ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

A simple validated UV Spectrophotometric method for quantitative analysis of Sitagliptin phosphate in pharmaceutical dosage form

P. Ravisankar*[1], G. Mounika[1], Ch. Devadasu[1], G. DevalaRao[2]
[*1]Vignan Pharmacy College, Vadlamudi, Guntur – 522213, A.P., India.
[2]KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, A.P., India.
***Corresponding Author: banuman35@gmail.com, Mobile: +91-9000199106, +919059994000**

ABSTRACT

A simple, precise, accurate, economical and reliable UV spectrophotometric method has been developed for the estimation of Sitagliptin phosphate in tablet dosage form. The drug shows maximum absorption at 267 nm in water and obeys Beer's law in the concentration range of 2-10 µg /mL with good correlation coefficient (R^2=0.9995). The results of analysis were validated by recovery studies. The recovery was found to be 99.53-100.41.The relative standard deviation was found to be < 2.0 % in all cases. The Proposed spectrophotometric method was validated as per the ICH Q2 (R1) guidelines. The proposed method can be used for the reliable quantification of Sitagliptin in bulk form and routine analysis of pharmaceutical formulations.

Key Words: Sitagliptin phosphate, Spectrophotometry, Validation.

INTRODUCTION

Sitagliptin phosphate (SITA) (Figure 1) is chemically((R)-4-oxo-4-[3(trifluoromethyl)-5,6 dihydro [1,2,4]triazolo [4,3-a]pyrazin- 7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine) is an oral anti hyperglycemic agent of the dipeptidyl peptidase-4 (DPP-4) inhibitor class competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4) involved in the breakdown of incretins such as glucagon likeparticle-1 (GLP-1) which potentiate insulin secretion in vivo. Inhibition of DPP-4 reduces the breakdown of GLP-1 and increases insulin secretion; this suppresses the release of glucagon from the pancreas and drives down blood sugar levels.

Literature review revealed that few methods have been reported to determine SITA individually in formulations by spectrophotometry by using acetonitrile, methanol or the combinations of both, NaOH, 0.1N HCl. In fact there was no analytical method developed for the estimation of SITA with water hitherto as solvent. (Bala sekaran, 2010) (G. Jeyabalan, 2013) (Madhuri B. Sarode, 2013) (Amruta B. Loni, 2012), (Sheetal Sharma, 2013), **(**Safaa M Riad, 2012) RP-HPLC (Rani Sirisha, 2013) (Lavanya, 2013) techniques. However the reported spectrophotometric methods are either poorly validated or uneconomical to be used for routine laboratory analysis. Hence the present study describes the simple and cost effective validated method for the determination of SITA in tablet dosage form.

MATERIALS AND METHODS

Selection of solvent: A number of trails were made to find out the ideal solvent system for dissolving the drug. The solvents such as water, methanol and acetonitrile were tried based on the solubility of the drug. Better absorption maximum was found to be 257 nm with water. So water was selected as optimized solvent in this spectrophotometric method.

Instruments used: UV-Visible Spectrophotometer (Systronics model 2203). The UV-VIS spectrophotometer achieves a resolution of 1 nm with matched quartz cells of 1 cm path length.

Reagents and Materials: SITA standards supplied by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. Januvia tablets containing 50 mg of Sitagliptin phosphate tablets are obtained from local pharmacy. Analytical grade triple distilled water was used throughout the experiment which was provided by Vignan Pharmacy College, Vadlamudi, Guntur Dist.

Selection of detection wavelength: Appropriate dilutions of SITA were prepared from the standard stock solution. Using UV- Spectrophotometer, the dilutions of SITA were scanned over a range of 200-400 nm. It was observed that the drug showed maximum absorbance at 267 nm which was selected as the wavelength for detection. The spectrum of SITA is shown in Figure 2.

