Estimation of Sitagliptin Phosphate in Pharmaceutical Dosage Form Using RP- HPLC

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Abstract

The method development and validation of RP-HPLC for the estimation of Sitagliptin Phosphate in pharmaceutical dosage form was developed using Qualisil C_{18} BDS Column (150X4.5mm; 5µ) as stationary phase and potassium dihydrogen phosphate and Acetonitrile in a ratio 60:40% v/v at a pH 4.5 as mobile phase was maintained at a flow rate of 1.0 ml/min, the retention time of the drug was found to be 2.70 min and detection was carried out a 228 nm. The significant recovery and minimal coefficients of variation validate the method's appropriateness for concurrent examination of the drug in tablet form. The verified procedure worked well for the tablet's quantitative analysis as per ICH guideline.

***Keywords*:** Sitagliptin, RP-HPLC, Qualisil C_{18} BDS Column, Method development, Validation.

1. Introduction

This oral antihyperglycemic medication belongs to dipeptidyl peptidase-4 (DPP-4) inhibitor class is sitagliptin phosphate. It inhibits an enzyme and is used to treat type 2 diabetes either on its own or in conjunction with other oral anti-hyperglycemic medications (such metformin or thiazolidinedione). Sitagliptin inhibits the DPP-4 enzyme in a competitive manner. The gastrointestinal hormones known as incretins, or GLP-1 and GIP, are broken down by this enzyme in response to a meal. They are able to decrease the pancreas' production of glucagon and boost insulin secretion by blocking GLP-1 and GIP inactivation. As a result, blood glucose levels approach normal.

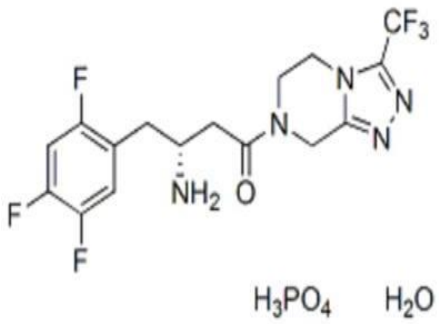

Figure 1. Chemical Structure of Sitagliptin Phosphate

Chemical Name of Sitagliptin Phosphate:- 7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl) butyl]-5,6,7,8 tetrahydro-3 (trifluoromethyl)-1,2,4-triazolo[4,3-a]pyrazine phosphate (1:1) monohydrate.

2. Materials and Methods

The strip of tablet, Sitagliptin were obtain from local market (Maker: Sun Pharma Laboratory) (Brand name: SITARED 100).

2.1 Chemicals and reagents Used: The following chemicals and reagents were used:

Table 1. Chemicals and reagents used

Sl. No.	Chemicals / Reagents	Grade	Company
1	Acetonitrile	HPLC	Purechem
2	Water	HPLC	HPLC-Grade
3	Potassium dihydrogen phosphate	Analytical	Rankem
4	Ortho phosphoric acid	Analytical	Qualigens

2.2 Instruments Used:

The method development was carried out by using Agilent HPLC (Model: 1100 Series) equipped with UV-Agilent detector and Qualisil C_{18} BDS Column (150×4.5mm; 5µm) was used. The mobile phase was consisted of Potassium dihydrogen phosphate: Acetonitrile in the ratio of 60:40 % v/v at pH 4.5. By using Orthophosphoric acid pH was adjusted with a flow rate of 1.0 ml/min and the effluent was detected at 228 nm. The volume of injection is 10.0 µl.

2.3 Preparation of Mobile phase:

The mobile phase was prepared by mixing 600.0 ml of Potassium dihydrogen phosphate and 400 ml of Acetonitrile (60:40) and its pH was adjusted to 4.5 with Orthophosphoric acid. The mobile phase was transferred to a 1000 ml bottle container of mobile phase and sonicated for 15 minutes in order to eliminate any remaining contaminants and undissolved gases which may cause undesired peaks in the chromatogram.

2.4 Preparation of Standard Solution:

About 64.25 mg of drug was precisely weighed and transferred to a clean and clear 100 ml volumetric flasks, the drug powder was dissolved by using some amount of the mobile phase and sonicated. The volume was adjusted with the mobile phase to yield solutions with a concentration of 500µg/ml.

