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NOVEL CHEMOMETRIC-DRIVEN GREENNESS ASSESSED SPECTROSCOPICAL METHOD DEVELOPMENT AND FORCED DEGRADATION STUDY OF SITAGLIPTIN PHOSPHATE IN ITS BULK DRUG AND OPHTHALMIC FORMULATION

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ABSTRACT
Background: Sitagliptin phosphate (SIT), an antidiabetic drug, requires reliable analytical methods for routine quality control and stability assessment. UV-visible spectrophotometry offers a simple and economical approach; however, method robustness and stability-indicating capability must be ensured using systematic development strategies such as Quality by Design (QbD). The objective of this work is to develop and validate a rapid, simple, economical, accurate, and precise UV-visible spectrophotometric method with a stability-indicating capability for the analysis of SIT in bulk drug and ophthalmic formulation. **Methodology:** Method development was performed using a QbD approach in a quality control laboratory, with a UV-visible spectrophotometer and 1-cm quartz cells. Various solvents (phosphate buffer, ethanol, acetonitrile, methanol, and water) were evaluated, with water selected due to superior solubility and spectral characteristics. SIT showed maximum absorbance at 265-267 nm. Forced degradation studies (acidic, basic, oxidative, thermal, and photolytic) were conducted. Validation was performed in accordance with ICH Q2(R1) guidelines. **Results and Discussion:** The developed method exhibited excellent linearity ($R^2 \geq 0.998$), accuracy (99-101% recovery), and precision (%RSD ≤ 2%). Recovery ranged from 98.68% to 99.46%. The method was robust and successfully indicated stability under stress conditions. The application of QbD ensured systematic optimization and robustness. Water proved to be an ideal solvent, enhancing method simplicity and cost-effectiveness. The method effectively distinguished SIT from degradation products, confirming its stability-indicating nature. **Conclusion:** A validated, reliable, and economical UV-visible spectrophotometric method was successfully developed for SIT analysis, suitable for routine quality control and stability studies in bulk drug and ophthalmic formulations.

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INTRODUCTION

Sitagliptin (SIT) is widely used for glycaemic control and can improve outcomes in Type 2 Diabetes Mellitus, either on its own or in conjunction with other antidiabetic medications [1-3]. Chemically, SIT is a triazolo-piperazine derivative belonging to the group of enzyme inhibitors known as Dipeptidyl Peptidase-4 (DPP-4), as shown in Figure 1. The IUPAC name of SIT is (2R)-4-Oxo-4-[3-(trifluoromethyl)-5,6-dihydro [1,2,4] triazolo [4,3-a] pyrazin-7(8H)-yl]-1-(2,4,5trifluoro phenyl) butan-2-amine. It has a molecular formula of $C_{16}H_{15}F_6N_5O$ and a molecular weight of 407.31 g/mol[3].

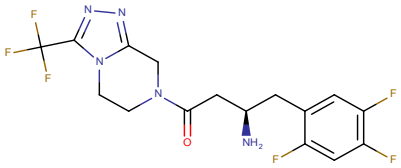

Figure 1: Structure of SIT

SIT exerts its antihyperglycemic effect by blocking the DPP-4 enzyme, thereby increasing the levels of the incretin hormones GLP-1 and GIP. This results in enhanced insulin secretion, suppression of glucagon release, reduction of hepatic gluconeogenesis, and improved glucose uptake by peripheral tissues. SIT also indirectly influences lipid metabolism by decreasing free fatty acid concentrations and promoting activation of an essential component of glucose and lipid metabolism, AMP-activated protein kinase (AMPK) [4]. A detailed review of the literature showed that various analytical techniques have been reported for estimating SIT in bulk drugs and ophthalmic formulations, including High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), UV-visible spectrophotometry, and High-Performance Thin-Layer Chromatography (HPTLC). However, many of these methods involve expensive instrumentation, high solvent consumption, longer analysis time, or lack cost-effectiveness and simplicity [5]. In view of these limitations, the present research developed and validated an accurate, precise, quick, affordable, and reliable UV-spectrophotometric technique for estimating SIT in bulk drugs and ophthalmic formulations and samples, utilizing water as the solvent and following the guidelines for method validation and stability studies [6]. QbD is a holistic and systematic strategy aimed at ensuring consistent quality and efficiency throughout pharmaceutical development. It aligns with the standards of the International Council for Harmonization (ICH), particularly ICH-Q8(R2), emphasizing clearly defined objectives, scientific understanding, and risk management. Introduced after the FDA's launch of "Pharmaceutical cGMPs for the 21st Century" in 2002, QbD has become a key strategy for developing reliable analytical methods. It involves six critical stages focused on building a robust, high-quality, and resilient process [7]. A fundamental element of QbD is the Design of Experiments (DoE), which helps to determine optimal method conditions and enhance efficiency. In this study, QbD principles were applied to develop a UV spectrophotometric method for estimating SIT in ophthalmic formulation [8]. Through FFD, it was easy to test a variety of CMVs simultaneously without increasing the number of experimental trials. The technique helped determine that the sampling interval and slit width were the two critical variables influencing reproducibility of the procedure and the absorbance pattern. After improving these two critical variables using CCD, it was possible to develop a model design space that produced reliable results despite slight deviations in the experimental environment. The implementation of QbD not only minimized variability but also ensured method robustness, resulting in a validated, cost-effective, and efficient analytical procedure in accordance with ICH guidelines [9].

