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Development and Validation of RP-HPLC Method for the Estimation of Sitagliptin Phosphate in Tablet Dosage Form

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Abstract:

A new simple, rapid, specific, accurate, precise and novel Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for the estimation of Sitagliptin Phosphate in the pharmaceutical dosage form. The chromatographic separation for Sitagliptin was achieved with mobile phase containing methanol, Thermoscientific C18 column, (250x4.6 particle size of 5µ) at room temperature and UV detection at 248 nm. The compounds were eluted in the isocratic mode at a flow rate of 1ml/min. The retention time of Sitagliptin was 1.91min. The above method was validated in terms of linearity, accuracy, precision, LOD and LOQ in accordance with ICH guidelines.

1. Introduction

Sitagliptin (Figure 1) is chemically (3R)-3-amino-1-[3-trifluoromethyl)-6,8-dihydro-5H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one (Jain Pritam et al., 2011, Baptist Gallwitz et al., 2007). It is an oral hypoglycemic agent which acts by inhibiting the proteolytic activity of dipeptidyl peptidase-4, thereby potentiating the action of endogenous glucoregulatory peptides, known as incretins (Daniel Ducker et al., 2007).

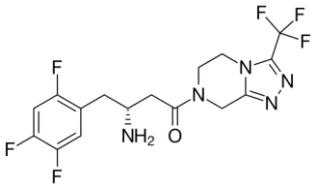

Figure 1. Structure of Sitagliptin

Literature survey revealed that there were few methods reported for estimation of sitagliptin individually and in combination with other drugs using spectrophotometric and RP-HPLC methods (A.S.K. Sankar et al., 2013, Nancy Veronica B et al., 2014, Geetha et al., 2015, K. Ganesh et al., 2016, T. Himabindu et al., 2016). Here is an attempt made to develop an economical RP-HPLC method for estimation of sitagliptin in tablet dosage forms.

2. Materials and methods

The API of sitagliptin phosphate was received from KP labs Hyderabad. A tablet strip (Brand name: Januvia) was purchased from local market. (Label Claim-100mg).

2.1 Chemicals and reagents used

All the chemicals and reagents were supplied by S.D. Fine Chemicals Ltd., India; Qualigens Fine Chemicals Ltd., Mumbai, India

2.2 Instruments used

Method development was carried out using Shimadzu HPLC (model SPD20A).

2.3 Selection of analytical wavelength

$\lambda_{max}$of the drug was determined by scanning the standard solution in the range of 200-400nm. Sitagliptin showed maximum absorbance at 248nm in methanol as solvent.

2.4 Selection of Mobile Phase

Several solvent systems (Figure 2-4) were tried to obtain symmetric peak for sitagliptin in the chromatogram. Peak of sitagliptin was optimum with the solvent system containing methanol.

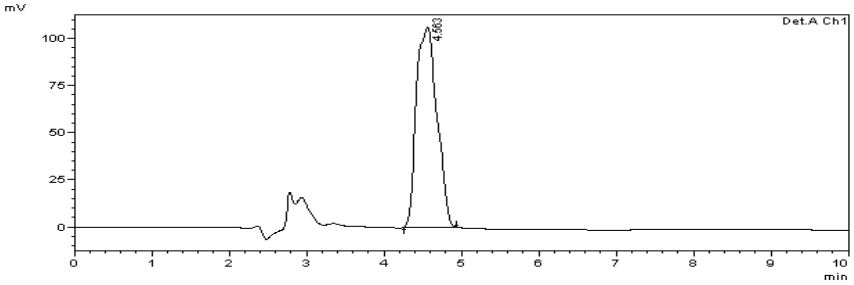

Figure 2. Acetonitrile and water (50:50)

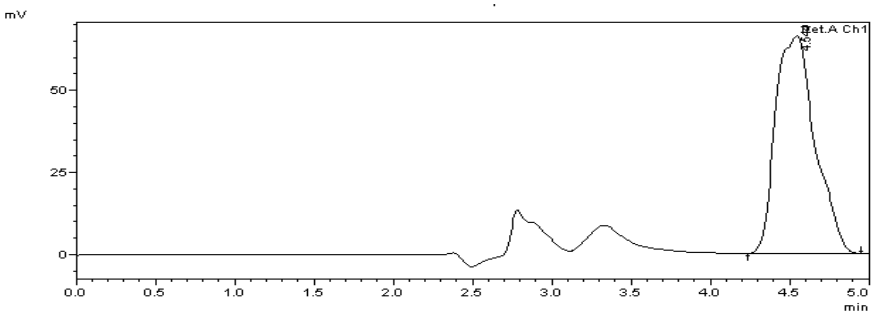

Figure 3. Methanol and water (50:50)

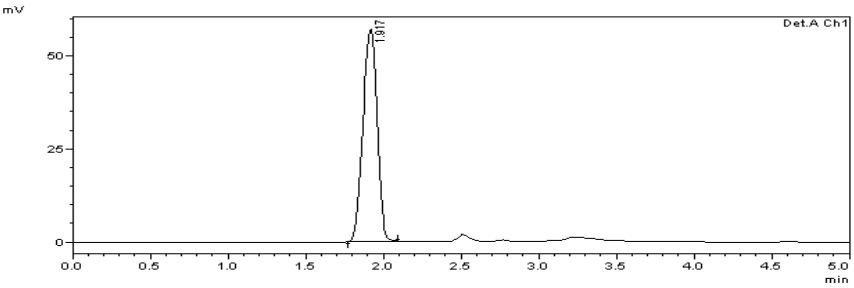

Figure 4. Methanol

2.5 Standard solutions of sitagliptin:

2.5.1 Standard stock solution:

Accurately weighed 100mg of sitagliptin was transferred into clean, dry 100ml volumetric flask and dissolved with sufficient volume of methanol. The volume was made up to 100ml with methanol and further dilutions were made for appropriate concentrations.

