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UV-Method for the quantitative determination of sitagliptin in bulk and pharmaceutical dosage form and its validation including stability studies

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Abstract

The estimation of sitagliptin in tablet and bulk dose forms has been created using a straight forward UV approach. In all experiments, water was utilized as the solvent. It was discovered that water had an absorption maximum of 267 nm. A linearity range of between 10-300 µg/ml was obtained after performing all the validation parameters. %Recovery of the proposed method was found to be between 97.12 and 99.46, and its %RSD was discovered to be within acceptable bounds, or less than 2. The thresholds for detection and quantitation were determined to be 3.397 and 10.295 µg/ml, respectively. Stress studies were also performed for the drug substance. In addition to checking the absorbance of the deteriorated product, stress investigations such as acid and alkali hydrolysis, oxidation, photolytic, and thermal were conducted.

1. Introduction

In US, sitagliptin received its initial medical approval in 2006. Initially created by Merk & CO (Gumieniczek *et al.*, 2019). Type 2 diabetes is the condition that it is used to treat and the drug is marketed under the name Januvia. In comparison to metformin and sulfonylureas, it is less popular in the UK (Kamal *et al.*, 2021). It functions by raising the concentrations of incretins, which are organic compounds. Specifically, after a meal, incretins will increase the production of insulin to regulate blood sugar levels (Herman *et al.*, 2005). Additionally, the liver's sugar level is decreased (Salim *et al.*, 2012). The drug will be administered according to the patient's health, kidney function and reaction to treatment. The drug should be taken along with a nutritious diet, frequent exercise and other prescription medications to control blood glucose levels (Zhao *et al.*, 2014). By controlling blood sugar, we can prevent kidney damage, heart attacks and strokes (Sai *et al.*, 2017). It has not yet received approval for usage during pregnancy or breastfeeding (Stricklin, 2012). Because, it belongs to the dipeptidyl peptidase-4 inhibitor class, it will cause the pancreas to produce more glucose while producing less glucagon (Salvo *et al.*, 2014). Some of this medication's negative effects include headaches, limb edema and upper respiratory tract infections (Parikh, 2014). One of sitagliptin's significant adverse effects is angioedema, which can also cause low blood sugar, kidney issues, pancreatitis and joint discomfort (Iqbal *et al.*, 2018). The adverse effects of sitagliptin are similar to those of a placebo, with the exception of a few unusual incidences of nausea, symptoms similar to the common cold, and photosensitivity. This medication does not raise the risk of diarrhea (Scherf-Clavel and Hogger, 2015). Sitagliptin and placebo did not significantly differ from one another when hypoglycemia occurs. Low blood sugar is more likely in people using sulphonylurea (Choy and Lam, 2007).The structure is shown in Figure 1.

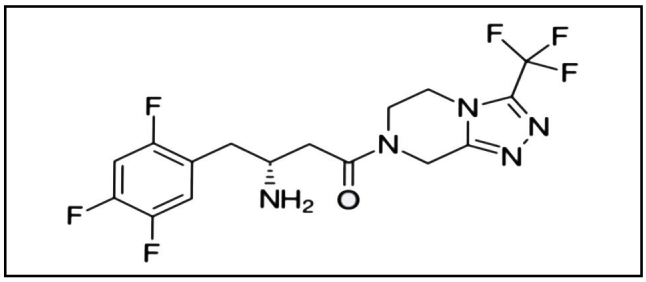

Figure 1: Structure of sitagliptin.

Molecular formula: $C_{16}H_{18}F_6N_5O_5P$.
Molecular weight: 505.31 g/mol.

2. Materials and Methods

2.1 Chemicals

Sitagliptin standard was obtained as a gift sample from a pharmaceutical company in Hyderabad, Januvia 25 mg tablets were purchased from the local drug store, distilled water.

2.2 Instruments

ELICO SL 210 double beam UV-Visible spectrophotometer, quartz cuvettes and analytical weighing balance were used.