MATERIALS AND METHODS

Instruments

A LABINDIA UV 3200 UV/VIS spectrophotometer with two identical 1 cm quartz cells was employed.

Chemicals and reagents

We received a gift sample of 99.8% pure SIT from ALKEM Laboratories LTD, Taloja, Panvel, Raigad 410208. All pure, analytical-grade chemicals and reagents used in the experiment were obtained from our organization's storage facility [10].

QbD: A Systematic and Science-Driven Approach in Pharmaceutical Development

Analytical Target Profile Incorporation

To create an effective, precise, and cost-effective UV spectrophotometric technique for SIT quantification in bulk drug & ophthalmic formulations, a thorough review of the literature and drug profiles was carried out to develop an Analytical Target Profile (ATP) [11].

Incorporation of Risk Management & Cause-Effect Relationship

Risk factors affecting method performance were identified using the Ishikawa fish-bone diagram and the CNX method. Critical Method Variables (CMVs), such as pH, sample integrity, wavelength, solvent, and scan rate, were evaluated for their impact on method variability [12].

Screening of CMVs by FFD

Fractional Factorial Design (FFD) using Design Expert software screened high-risk CMV scan speed, pH, and sampling interval that influence absorbance. Significant variables were identified through statistical analysis [13].

Optimization of Methods and Robustness Analysis (CCD)

Using Central Composite Design (CCD), the goal was to optimize critical parameters (pH, sampling interval) to maximize method reliability. Statistical modeling ensured accuracy and robustness using ANOVA and response surface methodology [14].

Method Control Strategy

A Design Space was established using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA) to develop control strategies ensuring method consistency, resilience, and reliable performance despite minor variations [15].

Method development

The solubility of the samples was evaluated in a variety of solvents, including 0.1N HCl, 0.1N NaOH, methanol, hexane, Acetone, Chloroform, acetonitrile & distilled water, under the solubility conditions illustrated in Table 1. The solubility was well determined with distilled water. Samples were scanned with distilled water in the UV range of 200-400 nm. At 265 nm, the highest/greatest absorption of SIT was observed.

Standard Stock Solution Preparation

After being weighed, 100 mg of SIT was put into 100 mL volumetric flasks. The medications were dissolved by sonication in 50 mL of distilled water, and the volume was adjusted with the same solvent to achieve a final concentration of 1000 µg mL-1.

Drug content uniformity

Uniformity of the drug content was assessed by testing 10 films, each containing 100 mg of sitagliptin. The mean weight of one film was determined as 502 mg. An amount of the film corresponding to 10 mg of sitagliptin was excised, placed in a 10 mL volumetric flask, and dissolved in distilled water to prepare a 1 mg/mL stock solution. A 5 mL aliquot was diluted to 50 mL with distilled water, yielding a solution at 100 µg/mL. Further dilution was performed in distilled water to obtain concentrations within the linearity range [16].

Working Standard Solution Preparation

SIT concentrations of 10, 20, 30, 40, 50, and 60 µg mL-1 were prepared in water to the appropriate levels. Absorbance was measured at 265 nm [17].

Wavelength selection

The UV spectrum at a wavelength of 10 ppm was chosen for the SIT study. The λmax of the SIT standard solution, as shown in Figure 2, was 265 nm versus water when scanned in the 200-400 nm range.

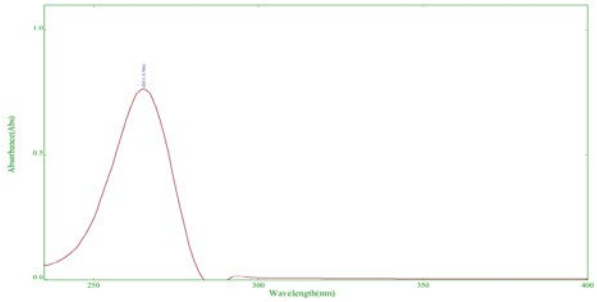

Figure 2: UV Spectra of SIT

Linearity

The method's ability to yield findings within a specific range that are directly proportional to the analyte concentration is known as linearity. To assess linearity, calibration standards are prepared at different concentrations and plotted as a calibration curve. A linear response indicates reliable measurement of different concentrations.

