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Research Article

UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF SITAGLIPTIN PHOSPHATE IN BULK AND TABLET DOSAGE FORM BY ABSORPTION RATIO AND AREA UNDER THE CURVE METHOD

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DOI: 10.7897/2321-6328.01407

Article Received on: 25/10/13

Accepted on: 20/11/13

Abstract

To develop two simple UV spectrophotometric methods for simultaneous estimation of Sitagliptin phosphate (STG) in bulk and tablet dosage forms and validates as per ICH guidelines. Method A involved Absorbance maxima method which based on the measurement of absorbance at λ max of Sitagliptin phosphate 267 nm and Method B involved Area under the curve (AUC) method which based on the measurement of AUC in the range of 261-270 nm. The developed methods were validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines. Both the methods were found to be linear within the conc. range of 20-160 µg/ml for Sitagliptin phosphate. The present methods were found to be simple, linear, precise, accurate and sensitive and can be used for routine quality control analysis for the estimation of Sitagliptin phosphate in bulk and tablet dosage form.

Keywords: Sitagliptin phosphate (STG), Absorbance ratio method, Area under curve method (AUC) and ICH guidelines.

INTRODUCTION

Sitagliptin phosphate (STG) is the first of a new class of drugs i.e. oral dipeptidyl peptidase-4 (DPP-4) inhibitor for the treatment of type II diabetes which improves glycaemic control by inhibiting DPP-4 inactivation of the increatin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin tropic polypeptide (GIP). This increases active increatin and insulin levels and decreases glucagon levels and post-glucose-load glucose excursion.[1-3] Chemically it is known as (2R)-1(2,4,5-triflourophenyl)-4-oxo-4-[3(trifluoromethyl)-5,6-dihydro-[1,2,4]-triazolo-[4,3-a]-pyrizin-7(8H)-yl]butan-2-amine (Figure 1). Sitagliptin phosphate can be estimated by different analytical techniques such as UV spectrophotometry[4-10], RP-HPLC[9-15] HPTLC[16-17] and LC-MS[18-20] alone or in combination with other agents. Because of cost-effective and minimal maintenance, UV spectrophotometry is always preferred at small scale industries. Literature survey reveals that so far many UV spectrophotometric methods have been reported for the estimation of Sitagliptin phosphate in alone or in combination with other drugs. But out of them only few methods included single estimation of Sitagliptin phosphate. Therefore the main objective of the proposed methods were to develop simple, new and economic UV spectrophotometric methods for the estimation of Sitagliptin phosphate in bulk and tablet dosage form and validate the same as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

The pure API sample of Sitagliptin phosphate was obtained as free gift sample from Getz Pharma Ltd; Thane while double distilled water used for whole experiment. The marketed combined pharmaceutical dosage form of Sitagliptin phosphate (100 mg) i.e. Januvia (MSD India Pvt. Ltd.) tablet was purchased from local market.

Instrumentation

A Jasco double beam UV–visible spectrophotometer, Model: V-630, with a fixed bandwidth (2 nm) and 1-cm quartz cell was used for Spectral and absorbance measurements.

Preliminary solubility studies of drug

1 g of Sitagliptin phosphate was weighed and solubility was checked in 10 ml distilled water, methanol, 0.1 N NaOH and 0.1 N HCl. The drug was found to be freely soluble in water, 0.1 N NaOH and 0.1 N HCl. Therefore distilled water was selected as diluent and Sitagliptin phosphate was also found to be stable in distilled water for 48 hours in stability studies.

Preparation of standard stock solutions

Transfer 25 mg of pure Sitagliptin phosphate in separate 25 ml of volumetric flask containing distilled water as diluent and then sonicated for 15 minutes and final volume made up to mark with same diluent to form 1000 µg/ml std. stock solution of Sitagliptin phosphate.