2.3 Method development

2.3.1 Standard stock preparation

Weigh precisely 10 mg of standard sitagliptin, transfer to a 10 ml volumetric flask and make up with water to get a 1000 µg/ml concentration.

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2.3.2 Preparation of working standard solution

Pipette 1 ml of the standard stock into a 10 ml volumetric flask, then mark the volume with water to equal 100 µg/ml. Pipette 1ml in a 10 ml volumetric flask from this 100 ppm solution, then add water to make up 10 ppm.

2.3.3 Determination of wavelength of maximum absorption

A prepared 10 ppm standard solution was used as the blank for a UV spectroscopy scan in the 200-400 nm region. The solution showed maximum absorbance at 267 nm, which is thought to be sitagliptin's lambda maximum. λ_{max} of sitagliptin is shown in Figure 2.

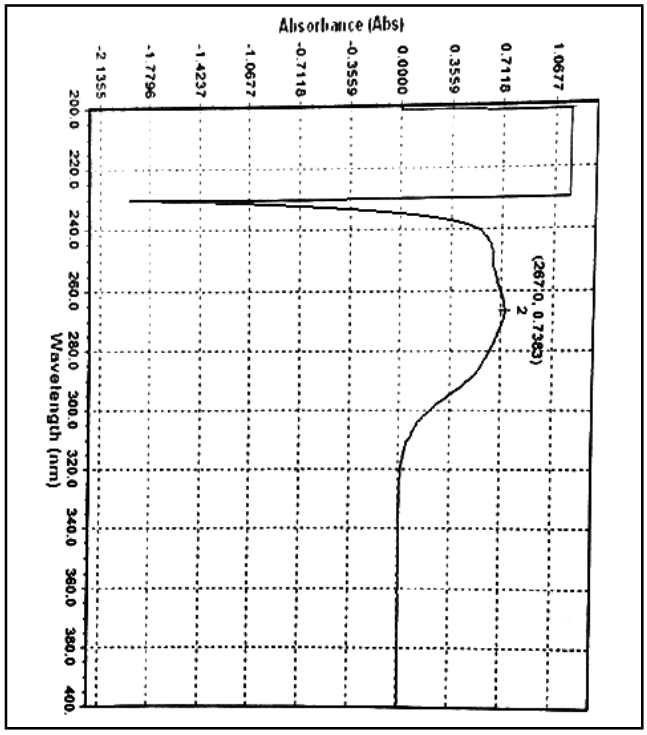

Figure 2: λ_{max} of sitagliptin.

2.4 Method validation parameters

2.4.1 Linearity

Linearity refers to the ability of analytical procedures to produce results in direct proportion to the concentration range of analyte in samples within the required concentration level.

2.4.2 Precision

Precision expresses the closeness of a series of measurements of the same sample under identical conditions. %RSD was calculated and %RSD should be less than 2:

$$s = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$$

where,

s = standard deviation

x = each value in the data set

x = mean of all values in the data set

n = number of values in the data set

%RSD = (standard deviation of the measurement/mean value of measurement)×100

Acceptance criteria: %RSD should be less than 2.

2.4.3 Accuracy

The degree to which the determined value of the analyte corresponds to the true value. Accuracy can vary over the expected concentration range. It should be determined using working or reference standards. % three thresholds of recovery were calculated: 50%, 100%, and 150%. Acceptance criteria: % recovery should be within the limits in the range of 98-102%

2.4.4 Limit of detection

The lowest amount of analyte that can be detected but not necessarily quantified is referred to as LOD. The limit of detection can be calculated by using the formula LOD = 3.3σ/S.

2.4.5 Limit of quantification

The lowest amount of the analyte that can be quantitatively determined with defined precision under the stated experimental conditions. The limit of quantitation can be calculated by using the formula LOQ = 10σ/S

2.4.6 Robustness

Variation should be deliberate but within a realistic range to study the robustness of the method. The results of the analysis after making the deliberate changes should be within the method's specified tolerance limits. The limit for robustness is %RSD should be less than 2%.