Accuracy (% Recovery)

Accuracy is the degree to which a measurement is precise and error-free. Recovery experiments, in which known quantities of analyte are introduced into the sample, are frequently used to evaluate the sample. High accuracy ensures the method can correctly quantify the analyte in real samples.

Precision

Precision is the closeness of agreement between measurements of the same sample under particular circumstances. It includes repeatability (same day) and intermediate precision (different days or analysts). Expressed as %RSD, a low value indicates that the method produces consistent, reliable results over repeated trials.

Ruggedness & Robustness

The repeatability of the technique during normal operating conditions, including the analysis performed by different analysts, at different times, and using different pieces of equipment or laboratory conditions, was considered explicitly in

the "Ruggedness" whereas, the "Robustness" has been assesed to consider the effects of deliberate small changes in analytical parameters such as the wavelength (±5 nm) of the already developed method of UV spectrophotometry.

Limit of Detection (LOD)

The minimum quantity of analyte that can be accurately identified but not always measured is known as the limit of detection, or LOD. It establishes the sensitivity of the procedure and is usually defined as a 3:1 signal-to-noise ratio.

Limit of Quantification (LOQ)

LOQ stands for the minimum possible analyte concentration that can be quantitatively measured with an exact level of accuracy and precision. It is often predicated on a signal-to-noise ratio of 10:1. With an established LOQ, the approach can accurately and statistically confidently assess low concentrations in a sample.

Forced degradation studies

To assess the durability of the established UV-spectroscopic approach, samples were subjected to oxidation, basic, acid, thermal, and photolytic deterioration. The percentage of degradation was calculated. The forced degradation study's limit ranges from 0.1 to 12%.

Acid degradation research

Approximately 10mg of SIT was weighed into separate 10 mL volumetric flasks for acid degradation. It was mixed with distilled water until the concentration reached 1000 μgml^{-1}. The concentration of the secondary stock solution of SIT was 100 μgml^{-1}, while the concentration of the working stock solution of SIT was 10 μgml^{-1}. This 10 μg ml-1 SIT solution was stressed for 2 hours at 80°C in a water bath containing 0.1N HCl. Spectra were recorded after scanning the samples in the UV range of 200-400 nm.

Base degradation study

A 10 μg ml-1 SIT solution was prepared as a stock and diluted with 0.1 N NaOH for the base degradation study. After two hours of stressing both solutions at 80°C, spectra were scanned.

Oxidation study

A solution containing 10 μg ml-1 of SIT was prepared from primary and secondary stock solutions and diluted with 30% hydrogen peroxide. After two hours of stressing both solutions at 80°C, spectra were scanned.

Photodegradation research

10 μg of the medications were exposed to UV light at 254 nm for 24 hours to assess photodegradation. Following that, solutions were prepared in distilled water to reach a final SIT concentration of 10 μg ml-1, and the appropriate spectra were obtained by scanning.

Thermal degradation research

Drugs were exposed to a hot air oven set at 40°C for four hours to perform thermal degradation. Subsequently, solutions were made with water (double-distilled) to reach a final concentration of 10 μgml^{-1}, and relevant spectra were obtained by scanning.

Formula to calculate percentage (%) degradation research

$$\%\ \boldsymbol{Degradation} = \frac{\boldsymbol{Initial\ degradation\ -\ Final\ degradation}}{\boldsymbol{Initial\ degradationa}}\ \boldsymbol{X\ 100}$$

Greenness of UV Spectroscopy method

The AGREE® program version 0.5 beta, available online from the University of Vigo in Spain, was used to assess the degree of greenness. The AGREE® program version 0.5 beta, available online from the University of Vigo in Spain, was used to assess the degree of greenness.

The goal of the scoring and penalties is to evaluate any approach in light of geo-safety and the human operating environment. Penalty points were shown as a pictogram and were subtracted according to preset standards. The ultimate greenness score obtained by subtracting penalty points is displayed at the center of the spherical pictogram. A higher level is indicated by a symbol with a green or almost green core color [18-20].

RESULTS AND DISCUSSION

Using a QbD approach to determine which variables needed to be included in a new variable-parameter method for spectrophotometric analysis, we began by creating an initial spectrophotometric analysis with each variable identified and in final form. The method variables were determined by establishing a schematic of the classic Ishikawa fishbone. The factors involved in the technique were physically evaluated.

The medicine was found to dissolve only partially in hexane. While SIT was somewhat soluble in acetone, alcohol, and chloroform, it was soluble in distilled water. However, distilled water was selected as the solvent to allow the experiments to proceed, as shown in Table 1.