Preparation of standard drug solutions: 10 mg of SITA pure drug was accurately weighed and transferred into a 100 mL volumetric flask containing distilled water. The volume was made up to the mark with distilled water to get the stock solution (100µg/mL). This solution was further diluted with the same to get the working standard solution.

Preparation of Calibration curve: Aliquots of standard drug (0.2 mL to 1.0 mL, 100 µg/mL) solution in triple distilled water were transferred into a series of 10 mL volumetric flasks and the solution was made up to 10 mL with water. After setting the instrument for its spectral properties the solutions were scanned in the wavelength ranging from 200 nm - 400 nm. The wavelength of maximum absorption for SITA was found at 267 nm. Calibration data is presented in Table 1. Calibration curve was prepared by plotting concentration of SITA on x-axis and their respective absorbance's on y-axis. The calibration curve is shown in Figure 3.

Procedure for assay of pharmaceutical formulations: 20 tablets of SITA marketed formulations were weighed and powdered .A quantity of tablet powder equivalent to 50mg of SITA was transferred to 50 mL volumetric flask and ultrasonicated for 20 minutes and volume was made up to the mark with distilled water. The solution was then filtered through a whatmann filter paper grade1. The filtrate was appropriately diluted further. The absorbance of the resulting solution was measured at 267 nm and the amount of SITA was computed from its calibration plot.

Validation of the developed method:

Linearity: The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations. A linearity correlation coefficient above 0.9995 is acceptable for most methods, especially for major components in assay methods. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Precision was determined by intra-day and inter-day study. The repeatability of the method was evaluated by carrying out the assay 3 times on the same day and intermediate precision was evaluated by carrying out the assay on 3 consecutive days for the sample solution. The percent relative standard deviation (% RSD) was calculated.

Accuracy (Recovery studies): The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. Accuracy studies were performed at three different levels (80%, 100% and 120%) by standard addition method and the samples were analyzed in triplicate by the proposed method. Known amount of standard SITA at 80%, 100% and 120% of predetermined sample was added to a pre quantified tablet sample.

Ruggedness: Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

Robustness: The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. The absorbance was measured and assay was calculated for six times. The results of robustness are presented in Table 6.

LOD and LOQ: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Limit of Detection and Limit of Quantitation were calculated using following formula LOD= 3.3(SD) / S and LOQ= 10 (SD) / S, where SD=standard deviation of response (absorbance) and S= slope of the calibration. The results of LOD and LOQ are shown in Table 2.

RESULTS AND DISCUSSION

The absorption spectra were recorded in the wavelength region of 200-400 nm in UV method, the absorption maxima curve was shown in Figure 2.The proposed method obeyed Beer's law in the concentration range of 2-10 µg/mL with good correlation coefficient of R2 =0.9995. Calibration data is presented in Table 1.

Beer's law range was confirmed by the linearity of the calibration curve of Sitagliptin was shown in **F**igure 3**.** The optical characteristics and the data concerning to the proposed method is represented in Table 2. Precision of the method was reported in terms of relative standard deviation and it should be evaluated by using a minimum of 3 determinations over which shows % RSD less than 2 indicates that the method was precise and the results are presented in Table 3**.** Recovery studies were carried out for the developed method by addition of known amount of standard drug solution of SITA to pre-analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed methods. The recovery (Table 4) was in the range of 99.53 to 100.41 percentages. The limit of detection and limit of quantitation for estimation of SITA were 0.2269 µg/mL, 0.6875 µg/mL respectively. Ruggedness was performed by two different analysts and two instruments under same experimental condition. The % RSD was calculated. The results were reported to be within the limits. In fact there was no difference in mean assay results of the method obtained from two instruments of different manufacturers. It reveals that the proposed method was found to be rugged and the results are tabulated in Table 5 for the determination of Sitagliptin. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influences of the variables were determined. The absorbance was measured and assay was calculated for six times. The results of robustness are presented in Table 6. The results are within the specified limits which states that this method is robust. The developed method was applied to the analysis of tablet formulations found to be within the proposed limits and the mean % assay value was found to be 98.36 %. The assay results are given in Table 7. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method.