2.4.7 Ruggedness

Ruggedness measures the reproducibility of test results under conditions such as results generated for the same sample under identical conditions by different laboratories, different analysts, different instruments, different environmental conditions, *etc*. The limit for ruggedness is % RSD should be less than 2%.

2.4.8 Assay

The assay is done to know the amount of API in the formulation. % assay was calculated by using the formula given below :

% Assay = (Sample absorbance/standard absorbance) × (standard concentration/sample concentration) x 100%

2.5 Forced degradation studies

Various forced degradation conditions: The following condition will be applied to carry out the study:

(i) Hydrolysis by acid

(ii) Hydrolysis by alkali

(iii) Oxidation

(iv) Photostability degradation

(v) Thermal degradation

2.5.1 Acid hydrolysis

Take 1 ml in a 10 ml volumetric flask to make a 100 ppm solution derived from the stock solution. Take 1 ml of the 100 ppm drug solution, put it in a 10 ml volumetric flask with 1 ml of 0.1N HCl, and let it sit for 24 h. Use 1ml of 0.1N NaOH to neutralize it after 24 h have passed. At 267 nm, the absorbance was then determined.

2.5.2 Alkali hydrolysis

By placing 1 ml of the normal stock solution in a 10 ml volumetric flask,100 ppm can be made. Take 1ml of the 100 ppm drug solution, put it in a 10 ml volumetric flask with 1 ml of 0.1 N NaOH and let it sit for 24 h. Use 1ml of 0.1N HCl to neutralize it after 24 h have passed. At 267 nm, the absorbance was then measured.

2.5.3 Photolytic degradation

The 10 mg of medication was placed in a petri dish and left in the UV chamber overnight to be exposed to UV radiation. After the specified amount of time had passed, the sample was diluted with 100 ml of water to obtain a concentration of 100 µg/ml, and absorbance was determined at 267 nm.

2.5.4 Thermal degradation

The smallest amount of the drug was placed in a petri dish and heated to dry heat for 3 h at 400ºC in a hot air oven. In order to create a 100 µg/ml solution, weigh 10 mg of the drug after 3 h and dissolve it in 100 ml of diluent in a volumetric flask. Calculate the percentage of degradation using the absorbance measured at 267 nm.

2.5.5 Peroxide degradation

A 100 ppm solution can be made by putting 1 ml of the regular stock solution in a 10 ml volumetric flask. Add 1 ml of the 3% hydrogen peroxide solution to 1ml of the medicine solution from the 100 ppm solution in a 10 ml volumetric flask. For 12 h, keep the combination. 12 h later, measure the absorbance at 267 nm. Figure 3 shows the results of forced degradation.

3. Results

3.1 Linearity

The working standard solution was used to create successive dilutions. Using linear regression analysis, calibration curves with a range of 10 ppm to 300 ppm were run to determine the linearity.

Diverse dilutions were made from the normal stock solution, including 10 ppm, 25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm, 150 ppm, 175 ppm, 200 ppm, 225 ppm, 250 ppm, 275 ppm and 300 ppm concentrations. Water was used as a blank to measure absorbance at 267 nm. The calibration graph was created by plotting the concentration on the x-axis and the absorbance on the y-axis, and it is displayed below in Figure 2. The developed approach was found to be linear in the range of 10-300 µg/ml.

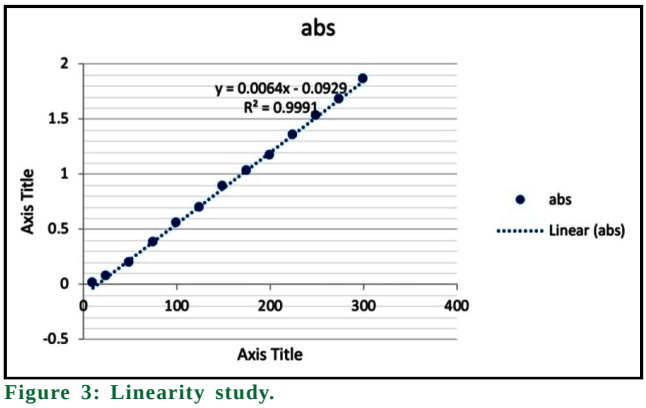

Figure 3: Linearity study.