Table 1: Solubility Assessment

S. No.	Solvent Investigated	Observed Solubility of Sitagliptin Phosphate	Interpretation/Remark
1	**Hexane**	Insoluble/Very slightly soluble	Inadequate dissolution observed; unsuitable for UV analysis
2	**Acetone**	Slightly soluble	Partial dissolution with poor spectral suitability
3	**Chloroform**	Slightly soluble	Limited solubility and unsuitable for routine analysis
4	**Methanol**	Partially soluble	Moderate solubility observed; organic solvent with less green compatibility
5	**Acetonitrile**	Partially soluble	Acceptable solubility but associated with higher solvent toxicity & cost
6	**0.1 N HCl**	Partially soluble	Solubility observed under acidic conditions
7	**0.1 N NaOH**	Partially soluble	Solubility observed under alkaline conditions
8	**Dist. Water**	Freely soluble	Exhibited maximum and consistent solubility with clear spectra; selected as optimized analytical solvent

At a wavelength of 265 nm, the standard SIT solution achieves its maximum absorption (max) in double-distilled water. This wavelength was selected as the detection wavelength for in-depth research to evaluate its impact on the approach's robustness. FFD simplifies screening CMVs by scan speed, sampling interval, and sample pH.

To show that the created model was representative/appropriate, analyses were performed using actual versus predicted values to assess how well they matched. To complete this analysis, there were three values used to determine how well they matched; the p-value of the created model was 0.0011; the R2 value indicated that 97.47% of the variability of actual data can be explained by predicted data; and RMSE indicated that the model was accurate, with only 0.0021 as a difference between actual and predicted.

When comparing these to the expected R2 value of 0.8198 and the modified R2 value of 0.9566, the model accurately represents the reaction and is therefore appropriate for measuring any reaction involving a CMV. To determine how the CMVs influence the reaction, the CCD was used to measure the absorbance of the sample in 13 trials, randomly ordered, using a UV spectrophotometer to obtain an unbiased result with a minimum of 5 center points.

Each experiment's findings are displayed in Table 2, and the examined spectrophotometric range led to the acceptance of the null hypothesis (H0) at a predefined significance level of 0.05 for the P-value. To draw insightful findings, a thorough analysis of the CCD model was conducted using a variety of statistical methods, including ANOVA and prediction profiles.

The predicted models and independent/nondependent factors' effects, data provided on a specific response when holding all other factors constant at a reference point, can be seen in Figure 3, part A of "Perturbation" plots. The sensitivity of the reaction to a particular component is indicated by the slope of the curve. In the sample pH analysis, factor B (the sampling interval) had the most significant effect on absorbance, as shown in Figure 3(A).

Table 2: Displaying Experimental Design matrix

Std	Run	Factor 1	Factor 2	Response
		A: Slit width (nm)	B: Sampling Interval (min)	Absorbance (Abs)
10	1	1.25	1.25	0.71
5	2	0.18934	1.25	1.18
12	3	1.25	1.25	0.71
9	4	1.25	1.25	0.71
7	5	1.25	0.18934	0.82
8	6	1.25	2.31066	0.64
3	7	0.5	2	1.06
2	8	2	0.5	0.61
6	9	2.31066	1.25	0.51
1	10	0.5	0.5	1.02
4	11	2	2	0.59
11	12	1.25	1.25	0.71
13	13	1.25	1.25	0.71

The straight and curved lines derived from the experimental research data lie smoothly/easily within Figure 3. Part B illustrates the "Actual" vs. "Predicted" differences between "Baseline" (Blue) and the corresponding model-created interval parameters.

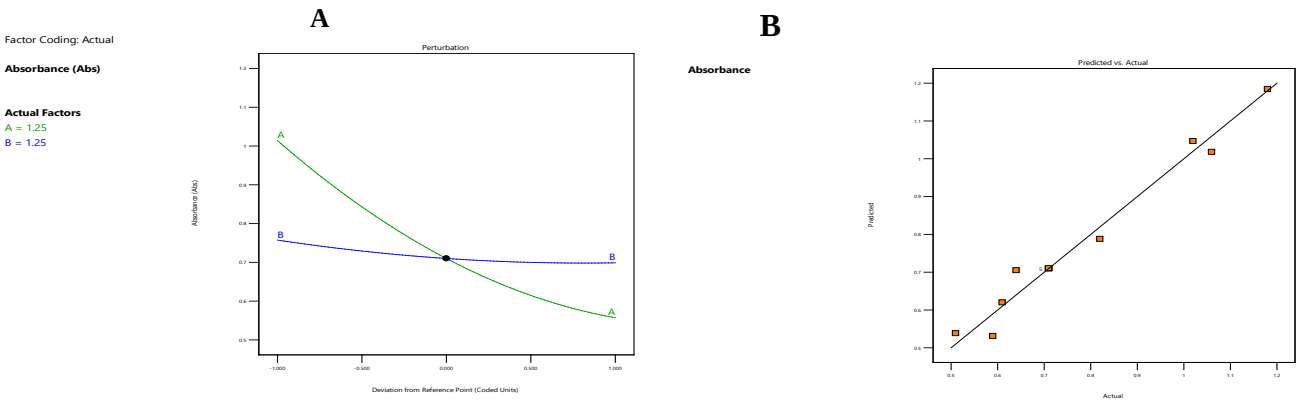

Figure 3: (A). Perturbation Plot, (B). Predicted vs Actual Plot

Figure 4 shows response surfaces plotted against slit width and sampling interval (slit width plotted against sampling interval). The optimization model perturbation and response plots show that these factors significantly affect the analyte's absorbance.