Preparation of calibration curve

From above std. stock solution of Sitagliptin phosphate (1000 µg/ml), pippete out aliquots 0.2 to 1.6 ml of Sitagliptin phosphate and transferred to series of 10 ml volumetric flasks and final volume made up to mark with distilled water as diluent to form solutions of 20 to 160 µg/ml of Sitagliptin phosphate. These solutions were then scanned in the range of 200-400 nm against diluent as blank. The absorbance maxima (λ max) were found to be 267 nm for Sitagliptin phosphate and then calibration curve was plotted as absorbance vs concentration.

Sample preparation for analysis of Tablet formulation

Twenty tablets (Januvia) containing 100 mg of Sitagliptin phosphate weighed, average weight calculated and triturated to fine powder and then weight equivalent 100 mg of Sitagliptin phosphate transferred to 100 ml of volumetric flask containing proposed diluent, then sonicated for 15 minutes and final volume made up to mark with diluent to form 1000 µg/ml of Sitagliptin phosphate stock solution and then filtered through Whatman filter paper no. 42. From this, 1 ml of aliquot transferred in 10 ml of volumetric flask containing diluent to form 100 g/ml of Sitagliptin phosphate stock solution and scanned in the range of 200-400 nm against distilled water as blank at 267 nm and then drug content of solution was calculated by using standard calibration curve.

Absorbance maxima method

For the selection of analytical wavelength, standard solution of Sitagliptin phosphate was scanned in the spectrum mode from 200 nm to 400 nm separately. From the spectra of drug, λ max of STG, 267 nm was selected for the analysis (Figure 2). Aliquots of standard stock solution were made and calibration curve was plotted.

Area under curve method

For the determination of Sitagliptin phosphate using the area under curve (AUC) method, suitable dilutions of the std. stock solutions (1000 µg/mL) of Sitagliptin phosphate were prepared in distilled water and scanned in the range of 200 - 400 nm. For Area under curve method, the sampling wavelength ranges from 261-270 nm. (Figure 3) selected for estimation of Sitagliptin phosphate and area were integrated between these selected wavelength range, which showed linear response with increasing concentration hence the same wavelength range were used for estimation of tablet formulations.

Validation

The present UV spectrophotometric methods were validated for linearity, precision, accuracy, LOD and LOQ as per ICH guidelines[21] for estimation of Sitagliptin phosphate in bulk and tablet dosage form.

Linearity

From std. stock solutions of Sitagliptin phosphate (1000 µg/ml), pipette out aliquots of 0.2 to 1.6 ml of Sitagliptin phosphate transferred to series of 10 ml volumetric flasks and final volume made up to mark with methanol as diluent to form solutions of 20 to 160 µg/ml of Sitagliptin phosphate. These solutions were then scanned in the range of 200-400 nm against diluent as blank at λ max of Sitagliptin phosphate and then calibration curve was plotted as absorbance vs concentration to check the linear relationship between absorbance and concentration of Sitagliptin phosphate.

Precision

Precision study expressed by carrying out Repeatability (intraday precision) and interday precision. The intraday (Repeatability) and interday precision study were carried out by estimating corresponding responses three times on the same day and on the three different days for the three different concentrations for (20, 40 and 60 µg/ml) for Sitagliptin phosphate. The results of precision study were reported in terms of % relative standard deviation.

Accuracy

The accuracy of developed method was carried out by calculating the % recovery of Sitagliptin Phosphate by standard addition method at three different levels i.e. 80 %, 100 % and 120 %. Known amount of standard solutions of STG (32, 40 and 44 µg/ml) were added to pre quantitated sample solutions of 40 µg/ml of STG.

LOD and LOQ

Limit of detection (LOD) is defined as lowest concentration of analyte that can be detected while limit of quantitation is defined as lowest concentration of analyte that can be quantitated. With suitable precision and linearity, LOD and LOQ can be calculated from the following formulas

$$LOD = 3.3 * r/S \text{ and } LOQ = 10 * r/S$$

Where r is the Standard deviation of y-intercept of the regression line and S is slope of the calibration curve.