Table 1: Results of sitagliptin's quantitative analysis

S. No.	Parameters	Results
1.	λ_{max}	267
2.	Linearity (µg/ml)	10-300 µg/ml
3.	Slope	0.0064
4.	r^2	0.9991
5.	Y-intercept	0.0929

3.2 Precision

The precision investigation was conducted with sitagliptin 100 ppm. Six times at 267 nm, the absorbance of a 100 ppm solution was checked to water as a blank. All of the absorbances were measured and recorded. Standard deviation and %RSD were calculated by using the formula and the results are shown in Table 2.

$$s = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$$

where,

s = standard deviation

x = each value in the data set

x = mean of all values in the data set

n = number of values in the data set

%RSD = (standard deviation of the measurement/mean value of measurement)×100 Acceptance criteria: %RSD should be less than 2.

Table 2: Results of precision studies

Concentration	Intra-day precision (% RSD)	Inter-day precision (% RSD)	
		Day 1	Day 2
100 µg/ml	0.65859	0.7935.	0.4165.

3.3 Accuracy

% Three thresholds of recovery were calculated: 50%, 100% and 150%. 50 ppm of the standard solution (2 ml), 100 ppm of the standard solution (2 ml), and 150 ppm of the standard solution (2 ml) were spiked with the corresponding amounts of the sample solution (2 ml). At 267 nm, the three different concentrations were all recorded. At 267 nm, the absorbance was measured three times. The absorbance of these was measured at 267 nm after three repetitions and the %recovery was calculated. The results of accuracy were reported in Table 3.

Table 3: Results of accuracy studies

Level	Amount of standard added	Pre-analyzed	Percentage recovery
50%	50 µg/ml	100 µg/ml	97.12%
100%	100 µg/ml	100 µg/ml	98.75%
150%	150 µg/ml	100 µg/ml	99.46%

Acceptance criteria: % recovery should be within the limits in the range of 98-102%.

3.4 Limit of detection

The detection limit (DL) can be written as DL=3.3σ/S, which is the response's standard deviation. The calibration curve for the analyte can be used to estimate S, which is the slope of the calibration curves.

LOD was determined to be 3.397 µg/ml.

3.5 Limit of quantification

It is possible to write the quantitation limit (QL) as follows: QL = 10 σ/S, where = the response's standard deviation. S is the calibration curve's slope, and the analyte's calibration curve can be used to estimate S.

The 10.295 µg/ml LOQ was discovered.

3.6 Robustness

The standard solution was made in 3 aliquots of 100 ppm each and it was scanned at a maximum wavelength of ± 1 nm. Table 4 shows the results of robustness.Acceptance criteria: % RSD should be within the limits of less than 2.

Table 4: Results of robustness

S.No.	Concentration	Wavelength	%RSD
1.	100 µg/ml	266 nm	0.1967%
2.		268 nm	0.0458%

3.7 Ruggedness

Six distinct analyzers and apparatus each performed a scan of a 100 ppm reference solution and the findings are shown in Table 5. Acceptance criteria: %RSD should be within the limits of less than 2.

Table 5: Results of ruggedness

S.No.	Concentration	Analyst	%RSD	Instrument	%RSD
1.	100 µg/ml	Analyst 1	0.04523%	Instrument 1 (ELICO)	0.0358%
2.		Analyst 2	0.02735%	Instrument 2 (SYSTRONIC)	0.04626%

3.8 Assay

3.8.1 Standard preparation

The previously generated 10 ppm solution was used as the benchmark. At 267 nm, absorbance was measured and absorbance was recorded.