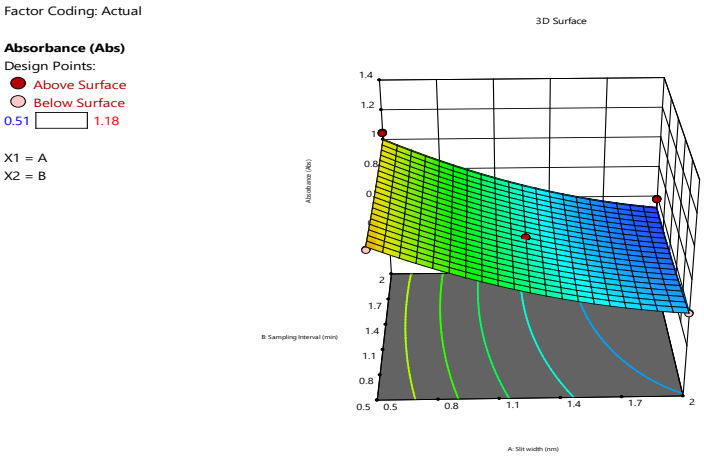

Figure 4: A plot of 3D Response surface for absorbance against slit width vs sampling interval

An analysis of variance (ANOVA) yielded a P-value of 0.0011, indicating that the model is appropriate for accounting for variability in the data and providing evidence to reject the Null Hypothesis (no effect). In addition, the lowest PRESS (Prediction Error Sum of Squares) value supports the model's appropriateness. Estimating variability risk across numerous factors requires re-evaluating parametric calculations. A P-value of less than 0.05 signifies a statistically significant non-zero slope. Two of the independent variable techniques that had the most influence were Slit Width (A) and Sample Interval (B2).

$$Absorbance\ (Y) = 0.7100 - 0.2284A - 0.0293B - 0.0150AB + 0.0756A^2 + 0.0181B^2$$

A-Slit Width and B-Sampling Interval are two important optical characteristics as outlined in Table 3. It was observed that the developed UV spectrophotometric method was selective for Sitagliptin phosphate in the range of 10-60 µg/mL, because the excipients present in the ophthalmic preparation did not interfere with the analysis. Regression analysis of the linearity data showed a high overall goodness of fit, as indicated by the R2, adjusted R2, and anticipated R2 values: R2 = 0.9747, adjusted R2 = 0.9566, and anticipated R2 = 0.8198.

The method used to evaluate linearity was deemed appropriate, as the ANOVA p-value was <0.05. The recovery percentage (%) from the bulk dosage form was verified with a sample size of 6 and found to be 100.2%, with a standard deviation of ±0.085.

Validation of the method

To assess linearity, accuracy, precision, LOD, LOQ, ruggedness, and robustness of the analyte, the technique was validated in accordance with ICH recommendations for the validation of analytical methods.

Linearity

Linearity was assessed by examining the various concentrations of the SIT standard solution. For SIT, the Beer-Lambert concentration ranges from 10–60 µgmL^{-1}. Plotting the SIT calibration curves, as shown in Figure 5 and Table 4, enabled determination of linearity.

Accuracy (% Recovery)

The usual addition method was used to assess accuracy. Additional amounts of 80%, 100%, and 120% of the standard SIT were added to the pre-quantified 10 µg mL-1 sample solution. Drug concentration was determined by measuring absorbance at 265 nm. The devised approach was used to examine these mixtures. Each experiment was conducted three times. Table 5 displays the percentage recovery, the percentage RSD, and the percentage that were computed at each concentration level.

Table 3: Validation parameters of SIT for UV spectro method

SNo	Parameter	Analytical Data
1	Linearity Range (µg/ml)	10-60
2	λmax (nm)	265 nm
3	Molar extinction coefficient	8.95×10^6 $L \cdot mol^{-1} \cdot cm^{-1}$
4	Sandell's sensitivity	4.55×10^{-5} µg/cm²
5	Slope	0.0212
6	Intercept	0.0094
7	Correlation coefficient (R^2)	0.9998
8	Limit of Detection (LOD, µg/ml)	0.063820755
9	Limit of Quantification (LOQ, µg/ml)	0.193396226
10	Intra-day Precision (%RSD)	0.147373722
11	Inter-day Precision (%RSD)	0.185144983

Table 4: Linearity study for SIT

Sl no.	Concentration	Absorbance
1	**10**	0.2198
2	**20**	0.4284
3	**30**	0.6546
4	**40**	0.8646
5	**50**	1.0643
6	**60**	1.2819
Mean		0.752266667
SD		0.39710054
Slop		0.0212
Correlation Coefficient		0.9998

