Research Article

UV Spectrophotometric Method Development and Validation of Sitagliptin in Bulk and Pharmaceutical Dosage Form

Namratha Sunkara[1]*, Kandala Neela Maneesha[1], B. Lavanya[1] and Sanapala Arunkumar[2]

[1]Bharat Group of Institutions, Ibrahimpatnam, Hyderabad, Telangana, India.

[2]Pulla Reddy Institute of Pharmacy, Annaram, Sangareddy District, Telangana, India.

ABSTRACT

A simple UV Spectrophotometric method was developed for the determination of Sitagliptin in bulk and its pharmaceutical formulations. Sitagliptin exhibited maximum absorption at 267 nm in Aqueous solvent as water and obeyed linearity in the concentration range of 2 to 30 µg /ml. The proposed method was statistically validated. From the results obtained for Precision, it was found that % RSD is less than 2%. It indicates that the proposed method has good reproducibility. From the results obtained for Accuracy, it was found that Percentage Recovery values of pure drug from the analyzed formulation was 99.75 which indicates that the method is accurate and commonly used excipients and additives present in the formulation was not interfering in the proposed method .

Keywords: Sitagliptin, Validation, UV-spectrophotometric, accuracy.

INTRODUCTION

Sitagliptin[1,6] is chemically known as (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 and its empirical formula is $C_{16}H_{15}F_6N_5O$ with a molecular weight of 407.31. Sitagliptin works to competitively inhibit the enzyme Dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal.[2] By preventing GLP-1 and GIP inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas. This drives blood glucose levels towards normal. As the blood glucose level approaches normal, the amounts of insulin released and glucagon suppressed diminishes, thus tending to prevent an "overshoot" and subsequent low blood sugar (hypoglycemia) which is seen with some other oral hypoglycemic agents. The chemical structure was shown in figure1. Literature review revealed that very few methods was reported for determining of sitagliptin in bulk and pharmaceutical dosage form by UV-spectrophotometric methods[1-6]. Hence in the present work an attempt was made to develop simple, precise and accurate analytical method for estimation of sitagliptin in bulk and pharmaceutical dosage form.

EXPERIMENT

Materials

Triple distilled water was used for the analysis. Sitagliptin pure gift sample provided by JANUVI (MERCK tablets was procured from local market and average weight was determined for 10 tablets and powdered and weight equivalent to 25 mg of Sitagliptin was taken and dissolved in 0.1N Hcl, sonicated to dissolve and from this various solutions of Sitagliptin was prepared and diluted to 10ml with 0.1N Hcl and estimated at 267 nm.

Instrumentation

Spectral and absorbance measured on an UV spectrophotometer – UV 1800- shimadzu. Shimadzu – type BL -220 H electronic balance was used for weighing the samples.

METHOD

Preparation of stock solution

Standard stock solution was prepared by dissolving 10 mg of drug in 100 ml of Aqueous solvent to get concentration of 1mg/ml (1000 µg/ml) solutions.

Preparation of Working Standard Solutions and construction of standard graph:
The prepared stock solution was further diluted with aqueous solvent to get working standard solutions of 100 µg/ml of Sitagliptin**.** To construct Beer's law plot for pure drug, different concentrations (2.0-30µg/ml) was taken and diluted to 10 ml with Aqueous solvent. The absorbance was measured maximum at 267nm against aqueous solvent as blank.. The standard graph was plotted by taking concentration of drug on x-axis and absorbance on y-axis and was shown in Figure2 the drug has obeyed Beer's law in the concentration range of 2.0-30µg/ml.

Estimation of sitagliptin in commercial formulation
10 tablets weighed and powder equivalent to 25 mg of Sitagliptin was taken and dissolved in 0.1N Hcl and filtered. The filtrate was considered as stock solution and from this various solutions of Sitagliptin was prepared and estimated at the 267 λmax.

RESULTS AND DISCUSSIONS
OPTIMIZATION
Scanning and determination of maximum wavelength (λ_{max})
In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, different solutions of the drug (2.0-30µg/ml) in Aqueous solvent was scanned using spectrophotometer within the wavelength region of 200 – 400 nm against aqueous solvent as blank. Sitagliptin shows λ_{max} at 267nm. The resulting spectra was shown in figure 2 and the absorption curve showed characteristic absorption maxima at 267nm for Sitagliptin
.
Precision
The precision of the proposed method was ascertained by actual determination of eight replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by proposed method. From the absorbance Mean, Standard Deviation, % R.S.D, % Range of errors (at 0.05 and 0.01 confidence limit) was calculated. The reading was shown in table 5.