Table 1: Results of regression analysis of STG

STG	Beer's Range (µg/ml)	Regression equation	Regression coefficient (r^2)
Method A	20-160	y = 0.0028x - 0.0621	0.9994
Method B	20-160	y = 0.0049x + 0.0309	0.9992

Table 2: Results of Intraday Precision Study

STG	Conc. taken (µg/ml)	Conc. found * (µg/ml)	%Amt. found	S.D.	% R.S.D.
Method A	20	19.85	99.25	0.112	0.112
	40	39.72	99.30	0.365	0.367
	60	59.49	99.15	0.256	0.258
Method B	20	19.82	99.10	0.124	0.125
	40	39.79	99.47	0.435	0.437
	60	59.42	99.03	0.326	0.329

*Average of three estimations, S.D. – Standard Deviation, R.S.D. - Relative Standard Deviation

Table 3: Results of Inter day Precision Study

STG	Conc. taken (µg/ml)	Conc. found * (µg/ml)	% Amt. found	S.D.	% R.S.D.
Method A	20	19.66	98.30	0.856	0.870
	40	39.54	98.85	0.231	0.233
	60	59.36	98.93	0.369	0.372
Method B	20	19.86	95.30	0.125	0.125
	40	39.87	96.67	0.381	0.382
	60	59.81	98.68	0.121	0.121

*Average of three estimations

Table 4: Results of Recovery Studies

STG	Conc. of drug taken (µg/ml) From Tablet	From API	% Recovery *
Method A	40	32	99.26
	40	40	99.52
	40	48	99.84
Method B	40	32	99.40
	40	40	100.05
	40	48	99.13

*Average of three estimations

Table 5: Results of LOD and LOQ

STG	LOD (µg/ml)	LOQ (µg/ml)
Method A	11.85	35.91
Method B	14.05	42.60

Table 6: Results of tablet Assay

STG	Label Claim (mg/tab)	Amount of Drug* Estimated (mg/tab)	% Assay
Method A	100 mg	99.26	99.26
Method B	100 mg	99.54	99.54