2.5.2 Sample stock solution:

Twenty tablets each containing 100mg of sitagliptin were weighed and powdered. A quantity of tablet powder equivalent to 50mg of sitagliptin was transferred to 100ml volumetric flask and dissolved in methanol. Volume was made upto the mark and filtered.

2.6 Validation Parameters

The method was validated according to ICH guideline for linearity and range, precision, accuracy, LOD and LOQ (ICH guideline, 1996, Rakesh K. Patel et al., 2012).

3. Results & discussion

3.1 Fixed chromatographic conditions

Stationary phase : Thermoscientific C18 ODS 250mm x 4.6mm, 5µm
Mobile phase : Methanol
Detection wavelength : 248 nm
Flow rate : 1 ml/ minute
Temperature : Room temperature
Mode : Isocratic elution
Retention time : 1.91min

3.2 Linearity And Range

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curve (Figure 10, Table 1) was linear over the concentration range of 5-15 µg/ml. Peak areas versus respective concentrations were plotted and linear regression analysis was performed on the resultant curve. The slope, intercept and correlation coefficient were found to be 32091, 4685, 0.997 respectively.

3.3 Precision

Precision was done by carrying out analysis of standard drug solution (Table 2) in the linearity range and %RSD was calculated. Low RSD value indicates that the method is precise.

Relative standard deviation – 0.25%

3.4 Accuracy

Recovery studies of the drug were carried out for determining accuracy parameter (Table 3). It was done by spiking the sample solution with standard solution at 80, 100 and 120**%**

Recovery: 98-102

3.5 Limit of detection (LOD) and Limit of quantification (LOQ)

LOD is used to describe the smallest concentration that can be reliably measured by an analytical procedure.

LOQ is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met.

LOD and LOQ of sitagliptin were calculated mathematically. The LOD and LOQ of sitagliptin were found to be 0.77µg/ml, 2.35µg/ml respectively (Fig 11).

3.6 Analysis of marketed formulation

The sample stock solution was taken and 1ml of filtrate was diluted to 100ml with methanol. By injecting into chromatograph, its peak area was determined. Using the calibration graph, percentage purity was calculated. It was found to be 98.84%

Table 1: Linearity data of sitagliptin

S No.	Concentration, µg/ml	Peak area
1	5	157860
2	7.5	250509
3	10	330089
4	12.5	410550
5	15	478979

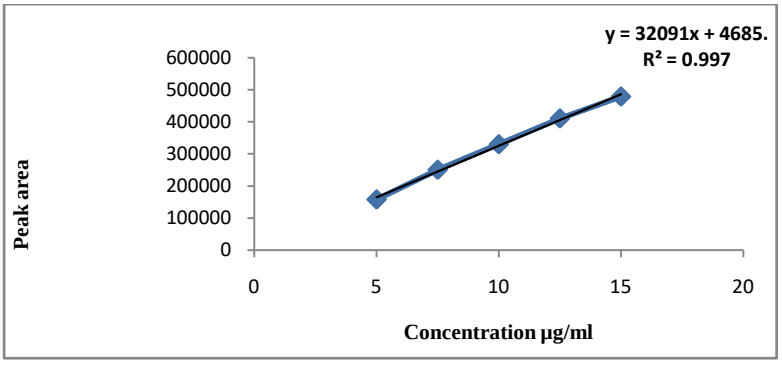

Figure 5. Calibration graph of Sitagliptin

Table 2: Precision data of sitagliptin

S No.	Concentration, µg/ml	Peak area
1	5	157860
2		157750
3		157926
4		158032
5		157386
6		**158620**

Table 3: Accuracy data of sitagliptin

S No.	Amount taken, µg/ml	Amount added, µg/ml	Peak area	Amount recovered, µg/ml	% Recovery
1	5	9ppm	293054	8.985977	99.52
2	5	10ppm	331089	10.1712	101.7
3	5	11ppm	357086	10.9813	99.06

Table 4: Linearity, LOD, LOQ

S.No.	Concentration (µg/ml)	Peak Area
1	5	157860
2	7.5	250509
3	10	330089
4	12.5	410550
5	15	478979
Parameters:		
Linearity range (µg/ml)	5-15	
Correlation coefficient	0.997	
Slope	32091	
Standard deviation	7571.3	
LOD (µg/ml)	0.77	
LOQ (µg/ml)	2.35	

4. Conclusion

The developed isocratic LC method is economic and offers simplicity, precision, and accuracy. In the proposed method symmetrical peaks with good resolution were obtained and this method was validated according to ICH guidelines. Hence, it can be applied for routine analysis of formulation.

Conflict of interest

None declared

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