Liquid Chromatography system, with LC solutions data handling system (Shimadzu-LC2010) with an auto sampler was used for the analysis. The data was recorded using LC 2010 solutions software. The purity determination performed on a stainless steel column 150 mm long, 4.6 mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5mm diameter (Inertsil C18, 5m, 150 mm x 4.6 mm, make: Shimadzu ltd, Japan) with the mobile phase containing acetonitrile and phosphate buffer in the ratio of 60:40 (v/v pH 7.0) at ambient temperature. Flow rate was kept at 0.8 ml/min and the elution was monitored at 260 nm. Standard stock solution (1mg/ml) of Sexagliptin and pioglitazone were prepared by dissolving 25 mg of drug in 25 ml of acetonitrile, separately. The solutions were suitably diluted with mobile phase to get mixed standard solution containing 25 µg/ml of Sexagliptin and 20 µg/ml of pioglitazone and 30 µg/ml of aceclofenac as internal standard

Twenty tablets (Trajenta, Aventis ltd, Mumbai) each containing 325 mg of pioglitazone and 400 mg of Sexagliptin were weighed, and powder equivalent to 25 mg of Sexagliptin was weighed accurately and taken into 25 ml volumetric flask. The drugs were extracted into acetinitrile volume was adjusted to 25 ml, vortexed and then filtered through 0.45 µ membrane filter. From this solution, further dilutions were made using mobile phase to get a final concentration of 25 µg/ml of Sexagliptin and 20 µg/ml of

pioglitazone, to this 30 µg/ml of aceclofenac as internal standard and this solution was used for the estimation. The concentration of the drugs was calculated (Table 1).

With the optimized chromatographic conditions, a steady baseline was recorded. The retention time of pioglitazone, Sexagliptin and aceclofenac was found to be 2.48, 4.45 and 6.34 min respectively. A typical chromatogram of sample solution is given in figure-2, figure 3&4 are individual injection and figure 5 is blank. Detection was done at 260 nm. The assay procedure was repeated for six times and mean peak area ratio and mean weight of standard drugs were calculated. The percentage of individual drugs found in formulation, mean, standard deviation in formulation were calculated and presented in table 1. The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

Table 1: Results of analysis of formulation and recovery studies

Drug	Amount mg/tab		% label claim	% Recovery
	Labeled	Found		
Sexagliptin	400	399.06 ± 1.045	99.80 ± 1.020	98.90 ± 0.815
Pioglitazone	325	324.01 ± 1.135	96.05 ± 1.095	96.01 ± 0.580