*Average of Six estimations

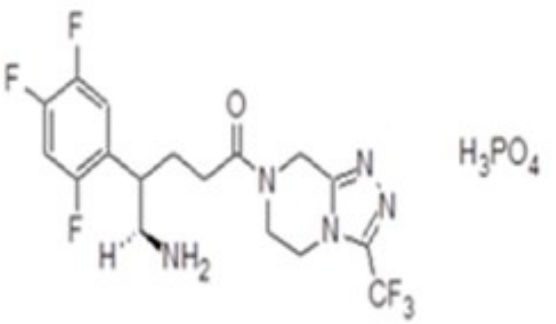

Figure 1: Chemical structure of Sitagliptin phosphate

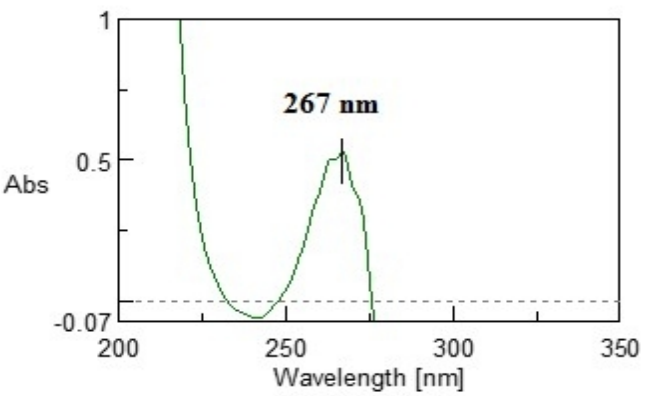

Figure 2: Absorption maxima of Sitagliptin phosphate

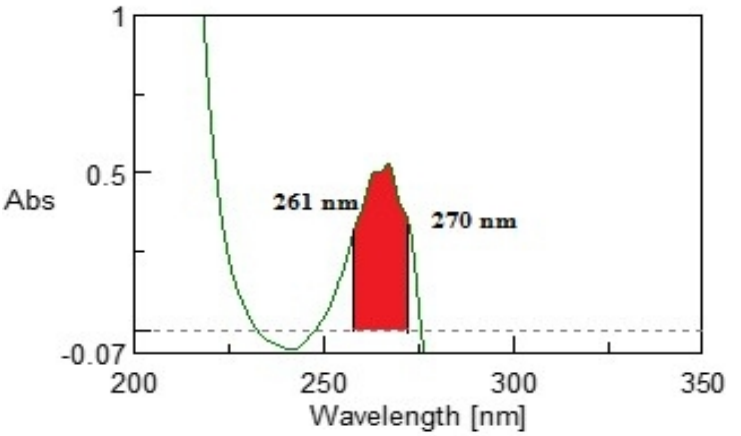

Figure 3: Area under the Curve Method

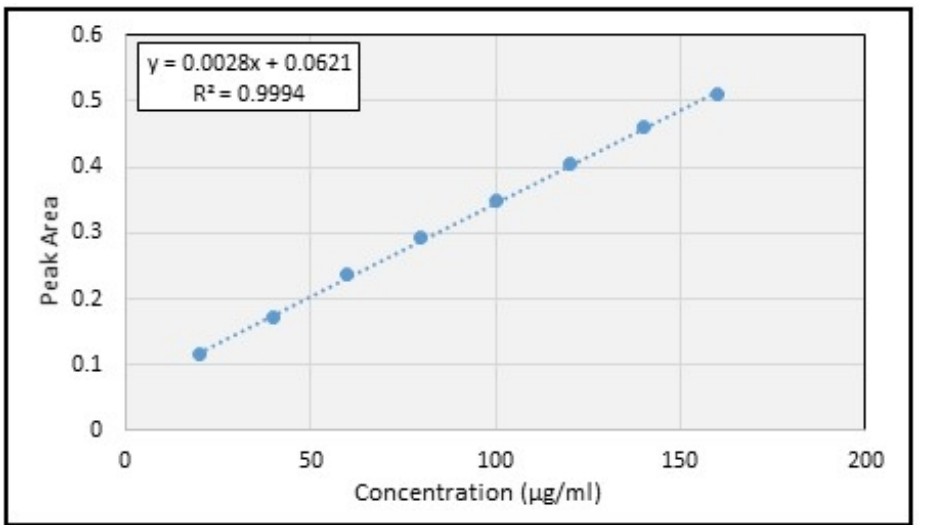

Figure 4: Linearity of STG by method A

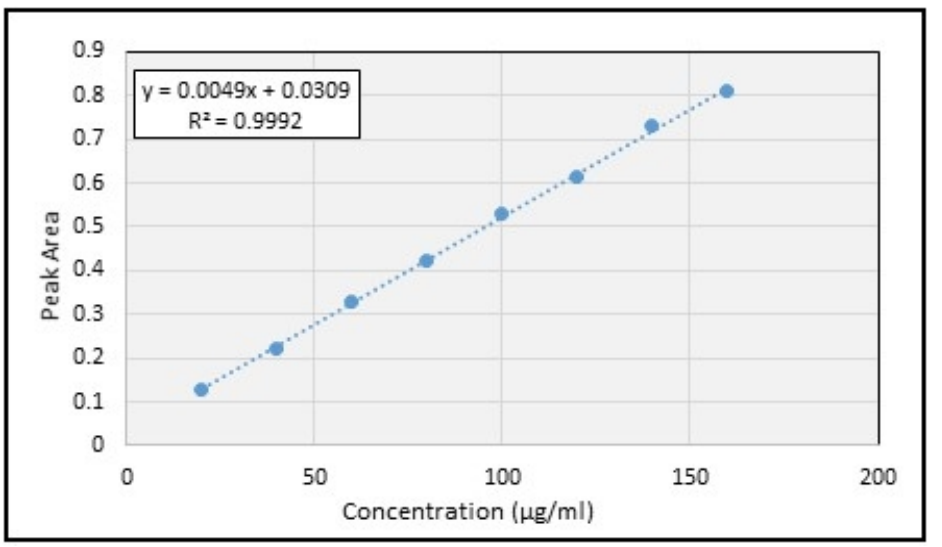

Figure 5: Linearity of STG by method B

RESULTS AND DISCUSSION

Method development and optimization

The present study describes development and validation of two simple UV spectrophotometric methods for the estimation of Sitagliptin phosphate in bulk and tablet dosage form using absorbance maxima method and area under the curve method. Solubility studies indicated that a Sitagliptin phosphate shows better solubility in distilled water as compared to other solvents and the λ max of Sitagliptin phosphate was found to be 267 nm. Because of cost-effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industries as compared to other reported methods.

Validation

Linearity

Linearity was evaluated by analysis of Std. stock solution of STG at Six different concentrations. STG found to be linear within conc. range of 20-160 µg/ml with regression coefficient of 0.9994 by the method A and 0.9992 by method B. The results of regression analysis are summarized in (Table 1). A result shows that within the concentration range mentioned above, there was an excellent correlation between peak area and concentration. (Figure 4 and 5)

Precision

The repeatability (intra-days precision) is expressed as percentage relative standard deviations (% RSD). The average % RSD values of intra-days precision for STG at the concentrations of 20, 40 and 60 µg/ml were 0.112, 0.367 and 0.258 for method A while 0.125, 0.437 and 0.329 µg/ml for method B and for inter-days precision, the average % RSD were 0.870, 0.233 and 0.372 µg/ml respectively for method A while 0.125, 0.382 and 0.121 µg/ml for method B respectively. The % RSD levels of intra-day and inter-day precision were less than 2 in all cases, which indicated that there were no significant variations in the analysis of STG at the concentrations and the proposed method was precise which are shown in (Table 2 and 3).