Table.1.Linearity data for Sitagliptin:

Concentration(µg/mL)	Absorbance
0	0
2	0.015
4	0.032
6	0.048
8	0.064
10	0.082

Table.2.Optical characteristics, regression data of the proposed method

Parameter	Result
λ_{max} (nm)	267
Beer's law limits (µg / mL)	2-10
Molar absorptivity (L. mole^{-1} cm^{-1})	3258.512
Detection limits (µg / mL)	0.2269
Quantitation limits (µg / mL)	0.6875
Sand ell's sensitivity (µg /cm^2/0.001 absorbance unit)	0.125
Regression equation (Y = a+ bc): Slope (b)	0.008
Standard deviation of slope (S_b)	9.29487
Intercept (a)	-0.005
Standard deviation of intercept (S_a)	5.628×10^{-4}
Standard error of estimation(S_e)	7.77×10^{-4}
Correlation coefficient (r)	0.9995
% Relative standard deviation*	1.72499

***Average of six determinations.**

Table.3.Results of precision study

Precision*	Intra-day	Inter-day		
		Day -1	Day -2	Day -3
Mean % recovery	0.047	0.05666	0.06866	0.72566
SD	0.001	0.00057	0.00057	0.00115
%RSD	1.2197	1.01885	0.84080	0.15912

***average of 6 determinations**

Table.4.Results of accuracy study

Level of recovery	Amount added (µg/mL)		Amount recovered (µg/mL)	Percent recovery (% w/w)	% RSD
	Amount of standard drug solution added (µg/mL)	amount of the drug formulation added (µg/mL)			
80 %	8	5	12.94	99.53	0.214
100 %	10	5	14.99	99.93	0.203
120 %	12	5	17.07	100.41	0.176

Table.5.Ruggedness results

Parameter	Instrument-1 (Systronics model 2203)	Instrument-2 (Elico SL 159)	Analyst -1	Analyst -2
Mean	0.6277	0.6244	0.6315	0.6258
SD	0.00200	0.00128	0.0007	0.0038
%RSD	0.319	0.205	0.110	0.616

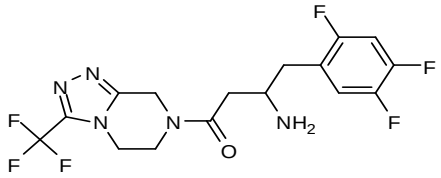
Table.6.Results for Robustness study

S.N0	λ_{max} 1	λ_{max} 2
Mean	0.9911	0.9912
SD	0.0073	0.0073
% R.S.D	0.73655	0.73648

Table.7.Assay results

Formulation	Labeled amount	Mean % ± SD	% Assay	% RSD
Januvia Rx	50mg	49.18 ± 0.8	98.36	1.626