3.8.2 Test preparation

Seven tablets were taken, weighed and the weight was recorded to estimate the amount of sitagliptin in the tablet formulation. In a mortar and pestle, the tablets were then ground into a fine powder. Calculate the equivalent weight and place it in a 10 ml volumetric flask together with 10 mg of sitagliptin. Then, add small amounts of water and sonicate the mixture for 10 min in an ultrasonic water bath. Make up the volume to 10 ml with water to obtain 1000 ppm once the powder has completely dissolved in the solution. Then run the solution through the Whatman filter paper. A measured volume was collected from this filtered solution and diluted to a volume of 10 ml in a volumetric flask. This solution's absorbance is measured at 267nm. Weight of 7 tablets: 0.731g.

Weight on average: 0.731/7

Each pill weighs on average 0.1044 gm (104.4 mg).

Sitagliptin is present in 25 mg per tablet.

104.4 mg in 10 ml of diluent equals the required weight for 1000 ppm.

Sample absorbance is 0.5437.

Standard absorbance is 0.5587

Standard concentration = 100

Concentration of sample=(Absorbance of sample/Absorbance of standard)×Concentration of standard

By substituting all the above values, the concentration of the sample was found to be

Concentration of sample = 97% assay = (Sample absorbance/ standard absorbance) × (standard concentration/sample concentration) x 100%

Assay= 100%

Results of sitagliptin's quantitative analysis are given in Table 1.

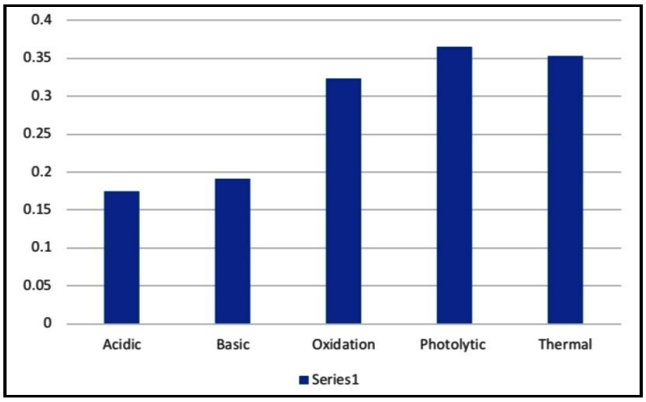

Figure 4: Degraded absorbance.

4. Discussion

A simple, rapid, precise, robust UV-spectrophotometric method has been developed for the estimation of sitagliptin in bulk and tablet dosage forms. The spectrum of 10 µg/ml of sitagliptin is shown in Figure. 2 indicated peak absorbance at 267 nm. Hence, 267 nm was considered as λmax of sitagliptin. A calibration curve is represented in Figure 3. The curve was obtained with satisfactory a correlation coefficient value of 0.9991, which indicated a positive correlation between concentrations of sitagliptin and the corresponding absorbance values.

All the validation parameters were performed according to ICH guidelines. The method obeyed Beer's law in the range of 10-300 µg/ml. Repeatability or intraday precision of sitagliptin was 0.65859% and inter-day precision was within the range of 0.4165%-0.7935%. Accuracy was within the range of 97-99%. The detection limit and

quantitation limits are 3.3 µg/ml and 10.2 µg/ml, respectively. All the validation parameters were found to be within the limits.

Forced degradation studies were also performed. Various studies such as Acid hydrolysis, alkali hydrolysis, peroxide, thermal, and photolytic degradation were performed. The forced degradation studies graph was shown in Figure 4.

5. Conclusion

It was discovered that the UV method using an aqueous solvent and a detection wavelength of 267 nm can efficiently analyze sitagliptin. The range of linearity was discovered to be 10-300 µg/ml. %RSD was found to be less than 2%, indicating that the method is highly reproducible. The accuracy research revealed a % recovery between 92.7-99.75%, indicating that the approach worked as intended and showing that the often-utilized excipients found in the pharmaceutical preparations do not affect the approach suggested. For sitagliptin, forced degradation studies were conducted and the amount of drug degradation was found to be within the limits. According to ICH guidelines, all the validation parameters were performed and all the parameters were found to be within the limits.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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