Accuracy
An Accuracy study was carried out by standard addition method. Pure Sitagliptin was added at different levels i.e. 80%, 100% and 120% to drug sample present in tablet dosage form (50 mg in each coated tablet). The reading was shown in table 4.

Limit of detection
It was calculated from the values of calibration curve and it was found to be 5.39µg/ml.

Limit of quantization
It was calculated from the values of calibration curve and it was found to be 19.68µg/ml.

CONCLUSION
It was found that sitagliptin can effectively be analyzed by the UV method with Aqueous solvent and detection wavelength of 267 nm. The linearity range was found to be 2.0-30 µg/ml. In the precision study, %RSD was found to be less than 1% which indicates that the method has good reproducibility. The accuracy studies showed % recovery in the range 99.75 %, which indicates that the method was accurate and also revealed that the commonly used excipients present in the pharmaceutical formulations do not interfere in the proposed method.

ACKNOWLEDGEMENT
The authors acknowledge Merck Pharmaceuticals for providing authentic gift sample of sitagliptin.

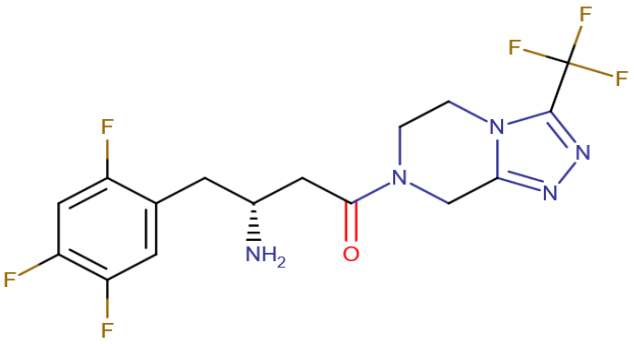

Fig. 1: Chemical structure of sitagliptin

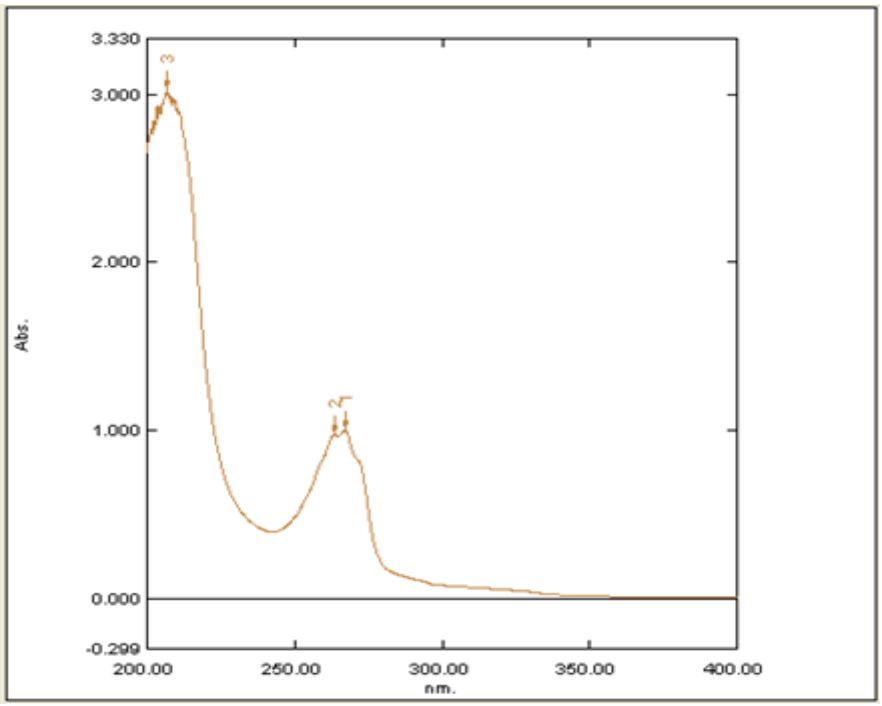

Fig. 2: Chromatogram of standard solution

R2 = 0.9950

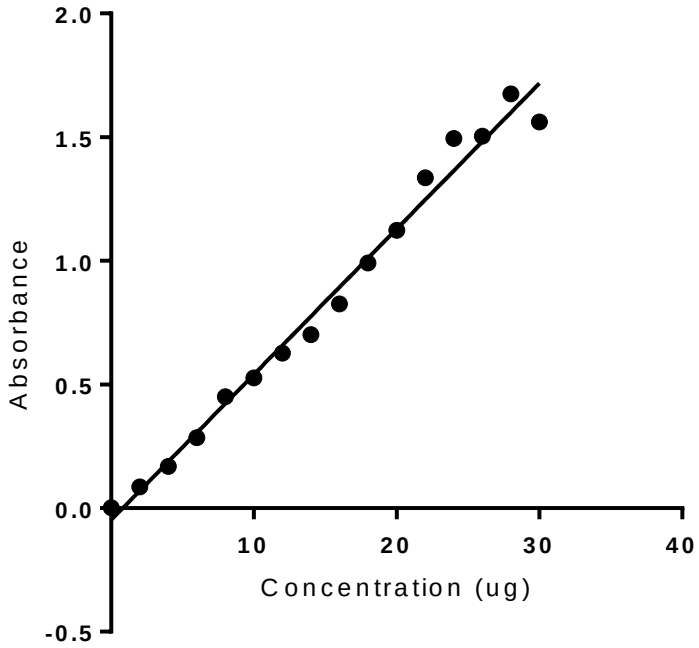

Fig. 3: LINAERITY GRAPH OF SITAGLIPTIN

Table 2: Optical characteristics

Parameters	Sitagliptin
Beer's Law limit (µg/ml)	0-30
Sandell's Sensitivity (µg/cm^2/0.001 absorbance unit)	0.014013
Molar Extinction Coefficient (1 mole^{-1}. cm^{-1})	2.421317 x 10^4
% Relative Standard deviation	±0.001288