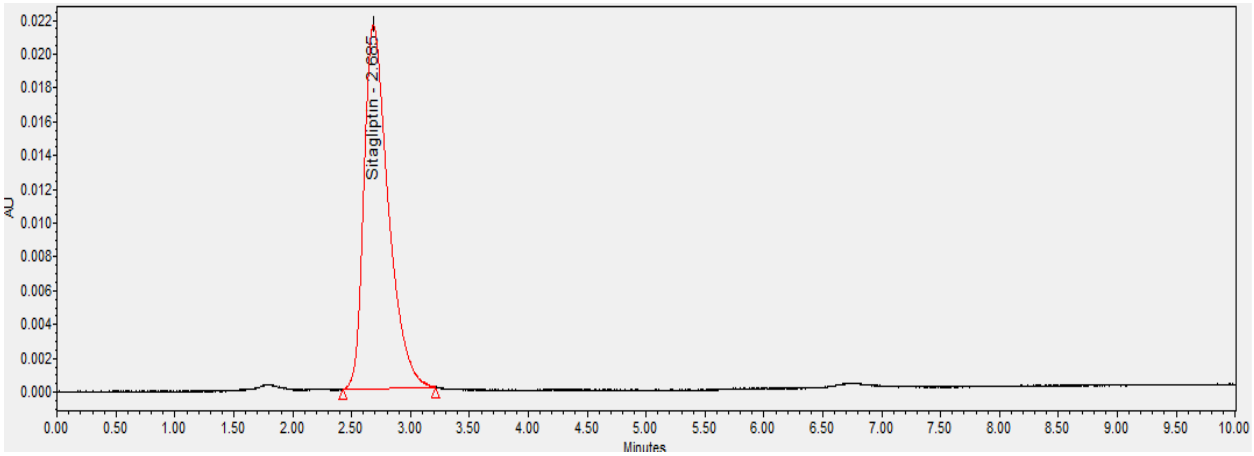

Figure 2. Chromatogram for Sitagliptin (Standard)

Table 2. Chromatographic Study of Sitagliptin (Standard)

Sl. No.	Standard Area	Retention Time
1	307920	2.685
2	310884	2.690
3	307636	2.684
Mean	308813	2.69

2.5 Preparation of Sample Solution:

Twenty tablets were weighed individually and powdered, equivalent to 50 mg of powdered drug was transferred to a 100 ml clean and clear. To this volumetric flask containing tablet powder was dissolved with mobile phase and allowed to sonication.

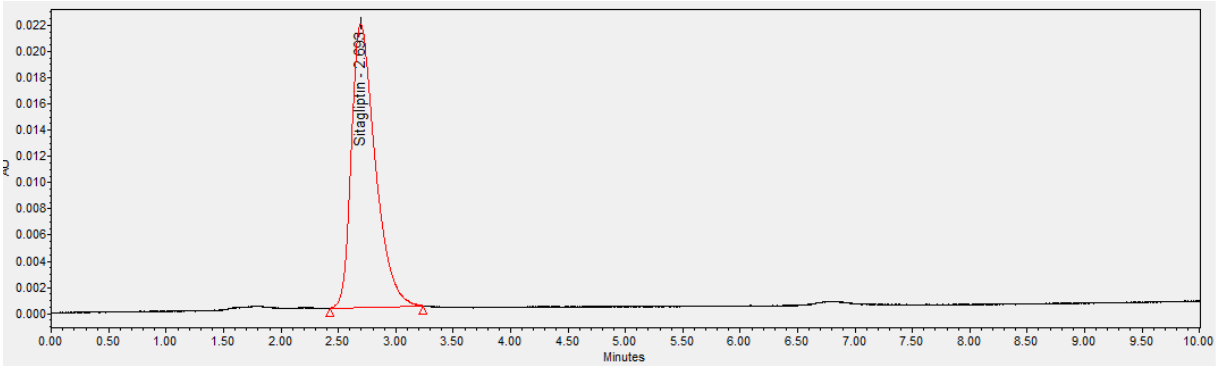

Figure 3. Chromatogram for Sitagliptin (Sample)

Table 3. Chromatographic Study of Sample

Sl. No.	Sample Area	Retention Time
1	311531	2.692
2	311906	2.693
3	308455	2.693
Mean	310631	2.7

2.7 System Suitability:

To perform the System Suitability test, 3 replicate injection of drug was injected into the system to observe the sharp peaks at retention time of 2.70 min.

Table 4. System Suitability Parameters

System Suitability Parameters	Result
Retention time (min)	2.70 min
Theoretical plates	2562
Tailing factor	1.5

3 Results and Discussion

3.1 Fixed Chromatographic Conditions:

Stationary phase	:	Qualisil C_{18} BDS Column (150×4.5 mm, 5µ)
Mobile phase	:	Potassium dihydrogen phosphate: Acetonitrile (pH adjusted upto 4.5) 60:40 %v/v
Detection wavelength	:	228nm
Temperature	:	Room temperature
Mode	:	Isocratic elution
Retention time	:	2.70 mins

3.2 Linearity

The calibration curve was analyzed using least squares linear regression to determine linearity. The conc. of 20.0 to 100.0 µg/ml was prepared and the calibration curve obtained which was found linear. Plotting peak regions against corresponding concentrations produced a curve that was subjected to linear regression analysis. It was discovered that the correlation coefficient was, respectively, 0.99961.