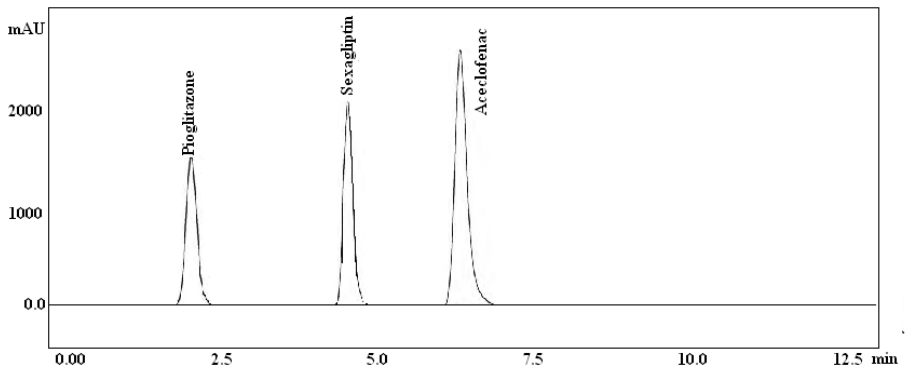

Figure 2: Typical Chromatogram of sample solution with internal standard

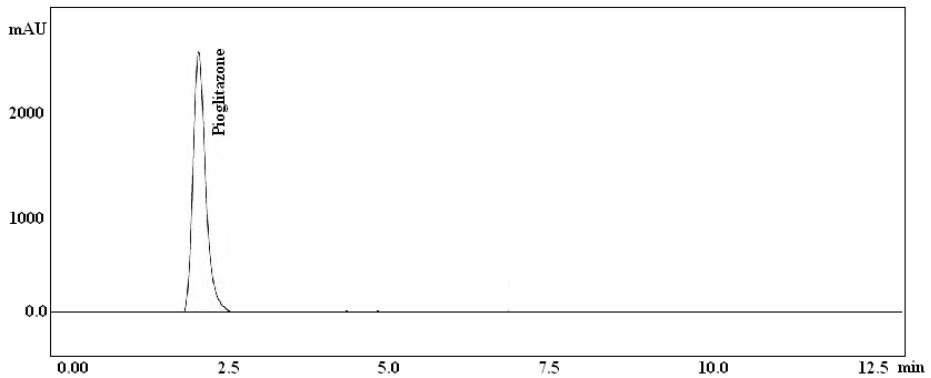

Figure 3. Chromatography of Pioglitazone

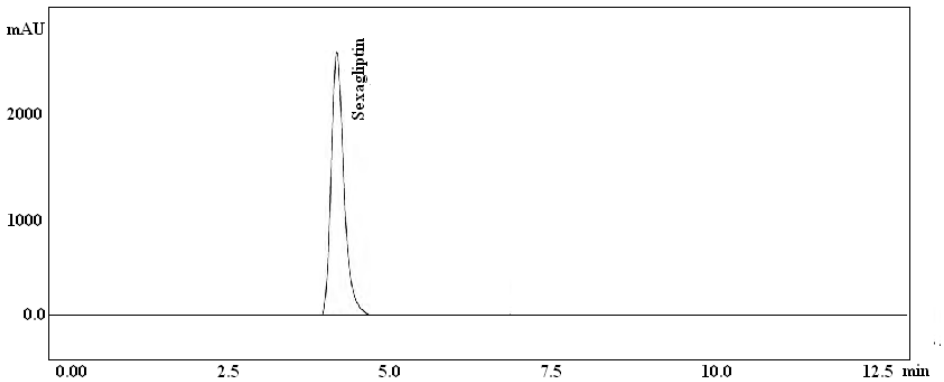

Figure 4. Chromatography of Sexagliptin

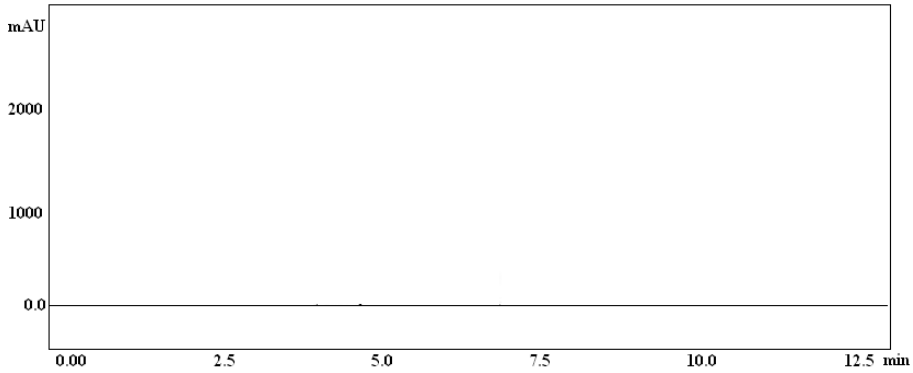

Figure 5. Blank

The method was validated as per ICH guideline. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery were calculated and presented in table 1. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated. From the data obtained, the developed HPLC method was found to be precise.

The linearity of the method was determined at seven concentration levels ranging from 20 to 80 µg/ml for Sexagliptin and 10 to 70 µg/ml for pioglitazone. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 0.0071x-0.001 (R2= 0.999) for Sexagliptin and y = 0.0061x+0.002 (R2= 0.998) for pioglitazone. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal-to-noise ratio of 3). The LOD for Sexagliptin and pioglitazone was found to be 6ng/ml and 10ng/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal-to-noise ratio of 10). The LOQ was 15 ng/ml and 25ng/ml for Sexagliptin and pioglitazone respectively (Table-2).

Table 2: Validation and system suitability studies

Parameters	Sexagliptin	Pioglitazone
Linearity and Range	20 to 80 µg/mL	10 to 70 µg/mL
Regression equation (y= mx + c)	y=0.0071x-0.001	y=0.0061x+0.002
Correlation coefficient	0.999	0.998
Theoretical plates	26458	28764
Resolution factor	1.30	1.30
Asymmetric factor	0.90	1.01
Tailing factor	1.2	1.2
LOD (ng/mL)	1.2	10
LOQ (ng/mL)	15	25

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC-2010), Agilent HPLC by different operators using different columns of similar type Intersil C18, Hypersil C18. Robustness of the method was determined by making slight changes in the chromatographic conditions. No marked changes in the chromatograms demonstrated that the HPLC method developed are rugged and robust.

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 hours at room temperature. The results show that for solutions, the retention time and peak area of Sexagliptin and pioglitazone remained almost unchanged(%RSD <2) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 hours, which was sufficient to complete the whole analytical process.

The system suitability studies were carried out to determine theoretical plate/meter, resolution factor, asymmetric factor and tailing factor. The results were given in the Table 2. The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ±3% standard deviation range during routine performance of the method. Thus the proposed RP-HPLC method for the simultaneous estimation of Sexagliptin and pioglitazone in combined dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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