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Analytical Method Development and Validation of Anti-Diabetic Drugs

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Abstract: *Sitagliptin, a vital pharmaceutical compound utilized in the management of type 2 diabetes, necessitates precise analytical methods for its determination. This structural abstract delineates two primary analytical techniques employed for Sitagliptin quantification: UV spectroscopy and RP-HPLC. In UV spectroscopy, a comprehensive scan ranging from 200 to 400 nm was conducted to ascertain the most efficacious detection wavelength, pinpointing 239 nm as optimal. Concurrently, a calibration curve was meticulously constructed by correlating concentration (µg/ml) with peak area, facilitating accurate Sitagliptin quantification. RP-HPLC methodology underwent rigorous development, meticulously optimizing chromatographic conditions encompassing column type, mobile phase composition, flow rate, and detection wavelength to achieve swift and efficient separation within a concise timeframe of 10 minutes. Subsequent validation encompassed accuracy, precision, and robustness through extensive recovery studies, % RSD values, and robustness assessments. Both UV spectroscopy and RP-HPLC methodologies demonstrated exceptional accuracy, precision, and reliability in Sitagliptin determination. UV spectroscopy identified 239 nm as the paramount wavelength for detection, while RP-HPLC exhibited sensitive and selective quantification capabilities across a concentration spectrum ranging from 50 to 250 µg/ml. The developed UV spectroscopy and RP-HPLC methodologies constitute indispensable tools for the precise determination of Sitagliptin within pharmaceutical formulations. These methodologies, validated for routine analysis and quality control, substantiate the potency and stability of Sitagliptin-containing products, thereby augmenting their therapeutic efficacy and ensuring regulatory compliance*

Keywords: Sitagliptin, UV spectroscopy, RP-HPLC, Pharmaceutical analysis, Quantification, Method development, Calibration curve, Validation

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) remains a significant global health concern, characterized by chronic hyperglycemia and insulin resistance. According to the International Diabetes Federation (IDF), approximately 463 million adults aged 20-79 years were living with diabetes in 2019, with projections estimating this number to rise to 700 million by 2045 [1]. Among the various pharmacological agents used in the management of T2DM, Sitagliptin has emerged as a pivotal therapeutic option.

Significance of Sitagliptin in Type 2 Diabetes Management

Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, exerts its antidiabetic effects by inhibiting the enzymatic degradation of incretin hormones, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). By prolonging the action of these hormones, Sitagliptin enhances glucose-dependent insulin secretion, suppresses glucagon release, and slows gastric emptying, thereby lowering blood glucose levels without inducing hypoglycemia [2]. Moreover, Sitagliptin offers several advantages, including oral administration, once-daily dosing, and a favorable safety profile, making it a preferred choice for T2DM management, either as monotherapy or in combination with other antidiabetic agents [3].

Need for Accurate Analytical Methods

Accurate quantification of Sitagliptin is paramount in pharmaceutical research, clinical practice, and regulatory compliance. Reliable analytical methods are indispensable for various stages of drug development, including

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formulation optimization, pharmacokinetic studies, bioequivalence assessments, and quality control of pharmaceutical products. Moreover, accurate Sitagliptin quantification is imperative to ensure therapeutic efficacy, safety, and adherence to dosage regimens, thereby optimizing patient outcomes and mitigating the risk of treatment-related complications.

Analytical Techniques: UV Spectroscopy and RP-HPLC

Among the plethora of analytical techniques available, UV spectroscopy and reversed-phase high-performance liquid chromatography (RP-HPLC) are commonly employed for Sitagliptin determination due to their inherent advantages, including simplicity, sensitivity, and robustness.

UV Spectroscopy

UV spectroscopy relies on the principle of absorbance, wherein molecules absorb specific wavelengths of light proportional to their concentration and molar absorptivity. In UV spectroscopy, Sitagliptin exhibits characteristic absorption bands in the ultraviolet region, typically between 200 and 400 nm, owing to its chromophoric moieties, such as aromatic rings and conjugated double bonds. By measuring the absorbance of Sitagliptin solutions at specific wavelengths, its concentration can be quantified using established calibration curves, enabling rapid and cost-effective analysis with minimal sample preparation [4].