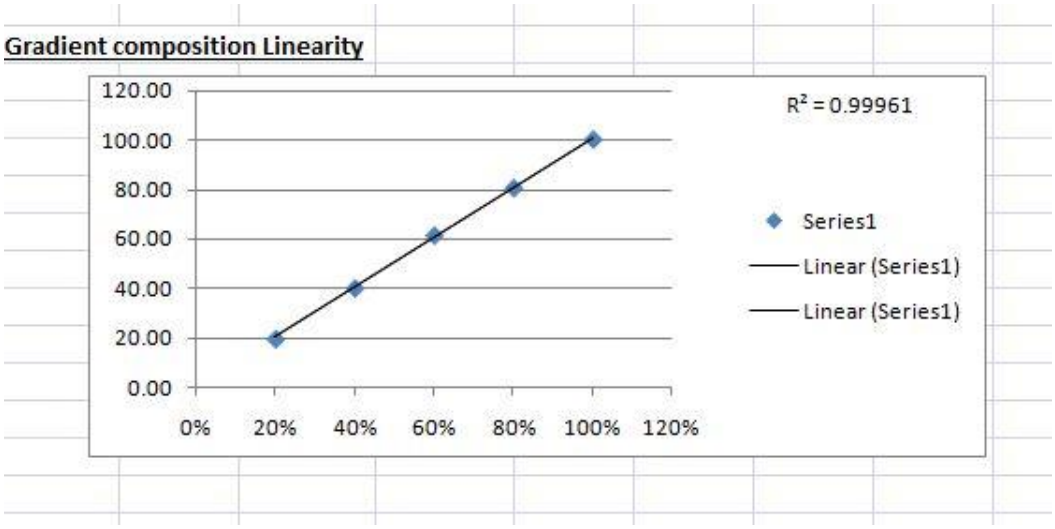

Figure 4. Linearity curve of Sitagliptin

Table 5. Result of chromatographic study for the sitagliptin linearity

Sl. No.	Concentration (µg/ml)	Peak Area
1	20	108290
2	40	220518
3	60	337195
4	80	441964
5	100	549338

3.3 Accuracy

The recovery studies were conducted by examining the samples by analyzing the added concentration of the drug as well as the measured concentration in order to assess the accuracy of the suggested approach. Every sample received three injections.

Table 6. Accuracy Data of Sitagliptin

Recovery	Average Area	% Average Recovery
80%	462691	98.49%
100%	569906	98.49%
120%	682806	98.49%

3.4 Precision

The precision include repeatability intraday and interday precision tests. The intraday (Repeatability) and interday precision experiments were finished by computing corresponding answers three times on the same day (Intraday) and three distinct days (Interday) for the three different concentrations of Sitagliptin. The precision study's findings were presented as a RSD%.

Table 7. Result of chromatographic study for the sitagliptin precision

Injection No.	Area of Sitagliptin	RT
1	308915	2.696
2	309756	2.700
3	312713	2.702
4	312808	2.705
5	310678	2.706
Mean	**310973.8**	
% RSD	**0.2**	
Std DV	**1746.4**	

Table 8. Sitagliptin Standard Solution for Inter-Day and Intra-day Precision

Sl. No.	Precision	Sitagliptin (%RSD)
1	Inter-Day	0.2
2	Intra-Day	0.2

3.5 Robustness

The physical parameters such as injection volume and wavelength, pH, Flow rate were varied, but the responses remained within the assay's bounds.

Table 9. Robustness Result of Chromatographic Study for Sitagliptin

Sl. No.	Condition	% RSD	RT
1	Flow rate 0.8 ml	0.1	0.0
2	Flow rate 1.2 ml	0.1	0.0

3	pH 3.5	1.1	3.2
4	pH 5.5	0.0	0.1

Summary and Conclusions:

The current study was aimed to offer an updated RP-HPLC method which will be sensitive, straight forward, accurate, and affordable. Without the interference of other formulation ingredients, it was effectively used for determination the concentration of Sitagliptin in pharmaceutical preparations. To provide a sufficient separation of eluted chemicals, this method's HPLC settings were optimized.

To obtain the best possible outcomes, a variety of mobile phase compositions were initially tried. Peak parameters-height, tailing, theoretical plates, run time, etc. were used to determine the mobile phase and flow rate. This system, which uses a flow rate of 1.0 ml/min and a mixed phosphate buffer with Acetonitrile (60:40) at pH 4.5 using Orthophosphoric acid, is very reliable. A better drug detector response was observed at 228 nm, which was the ideal wavelength for detection. It was shown that the average retention time for sitagliptin was 2.70 minutes. The calibration curve was linear at the concentration range of 20–100 µg/ml. The methods were précised and accurate at the low percentage R.S.D. For Sitagliptin, the mean recoveries were found to be 100.03%. By analyzing aliquots from homogeneous slots under similar operational and environmental settings by several analyzers, the robustness of the suggested procedures was assessed; the reported percentage R.S.D was shown to be less than 2%.

The proposed method was validated using ICH guidelines, and all of the approaches outcomes were extremely similar to one another and to the label value of pharmaceutical formulations used in commerce. As a result, the outcomes produced by the suggested strategy do not differ much. It is therefore recommended that the indicated isocratic RP-HPLC procedures be used successfully for the regular analysis of sitagliptin in tablet formulation.

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Conflict of Interest:

The authors report no conflicts of interest.

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