Precision

The repeatability of six SIT solutions at a constant concentration of 10 parts per million was determined by calculating the relative standard deviation (% RSD) of those six values. By performing multiple replicates of SIT, the method's repeatability was confirmed. The precision of this method was further validated through both interday and intraday variability testing. The interday variability was measured by sequentially testing the samples on a single day; therefore, the concentration of SIT (10 ppm) was used to determine both the intraday and interday precision estimates. A solution (10 ppm) was chosen for the intraday variation research, which was conducted six times on the same day. We computed the mean, standard deviation, and percentage % RSD. For a solution (10 ppm), interday precision was established and examined six times on separate days. A percentage % RSD was computed. Table 6 displays the intraday and interday data.

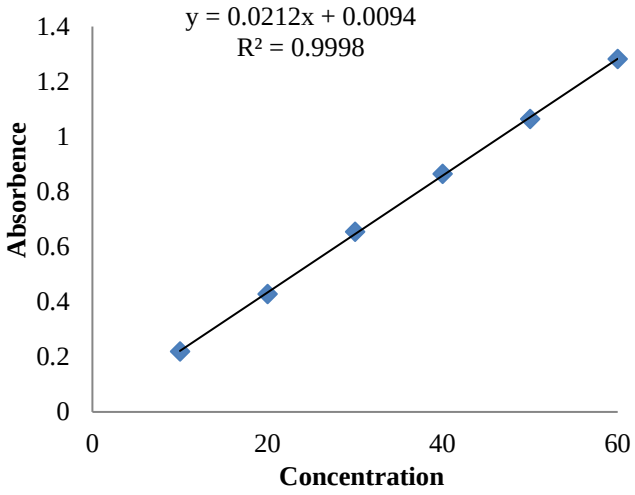

Figure 5: Calibration curve of SIT at 265

Table 5: Accuracy study for SIT

API	Formulation	Abs	%REC	Mean	SDV	%RSD
10	**20**	0.6199	98.94654088	98.68448637	0.267244209	0.270806708
10	**20**	0.6159	98.31761006			
10	**20**	0.6189	98.78930818			
20	**20**	0.8327	99.30424528	99.46147799	0.400866314	0.403036756
20	**20**	0.8387	100.0117925			
20	**20**	0.8307	99.06839623			
30	**20**	1.0423	99.21698113	99.3427673	0.247610311	0.249248453
30	**20**	1.0473	99.68867925			
30	**20**	1.0413	99.12264151			

Ruggedness

By employing various UV spectrophotometers across different labs and having different analysts conduct the analysis, the method's robustness was assessed. A % RSD was computed. Table 7 displayed SIT ruggedness data & analytical performance metrics.

Robustness

To verify the technique's robustness to minor intentional changes in spectrometric parameters, a robustness study was conducted. Table 8 displays SIT analytical performance characteristics, absorbance & robustness data for wavelength-of-detection fluctuations (±5 nm).

Table 6: Precision study for SIT

Interday		Intraday	
Conc	Abs	Conc	Abs
10	0.2203	10	0.2192
10	0.2192	10	0.2199
10	0.2204	10	0.2201
10	0.2198	10	0.2194
10	0.2202	10	0.2196
10	0.2198	10	0.2193
Mean	0.21995	Mean	0.219583
SDV	0.000407	SDV	0.000324
%RSD	0.185145	%RSD	0.147374

Table 7: Statistical validation for Ruggedness studies for SIT

S. No	Parameter	Set I	Set II
1	**System**	**LABINDIA UV 3200 UV/VIS Spectro.**	
2	**Sample**	A1	A2
3	**Day**	Tuesday	Tuesday
4	**Date**	25-03-2025	25-03-2025
5	**Time**	9:30 AM	2:30 PM
6	**Lab**	PG LAB I	PG LAB I
7	**Analyst**	Debashis Mishra	Dr. Kirtimaya Mishra
8	**Sample Conc**	10 μgml^{-1}	10 μgml^{-1}
9	**Absorbance**	0.2198	0.2198
10	**Assay**	0.193286592	0.193286592

Table 8: Statistical validation for Robustness studies for SIT

Sl. No.	Set. No.	Wavelength	Conc. (µg/ml)	Absorbance	Mean	Std dev	%RSD	%Assay
1	**1**	260	10	0.1992	0.19647	0.00526245	0.02678544	88.24
2		260	10	0.1998				
3		260	10	0.1904				
4	**2**	265	10	0.2192	0.2198	0.0006	0.00272975	99.24
5		265	10	0.2198				
6		265	10	0.2204				
7	**3**	270	10	0.2092	0.20647	0.00526245	0.02548811	92.95
8		270	10	0.2098				

LOD and LOQ

The limit of detection and quantification were calculated using statistical methods and the following formula, and tabulated in Table 9.

$$LOD = SDV/Slop \times 3.3 \quad (1)$$

$$LOQ = SDV/Slop \times 10 \quad (2)$$

Standard deviation of intercept LOD Slope of the calibration curve 10 standard deviation of intercept LOQ Slope of the calibration curve shown the formula in Eq. 1 & 2 respectively.