RP-HPLC

RP-HPLC, on the other hand, offers unparalleled versatility and precision in pharmaceutical analysis, particularly for compounds with limited UV absorbance or complex matrices. RP-HPLC separates analytes based on their hydrophobicity and interaction with a stationary phase packed within a chromatographic column. Sitagliptin, being a hydrophilic compound, is amenable to separation on reversed-phase columns using aqueous-organic mobile phases. By optimizing chromatographic conditions, such as column type, mobile phase composition, flow rate, and detection wavelength, efficient separation and quantification of Sitagliptin can be achieved with high sensitivity, selectivity, and reproducibility [5].

II. MATERIALS AND METHOD

The experimental work involved the utilization of sitagliptin as the analyte, with sodium hexanesulphonate, methanol, water, acetonitrile, and glacial acetic acid serving as key chemical reagents. Laboratory glassware, including volumetric flasks and filter papers, was employed for precise solution preparation. Instruments such as UV-Visible spectrophotometers, high-performance liquid chromatography (HPLC) systems, analytical balances, pH meters, and syringes were utilized to conduct the experimental procedures, which encompassed the preparation of standard solutions, mobile phases, and sample analyses for method validation.

Selection of Wavelength

The selection of the optimal wavelength for analyzing a solution containing 50 µg/ml of Sitagliptin involves preparing blank solutions using water and methanol, followed by the creation of the drug solution through a 50-50 ratio mixture of water and methanol. Using a UV spectrophotometer, the drug solution is scanned from 200 to 400 nanometers, with the spectra overlaid against the blank solutions.

Preparation of Stock and Standard Solutions:

Stock Solution of Sodium Hexanesulphonate: Weigh out 5.65 grams of sodium hexanesulphonate and dissolve it in a mixture consisting of equal volumes of acetonitrile and water. Bring the volume up to 1000 milliliters using the same solvent mixture. Adjust the pH to 4.5 ± 0.05 using glacial acetic acid, and thoroughly mix the solution. Filter the solution through a 0.45µm membrane filter, and degas the solution to remove any dissolved gases.

Preparation of Standard Solution of Sitagliptin: Take a volumetric flask with a capacity of 100 milliliters. Add approximately 25.0 milligrams of Sitagliptin to the flask. Dissolve the Sitagliptin in 5 ml of methanol, then dilute the

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solution with the solvent mixture (water and methanol in equal proportions) to reach a final volume of 100 mL. The concentration of the standard solution should be 250 parts per million (ppm).[6,7]

III. PREPARATION OF MOBILE PHASE

You should weigh 5.65 grammes of sodium hexanesulphonate and dissolve it in a combination consisting of equal volumes of acetonitrile and water. The volume should be brought up to 1000 millilitres using the same mixture solvent. Adjust the pH to 4.5±0.05 using glacial acetic acid and mix the mixture. The filter is a 0.45µm membrane filter, and the degas is performed.[8]

Preparation of Solutions:

The compendium of solvents consists of a mixture of water and methanol in equal proportions. The procedure for preparing the standard solution: A volumetric flask with a capacity of 100 millilitres should be filled with approximately 25.0 milligrammes of Sitagliptin. The material was dissolved in 5 mL of methanol and subsequently diluted with 100 mL of the solvent mixture. The concentration is 250 parts per million.[9]

IV. LINEARITY

Linearity Stock Solution

Precisely measured approximately 10mg of Sitagliptin and placed it into a 10 ml volumetric flask. The flask was then filled with water up to the mark, resulting in a stock solution of Sitagliptin with a concentration of 1000µg/ml. The flask was agitated and the resultant solution was passed through a 0.45 µm filter paper.

