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Development and validation of area under curve method by using first order derivative for estimation of Sitagliptin in tablet dosage form

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ABSTRACT

Simple, precise and economical UV spectrophotometric methods have been developed for the estimation of Sitagliptin in pharmaceutical dosage form. Method applied was area under curve (AUC) in which area under curve was integrated in the wavelength range of 271.60 - 278.20 nm. Calibration curves were plotted for method by using instrumental response at selected wavelength and concentrations of analyte in the solution. Linearity for the detector response was observed in the concentration range of 10-60 µg/ml for the method. Tablet formulation was analyzed and % assay determined was 99.44% – 101.68%. Accuracy and precision studies were carried out and results were satisfactory. The results of the analysis were validated statistically. Limit of detection and limit of quantitaiton were determined for methods. The methods were validated by following the analytical performance parameters suggested by the International Conference on Harmonization. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Sitagliptin in pharmaceutical formulations.

KEYWORDS

Sitagliptin;
UV-spectrophotometry;
Derivative spectrophotometry;
AUC-spectrophotometry.

INTRODUCTION

Sitagliptin Phosphate Monohydrate is chemically known as 1,2,4-triazolo[4,3-a]pyrazine,7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl), phosphate Monohydrate[1]. The molecular formula is $C_{16}H_{15}F_6N_5O.H_3PO_4.H_2O$, which corresponds to a molecular weight of 417.2. It is used in the treatment of diabetes. It is an oral antihyperglycemic (anti-diabetic) drug of the dipeptidyl peptidase -4 (DPP-4) inhibitor class. DPP-4 inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes that functions to stimulate glucose-dependent insulin release and reduce glucagon's levels. This is done through inhibition of the Inactivation of incretins, particularly glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), thereby improving glycemic control[2,3].

Sitagliptin is not official in USP. A detailed literature survey for Sitagliptin revealed that several analytical methods such as Spectrophotometric and fluorometric methods[3-6] were reported for the quantification of Sitagliptin. There are few RP-HPLC, LC-MS methods were reported for the determination of sitagliptin phosphate in plasma and urine of humans, rats and dogs[7-9].

To develop a simple, precise and accurate spec-

troscopic method for the estimation of Sitagliptin in Pharmaceutical formulation by first derivative area under curve. The method was validated according to ICH guidelines[10]. Thus the objective of present study was developed and applicable method for the routine analysis of Sitagliptin in tablet formulations.

EXPERIMENTAL WORK

Material and method

Sitagliptin working standard was obtained as gift sample from Watson Pharma. The drug was used without further purification. A tablet formulation containing 50 mg (Januvia) of Sitagliptin was purchased from local market. Analytical grade solution was used for the experiment.

Instrument

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager softwre UV Probe with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; derivative mode: 1D (first order derivative, $dA/d\lambda$); band width ($\Delta\lambda$):10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Preparation of standard stock and working standard solution

The standard stock solution of Sitagliptin was prepared by dissolving accurately weighed 10 mg of the drug in Distilled water and diluted to 100 mL with same solvent to obtain a final concentration of 100μg/mL.

Method: Area under curve

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The spectrum obtained of first order derivative was used to calculate AUC. The calibration curve was constructed by plotting concentration (10-60 μg/mL) versus AUC.

Preparation of sample solution

Ten Sitagliptin tablets (50 mg each) were weighed, transferred to a clean dry mortar and ground into a fine powder using a pestle. Tablet powder equivalent to 10 mg of drug was transferred to a 100 mL volumetric flask and 50 mL distilled water was added. After ultrasonic vibration for 10 min, the mixture was diluted to volume with distilled water and filtered through Whatman filter paper (No. 41). From the filtrate an appropriate aliquot was taken in such a way that the final concentration in 10 mL lies within the linearity range tested. The responses were measured and concentration in the sample was determined by comparing the response of sample with that of the standard.

Validation of method

The proposed methods were validated as per ICH guidelines[10].