% Range of error 0.05 confidence limits 0.01 confidence limits	 0.109412 0.138322
Correlation Coefficient	0.9950
Regression equation (Y)* Slope (a) Intercept (b)	 0.05886 0.8240

Table 3: Results of assay and recovery studies

Formulation	Labelled amount	Observed amount* (± S.D) mg	%Recovery by proposed method	%R.S.D
		SITA	SITA	SITA
JANUVIA (MERCK)	25mg	24.96±0.005	99.6	0.10142

Table 4: Results of recovery studies

Drug	Level of Addition (%)	Amount Added (µg/ml)	Amount recovered (µg/ml)	%Recovery± SD
Sitagliptin	80	8	7.98	99.75 ± 0.02
	100	10	9.97	99.70 ± 0.30
	120	12	11.91	99.25 ± 0.75

Table 5

Concentration in µg/ml	Absorbance at 267 nm	Statistical analysis Sitagliptin
10	**0.525**	**Mean : 0.528**
10	**0.526**	
10	**0.526**	
10	**0.527**	**S.D : 0.00138**
10	**0.526**	
10	**0.526**	
10	**0.525**	**%RSD : 0.216**
10	**0.525**	
10	**0.526**	
10	**0.526**	

REFERENCES

1. Indian Pharmacopoeia (2007). The Indian pharmacopoeia commission Ghaziabad 2, 685.
2. National Prescribing Service (August 2010). "Sitagliptin for Type 2 Diabetes". Retrieved 27 August 2010.
3. Ghazala Khan (2011). Asian Journal of Biochemical and Pharmaceutical Research, Issue 2, Vol. 1, 352-58.
4. Validation of Analytical Procedures: Methodology (in Q2 (R1)) http://www.ich.org/cache/compo/ 276-254- 1.html.
5. Anildubala et.al. (2012). RP-HPLC method for the estimation of Sitagliptin Phosphate in human plasma by protein precipitation technique. International journal of pharmacy and pharmaceutical sciences. 4(2): 691- 694.
6. Tian Yan Zhou and Wei Lu (2011). RP-HPLC method for the Quantitative determination of sitagliptin in rat plasma. Journal of Chinese Pharmaceutical Sciences. (20): 63-69.