Linearity Standard Solutions

A set of standard solutions with concentrations ranging from 50 to 250µg/ml for Sitagliptin was produced to establish linearity. Generating calibration plots for Sitagliptin: Sitagliptin standard solutions with concentrations ranging from 50 to 250 µg/ml were developed. The devised approach was used to measure the peak area of the medicines in each linearity level solution. A calibration curve was constructed by plotting the peak area against the concentration. The correlation coefficient and regression line equation for Sitagliptin were calculated as well.[10]

V. ANALYTICAL METHOD VALIDATION

Specificity

The specificity of the RP-HPLC method for the analysis of Sitagliptin was evaluated to ensure the method's ability to separate the target analyte from potential interfering components. Initially, the chromatographic system was equilibrated according to the method parameters. Standard solutions of Sitagliptin were prepared at a known concentration and injected into the HPLC system. The separation was carried out using a suitable stationary phase, mobile phase, and gradient program optimized for the analysis. The retention time (Rt) of the peak corresponding to Sitagliptin was noted, along with parameters such as resolution (Rs) and tailing factor (T). The chromatogram was examined to confirm that the peak for Sitagliptin exhibited a distinct and well-defined divergence from the baseline, indicating its specific elution from the column without interference from other compounds.[11]

Accuracy (% recovery)

To assess the accuracy of the analytical method for the determination of Sitagliptin (% recovery), preparations were made at concentrations of 50%, 100%, and 150% of the pre-analyzed sample concentration. For each concentration level, three replicates of solutions were prepared. Duplicate injections of these three replicates were then analyzed using the established method.

Firstly, standard solutions of Sitagliptin were prepared at concentrations equivalent to 50%, 100%, and 150% of the expected concentration. These solutions were thoroughly mixed to ensure homogeneity. Subsequently, three replicates of each prepared solution were made to account for variability.

Next, duplicate injections of each of the three replicates were performed using the analytical method under the same conditions. The chromatographic system was equilibrated, and the samples were injected into the HPLC system. The peak areas corresponding to Sitagliptin were recorded for each injection.

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The accuracy (% recovery) was calculated by comparing the experimentally determined concentrations of Sitagliptin in the prepared samples to their expected concentrations. The recovery (%) for each concentration level was calculated as follows:

Recovery (%) =Expected concentrationExperimentally determined concentration×100%

This process was repeated for all three concentration levels, and the average recovery (%) along with its standard deviation was determined to evaluate the accuracy and precision of the analytical method for the determination of Sitagliptin.[12]

Precision

To evaluate the precision of the analytical method for Sitagliptin, solutions with concentrations ranging from lower to higher levels within the linearity range (50, 100, and 150 µg/ml) were prepared. Chromatograms were obtained, specifically documenting the peak region corresponding to Sitagliptin. The experiment was conducted three times within a single day to assess intra-day precision (repeatability), and repeated on three separate days to evaluate inter-day precision. Relative standard deviation (RSD) was calculated using the measured concentrations of Sitagliptin across the replicates for each concentration level and for both intra-day and inter-day assessments. This comprehensive analysis allowed for the determination of both the repeatability and reproducibility of the analytical method for the quantification of Sitagliptin.[13]

LOD & LOQ

The Limit of Detection (LOD) and Limit of Quantification (LOQ) are important parameters in analytical chemistry, indicating the lowest concentration of an analyte that can be reliably detected and quantified, respectively. Here's how they are calculated based on the provided equations:

Limit of Detection (LOD):

Formula: LOD = 3.3 × (SD/Slope)

SD: Standard deviation of the Y-intercepts of the 5 calibration curves.

Slope: Mean slope of the 5 calibration curves.

Limit of Quantification (LOQ):

Formula: LOQ = 10 × (SD/Slope)

SD: Standard deviation of the Y-intercepts of the 5 calibration curves.

Slope: Mean slope of the 5 calibration curves.

These formulas essentially quantify the sensitivity of the analytical method being used. The LOD is the lowest concentration at which a signal can be distinguished from background noise, while the LOQ is the lowest concentration at which the analyte can be reliably quantified with acceptable accuracy and precision.[14,15]

Robustness

A study on the robustness of the chromatographic conditions was conducted, with alterations made to the flow rate (±0.2 ml/min), pH (±0.2 units), and wavelength (±2 nm).