Linearity

For all the methods, calibration curve was prepared on 3 different days. The calibration curve was constructed by plotting the response (*y*) versus the theoretical concentrations of standards (*x*), by using linear regression analysis. Linearity was expressed as a correlation coefficient; the value must be > 0.999.

Precision

The intraday and interday precisions of the proposed Spectrophotometric methods were determined by estimating the corresponding response 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of Sitagliptin for area under curve 30.0, 40.0, and 50.0 μg/mL and the results are reported in terms of percent relative standard deviation.

Accuracy

The accuracy of the method was determined by calculating recoveries of Sitagliptin by the method of standard additions. The study was performed by spiking three known amount concentration of Sitagliptin (8.0, 10.0, and 12.0 μg/mL; ranging from 80% to 120%)

into a prequantified sample solution (10 µg/mL). Three samples were prepared at each of these concentrations. The recovery of added drug was estimated by measuring the response and by fitting these values to the straight-line equation of calibration curve.

Specificity

Results of tablet solution showed that there is no interference of excipients when compared with the working standard solution. Thus, the methods were said to be specific

Ruggedness

Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous slot (30.0µg/mL) in different laboratories by different analyst using similar operational and environmental conditions. The results are reported in terms of percent relative standard deviation.

RESULTS AND DISCUSSION

The molecular structure of the Sitagliptin is presented in Figure 1. Sitagliptin was completely soluble in methanol and distilled water. Distilled water was selected as the solvent for Sitagliptin because provides the highest solubility and first order derivative and AUC measurements. Area under curve method was developed because Sitagliptin produces broad spectrum in fundamental spectrum. Results showed that, area under curve method measurements are feasible for the analysis of Sitagliptin without interference from sample matrices. Figure 2 shows overlaid first-derivative (10-60 µg/mL) absorption spectra of Sitagliptin respectively, and the spectra were found to be similar in nature and overlapping. Figure 3 shows the absorption spectrum of Sitagliptin (10µg/mL) in Water for the method. Optical characteristics of Sitagliptin were calculated by the proposed methods and presented in TABLE 1.

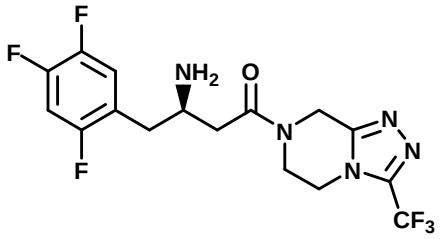

Figure 1 : Chemical structure of Sitagliptin

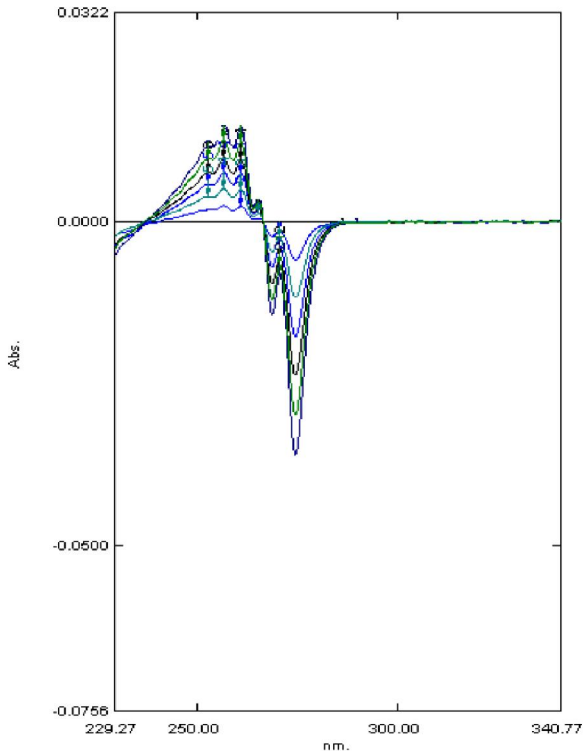

Figure 2 : First- derivative absorption spectrum of Sitagliptin in water (10–60µg/mL)