Table 9: LOD and LOQ data study for SIT

Analytes	SIT at 265 nm
LOD	0.063820755
LOQ	0.193396

Several circumstances were explored to improve the UV parameters and obtain satisfactory absorption and peak shape for SIT. Several solvents with varying compositions were attempted to offer adequate drug selectivity. Components of distilled water produced improved sensitivity. With a correlation coefficient (R^2) of 0.9998, the proposed method exhibited excellent linearity over the concentration range of 10-60 µg/ml, thereby indicating compliance with Beer-Lambert's law. The SIT correlation coefficient, as shown in Table 3 and Figure 5, was 0.9998. The standard addition method was employed to measure accuracy. The percentage recoveries for SIT were found in the range of 98.68-99.46 %. Table 5 presents the recovery (%) and %RSD data, demonstrating the validity of the proposed approach. The relative standard deviation (%RSD) values for the analytes indicated that the method performed within acceptable limits, as shown in Table 6 (< 2% RSD). Repeatability was demonstrated with low %RSD values. Ruggedness was confirmed through multiple sets of analyses conducted under identical circumstances on various days, by various analysts, using various tools, and at various times, with results ranging from 99-101%, as listed in Table 7.

Forced degradation research

The UV spectrophotometric studies revealed marked differences in spectra, decreased absorbance, and dissimilar absorption profiles between the unstressed and stressed samples, despite the method's inability to resolve individual degradation products or assess peak purity, as with HPLC and LC-MS. Comparison of overlaid spectra revealed visible changes in the drug's absorbance pattern under acid, alkali, and oxidative stress

conditions. Among all the stress conditions considered, acidic degradation exhibited the maximum percent degradation (11%), while alkali degradation was 9% and oxidation degradation was 5%. Therefore, it can be concluded that Sitagliptin phosphate was relatively more vulnerable to acidic stress conditions. (Table 10 & Fig. 6). These results indicate that, considering the limitations of UV spectrophotometry analysis, the developed method is suitable for stability monitoring/indicating method.

Table 10: Data of forced degradation study of SIT at 265 nm

Type	concentration (µg)	before degradation	after degradation	% degradation
Base	10	0.2198	0.2000	9.00
Acid	10	0.219833333	0.1957	11.00
Oxidation	10	0.2198	0.2088	5.00
Dry heat	10	0.219833333	0.2190	0.40000
Photolytic	10	0.2198	0.2194	0.20000