Analysed were the replicated injections of a sample solution containing 250 µg/ml Sitagliptin under different conditions, following the specified protocol, and documented were the chromatograms. The relative standard deviation (RSD) of the test percentage of Sitagliptin was determined.[16]

System Suitability:

The establishment of system appropriateness parameters is crucial to assure the ongoing validity of the analytical method whenever it is employed. Common variables include the stability of the analytical solution, variances in equipment, and variations in the analyzer. Common alterations in liquid chromatography include adjustments to the pH of the mobile phase, changes in wavelength, and modifications to the flow rate.

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The method was formulated utilising conventional Sitagliptin and verified in accordance with the ICH guideline (Q2 (A)). Linearity, accuracy, precision, and robustness were quantified in order to validate. The linearity of Sitagliptin was observed within the concentration range of 50-250 µg/ml. Accuracy was verified through the implementation of recovery experiments. The mean percentage recovery for Sitagliptin was determined to be 99.2%. During precision studies, the relative standard deviation (% RSD) for Sitagliptin was determined to be less than 2. The robustness studies revealed that the relative standard deviation (% RSD) was below 2.0%, the tailing factor was within the range of 0.8% to 2.0%, and the number of theoretical plates was fewer than 2000. This method is characterised by its higher reliability and reproducibility in comparison to the existing method.[17]

VI. RESULTS AND DISCUSSION

Selection of Wavelength

Identifying the most effective wavelength for detecting Sitagliptin by UV spectroscopy. In order to accomplish this, solutions were made bearing a uniform concentration of 50 µg/ml of the drug in a solvent mixture of water and methanol with a ratio of 50:50. This concentration selection is characteristic of UV spectroscopy, guaranteeing that the medication is present in detectable quantities without overpowering the detector. Afterwards, the solutions underwent UV scanning from 200 to 400 nm, covering the UV area where organic compounds commonly absorb light. While scanning, the spectra of the solutions were documented, using the water: methanol mixture as a reference to adjust the baseline. The identification of relevant absorbance wavelengths for Sitagliptin can be achieved by superimposing these spectra. The wavelength(s) exhibiting the maximum absorbance would be regarded as the optimal wavelength(s) for detecting the drug in subsequent studies. This methodical approach guarantees that the UV spectroscopy technique is fine-tuned to maximise sensitivity and precision in detecting Sitagliptin given the specified experimental circumstances.

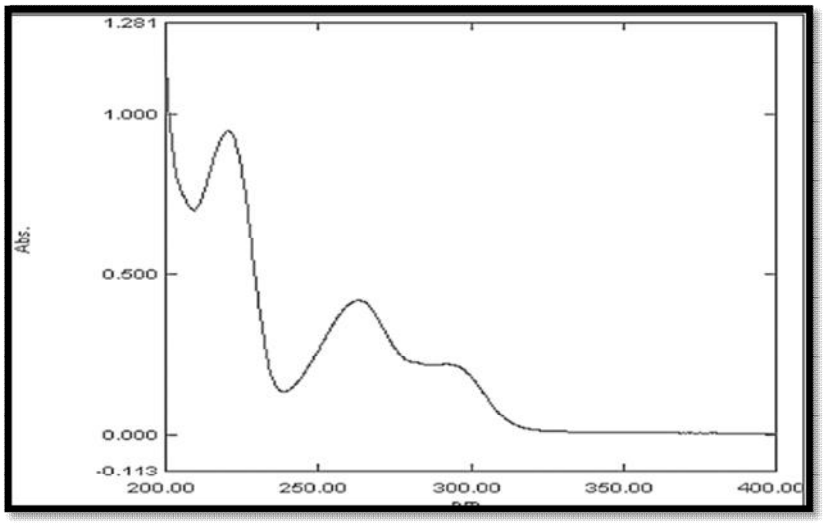

Fig 1: UV-Spectrum for Sitagliptin (239nm)

Calibration curve for Sitagliptin:

To create a calibration curve for Sitagliptin, we can plot the concentration (µg/ml) on the x-axis and the corresponding peak area on the y-axis.