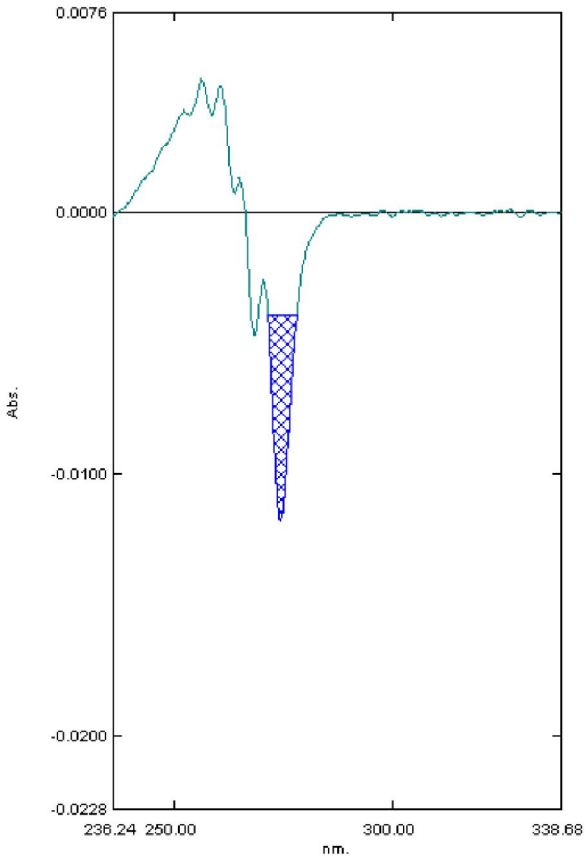

Figure 3 : Area under curve spectrum of Sitagliptin in water

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TABLE 1 : Optical characteristics of Sitagliptin.

Parameters	Sitagliptin
Beer-Lambert's range (μg/mL)	10-60
λ max (nm)/ wave length range (nm)	267
Slope	0.001
Intercept	0.001
Correlation coefficient	0.999
Limit of detection (μg/mL)	0.88
Limit of quantitation (μg/mL)	2.67

TABLE 2 : Assay results of commercial Sitagliptin tablet.

Sitagliptin marketed formulation	Label claim/Tablet (JANUVIA)	% Recover*	% RSD
Tablet	50 mg	99.44%	1.74

***Average of three determinations**

The intra-day and inter-day precision values (%RSD) were calculated (TABLE 3) and lying in the acceptable limits (=2%) for Sitagliptin. The accuracy of Sitagliptin which was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120% were found to be in the acceptable limits (=2%) (TABLE 4). This indicates that there was no interference from the excipients present in the dosage form. Ruggedness of proposed methods was determined with the help of two different analysts and results were evaluated by calculating the %RSD value and lying within the range (TABLE 5).

TABLE 3 : Precision

Conc. mg/mL	Intraday		Interday	
	% Recovery	% RSD	% Recovery	% RSD
30	98.46	1.5052	96.78	1.1070
40	99.67	0.5479	98.79	0.9488
50	98.90	1.0605	99.37	1.7220

TABLE 4 : Accuracy

Nominal Value %	Initial amt.	Added amt.	% Recovery	% RSD
80	10	8	99.44	1.05
100	10	10	98.08	1.12
120	10	12	101.36	1.23

TABLE 5 : Ruggedness

Analyst	Amount found of Sitagliptin [%]	%RSD [n=3]
I	99.80	1.04
II	99.60	1.25

n= no. of estimations

CONCLUSION

Method that was developed for the determination of Sitagliptin based on different analytical techniques, UV-Spectrophotometric, first order derivative and AUC method. The method was validated and found to be simple, sensitive, accurate, and precise. Hence, the methods can be used successfully for routine analysis of pharmaceutical dosage forms of Sitagliptin. The proposed Spectrophotometric methods will not replace the presently known methods available for the analysis of Sitagliptin. However, it can serve as an alternative where advanced instruments (e.g. HPLC) are not available for routine analysis.

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