** The result is the average of 3 conjunctive samples.*

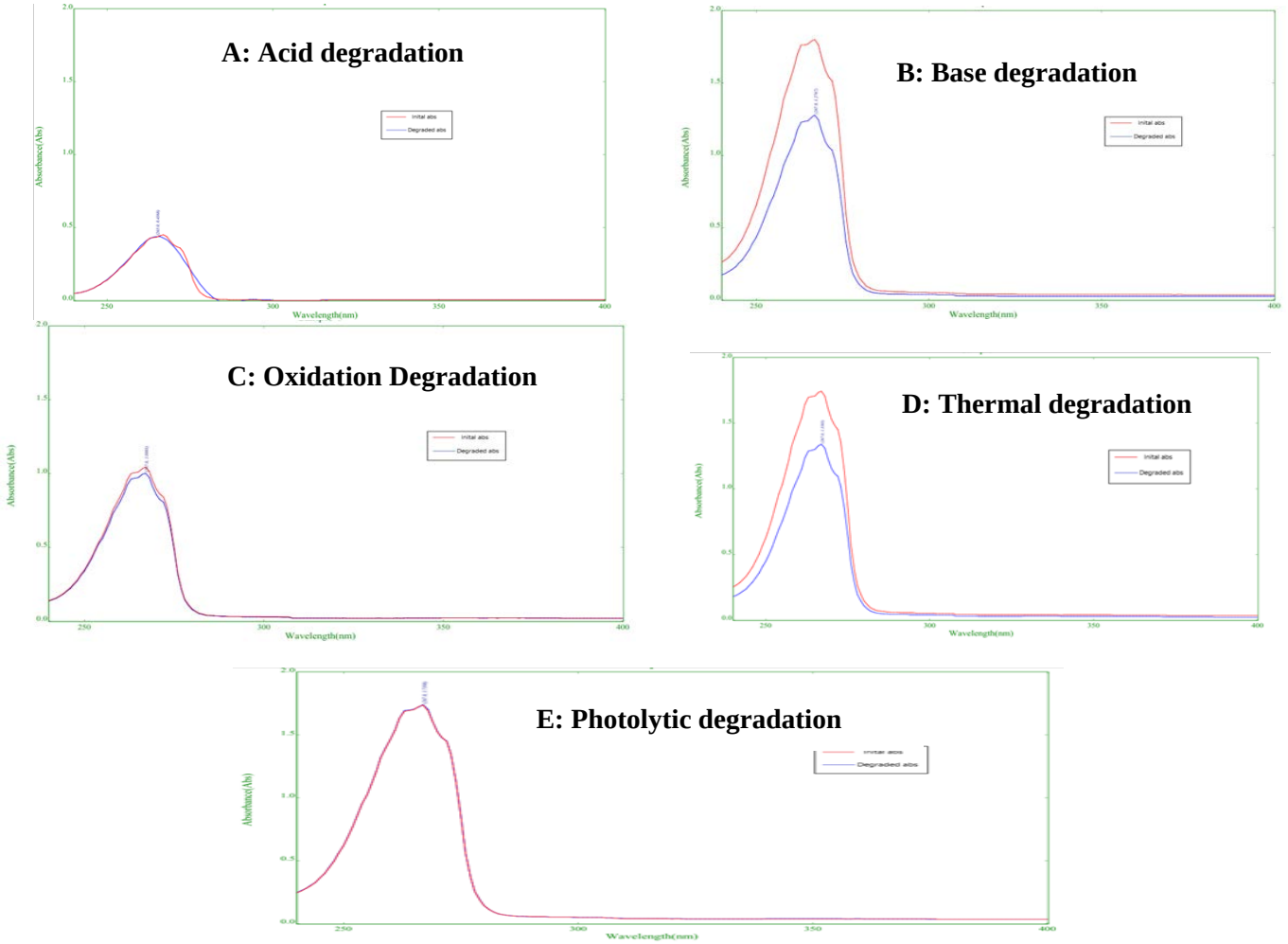

Figure 6: Degradation Study for SIT (A) Acid (B) Base (C) Oxidation (D) Thermal (E) Photolytic

Green assessment of the proposed method

The AGREE tool (Analytical Greenness Metric Approach) is a scale that ranges from 0 to 1, with 1 indicating more greenness & 0 for the least. Based on this, the proposed method achieves 0.87, which is also greener on the 0 to 1 scale, indicating that the process is eco-friendly and safe to analyze, as shown in Figure 7. The present UV spectrophotometry technique employs distilled water alone as the solvent throughout the development, validation, and forced degradation process. In contrast, most HPLC and other chromatographic techniques reported in the literature for SIT use toxic organic solvents such as methanol, acetonitrile, and tetrahydrofuran. The environmental and sustainability of the proposed analytical technique is validated by the absence of toxic organic solvents, which significantly reduces environmental impact, potential risks, solvent costs, and overall expenses. Moreover, the environmental sustainability of the proposed analytical method is supported by an AGREE-based greenness score of 0.87.

Figure 7: AGREE (Analytical Greenness Metric Approach) software

CONCLUSION

The newly developed UV-spectrophotometric technique exhibited high levels of linearity, accuracy, precision, robustness, ruggedness, and recovery. These attributes provide evidence that the method is reliable for the quantitative measurement of SIT in both its raw form and as an ingredient in ophthalmic pharmaceutical formulation. The simplicity, sensitivity, low cost, and readily available solvent system make this a viable option for routine quality control analysis using a UV detector. This technique has limited selectivity in complex matrices or in samples containing potential interfering substances and may not yield satisfactory results when applied to such samples. However, the validation process indicated that this approach is environmentally compatible, as determined by greenness assessment tools. This means using this method in the pharmaceutical sector will help reduce chemical-related risks and minimize the volume of solvent used during analyses. As the pharmaceutical sector continues to focus on developing sustainable, environmentally friendly analytical techniques, the method described here offers a viable, green alternative for routine drug analysis. It supports the pharmaceutical industry's regulatory and environmental goals.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Debashis Mishra contributed to the conceptualization of the study, the development of the methodology, & preparation of the original manuscript draft. Kirtimaya Mishra was responsible for conceptualization, investigation, and overall supervision of the work. Diptimayee Jena handled validation and data collection support and contributed to the review, editing, and interpretation of the results. Data curation was carried out by Debashis Mishra, Diptimayee Jena, and Himansu Bhusan Samal, who also contributed to visualization. Kirtimaya Mishra and Debashis Mishra provided essential resources required for the study. Each author has approved the final version for publication and has contributed significantly, directly, and intellectually to the work.

DECLARATION OF USE OF AI TOOLS

The authors used QuillBot and Grammarly to assist with language editing and readability. All content, analysis, and conclusions were reviewed and verified by the authors. The authors take full responsibility for the originality, accuracy, and integrity of the work.

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