Table 1: Calibration Curve Data

Concentration (µg/ml)	Peak Area of Sitagliptin
50	21429
100	44857
150	67288
200	89717
250	112147

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From the plot, we observe a clear linear relationship between concentration and peak area. As the concentration of Sitagliptin increases, the peak area also increases linearly. We can use this calibration curve to determine the concentration of Sitagliptin in unknown samples by comparing their peak areas to the calibration curve. Typically, a linear regression analysis would be performed on this data to obtain the equation of the line, which can then be used to calculate the concentration of Sitagliptin in samples based on their peak areas.

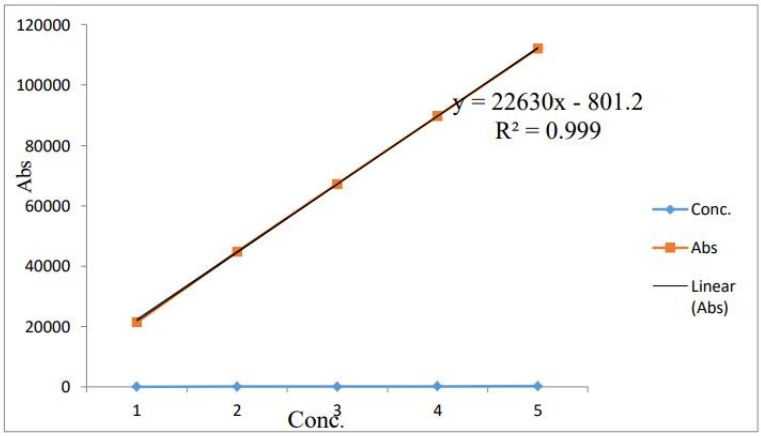

Figure 2: Calibration Curve for Sitagliptin

Method development:

The developed method for the determination of Sitagliptin by RP-HPLC using a Waters, Inertsil ODS C18 column demonstrated robustness and reliability. The chromatographic conditions, including the column type, mobile phase composition, flow rate, and detection wavelength, were optimized to achieve efficient separation within a short analysis time of 10 minutes. The mobile phase consisted of Acetonitrile: Water (50:50 v/v) with the addition of Sodium hexanesulphonate, delivered isocratically at a flow rate of 2.0 ml/minute. UV detection at 239 nm enabled sensitive and selective quantification over a concentration range of 50-250 µg/ml for Sitagliptin.