Accuracy (Recovery Study)

The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing 32, 40 and 48 µg/ml of STG. The average % recoveries for three different concentrations were found to be 99.54 for method A and 99.52 for method Busting proposed UV spectrophotometric methods. The higher values indicated that the proposed UV spectrophotometric method was accurate for the determination of STG in pharmaceutical dosage form. Results of recovery studies are summarized in (Table 4).

LOD and LOQ

The limit of detection was found to be 11.85 µg/ml and 14.05 µg/ml for method A and for method B respectively. The limit of quantification was found to be 35.91 µg/ml for method A and 42.60 µg/ml for method B respectively. Low values of LOD and LOQ indicates that the developed method was sensitive for the estimation STG in bulk and tablet dosage form. Results of LOD and LOQ are summarized in (Table 5).

Assay

Analysis of sample of marketed tablet containing 100 mg Sitagliptin phosphate was carried out and the amounts recovered were expressed as a percentage amount of the label claims. The percentage recovery of Sitagliptin phosphate was 99.26 for method A and 99.54 for method B respectively. Results of tablet assay are summarized in (Table 6).

CONCLUSION

Simple UV spectrophotometric methods have been developed and validated for the determination of Sitagliptin phosphate in bulk and tablet dosage form. The results of the validation parameters show that the UV spectrophotometric methods were found to be accurate, precise and sensitive. Because of cost-effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of Sitagliptin Phosphate in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

ACKNOWLEDGEMENT

Authors thankful to Getz Pharma Pvt. Ltd. for providing the free gift sample of Sitagliptin phosphate.

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Cite this article as:
Vinit Chavhan, Atul Kadam. UV spectrophotometric method development and validation for estimation of sitagliptin phosphate in bulk and tablet dosage form by absorption ratio and area under the curve method. J Biol Sci Opin 2013; 1(4): 317-322 http://dx.doi.org/10.7897/2321–6328.01407

Source of support: Nil; Conflict of interest: None Declared