Figure.1.Chemical structure of Sitagliptin



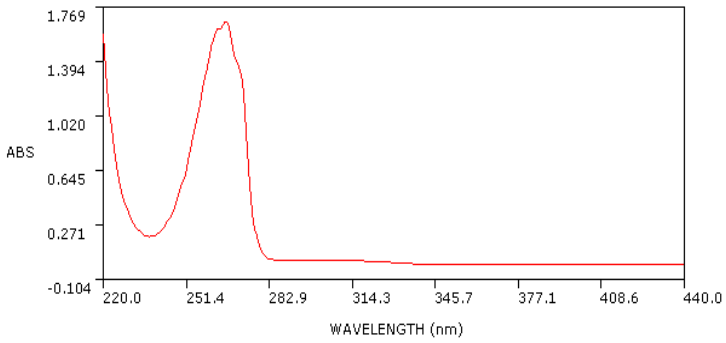


Figure.2.UV spectrum of Sitagliptin in distilled water

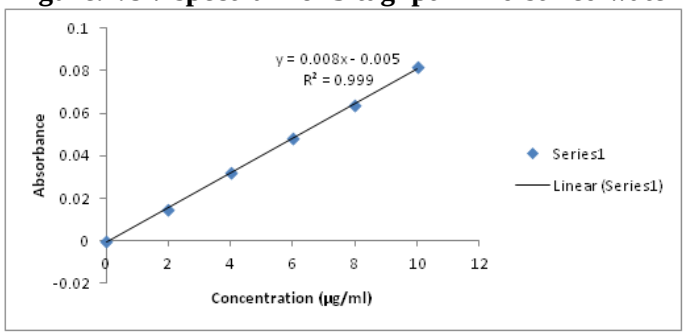


Figure.3.Calibration curve of SITA by UV method

CONCLUSION

The present study demonstrated an UV spectrophotometric method for the estimation of SITA available as tablet dosage form. The developed and validated UV spectrophotometric method was found to be economical due to the use of triple distilled water as a solvent throughout the experiment. From the above experimental data results and parameters, the developed method has advantages like the time taken for preparation of standard and sample solutions is less and hence suitable for the analysis of SITA raw material and its pharmaceutical dosage form. The system suitability parameters and system precision are determined and found within the limits. The plot is drawn between the concentration and absorbance which is found to be linear in the concentration range of 2-10 µ/mL with good correlation coefficient greater than r^2= 0.9995. The precision and accuracy of the proposed method are expressed in % RSD and % of recovery of the API respectively. Low % Relative standard deviation and high percent of recovery indicates that the method is highly precise and accurate. In fact the method developed for SITA was found to be simple, precise, accurate and cost effective and it can be effectively applied for routine analysis.

ACKNWOLEDGEMENTS

The authors would like to thank Hetero Labs for providing the samples of SITA. We are highly grateful to Dr.L.Rathaiah, Honourable Chairman, Vignan group of institutions, Vadlamudi, Guntur, for providing the necessary facilities to carry out this research work.

REFERENCES

Amruta B.Loni, MinalR.Ghante, S.D.Sawant, Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin phosphate and Metformin hydrochloride in Bulk and Tablet Dosage Form, Der Pharma Chemica, 4(3) 2013.

Bala Sekaran, Prameela Rani, Development and validation of Spectrophotometric method for the determination of Sitagliptin, in Pharmaceutical dosage forms, International Journal of Pharmacy and Pharmaceutical Sciences, 2 (4), 2010.

G. Jeyabalan and Narendra Nyola, Analytical method development and validation of Sitagliptin phosphate monohydrate in pure and tablet dosage form by derivative spectroscopy, Journal of Applied Pharmaceutical Science, 3(1), 2013.

K. N. Tarkase, Madhuri B. Sarode, Sumit A. Gulve and Ashwini Gawade Development and validation of UV spectrophotometric method for estimation of Sitagliptin phosphate, Der Pharmacia Lettre, 5(3), 2013.

R. Lavanya, Md. Yunoos, Development and Validation of RP HPLC method for the Estimation of Sitagliptin Phosphate in bulk and its tablet dosage form, Journal of Advanced Pharmacy Education & Research, 3(4), 2013.

Rani Sirisha ,Vasanth, Ramesh ,Ramesh Malothu, RP-HPLC method development and validation for simultaneous estimation of metformin and Sitagliptin in tablet dosage forms, International Journal of Pharmaceutical Research & Analysis, 3 (1), 2013.

Safaa M Riad, Mamdouh R Rezk, Ghada Y Mahmoud and Abdel-Aziz El Bayoumi Abdel Aleem, Spectrophotometric determination of Sitagliptin and metformin in their pharmaceutical formulation, International Journal Of Comprehensive Pharmacy, 3(5), 2012.

Sheetal Sharma, Nimita Manoch, Estimation of Sitagliptin phosphate and simvastatin and validation of the proposed method in a combined marketed tablet dosage form by simultaneous equation method, International Journal of Current Pharmaceutical Research, 4(3), 2012.