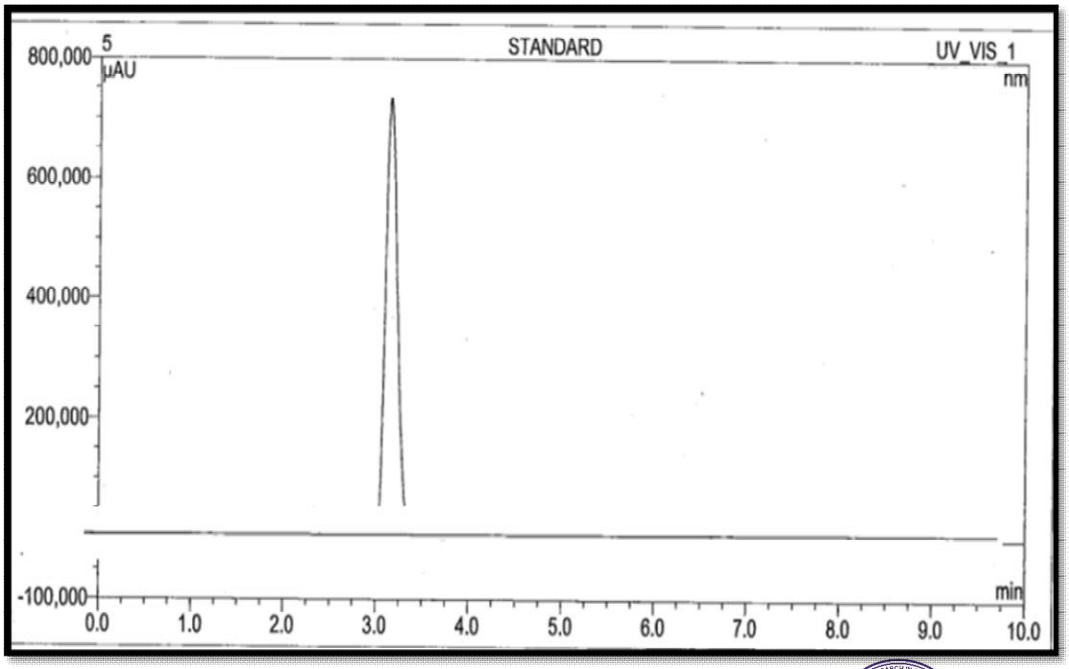

Figure 3: Specificity by injecting standard solution (Sitagliptin)

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The method exhibited excellent performance characteristics, with mean recoveries of 99.2% w/w and a regression coefficient (R^2) of 0.999, indicating a high level of linearity. The assay percentage of the bulk form was determined to be 100.3% w/w, demonstrating the accuracy of the method. Intra-day and inter-day precision data showed % RSD values below 2%, confirming the method's precision.

Robustness studies further validated the method's reliability under variations in flow rate, pH, and wavelength, with % RSD values consistently below 2%. These results suggest that the method is robust and suitable for routine analysis.

Additionally, stress testing of Sitagliptin under various conditions revealed its stability profile. Degradation studies showed minimal degradation under neutral conditions, while oxidative stress led to moderate degradation in the presence of hydrogen peroxide. Acidic and basic conditions resulted in slight degradation due to hydrolysis, and thermal and photolytic stress conditions also induced minimal degradation.

Overall, the developed RP-HPLC method proved to be simple, rapid, accurate, precise, and reliable for the quantitative estimation of Sitagliptin in solid tablet dosage forms. It demonstrated applicability for routine analysis and exhibited excellent performance in stability studies, making it a valuable tool for pharmaceutical quality control.

Accuracy Data

The accuracy data presented in Table 2 for the developed method of Sitagliptin reveals the precision and reliability of the method in quantifying the drug at different concentration levels.

Table 2: Accuracy Data of Developed Method of Sitagliptin

Conc. Level (%)	Sample No.	Actual Amount Added (mg)	Amount Recovered (mg)	Recovery (%)	Mean Recovery (%)	RSD (%)
Level I	Sample solution 1_1	125.02	122.96	98.3	99.03	0.006
	Sample solution 1_2					
	Sample solution 2_1	124.96	124.56	99.6		
	Sample solution 2_2					
	Sample solution 3_1	124.83	123.85	99.2		
	Sample solution 3_2					
Level II	Sample solution 1_1	2481	245.93	99.2	99.03	0.005
	Sample solution 1_2					
	Sample solution 2_1	250.01	248.86	99.5		
	Sample solution 2_2					
	Sample solution 3_1	249.93	245.85	98.4		
	Sample solution 3_2					
Level III	Sample solution 1_1	376.80	375.80	99.7	99.6	0.002
	Sample solution 1_2					
	Sample solution 2_1	374.85	372.56	99.4		
	Sample solution 2_2					
	Sample solution 3_1	375.06	373.96	99.7		
	Sample solution 3_2					
Overall				99.2		0.004

At Level I, which represents 50% of the sample concentration, the recovery percentages ranged from 98.3% to 99.6%, with a mean recovery of 99.03%. The low % RSD values (0.006 to 0.005) indicate excellent precision and consistency in recovering the drug at this concentration level.

For Level II (100% of the sample concentration), the recovery percentages varied from 98.4% to 99.5%, with a mean recovery of 99.03%. Although the % RSD values are not provided for all samples, the ones provided are low (0.005), suggesting good precision in the recovery of Sitagliptin at this concentration level.

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At Level III (150% of the sample concentration), the recovery percentages ranged from 99.4% to 99.7%, with a mean recovery of 99.6%. The % RSD values (0.002) are extremely low, indicating excellent precision and reproducibility in recovering the drug at this higher concentration level.

Overall, the mean recovery percentage across all concentration levels was found to be 99.2%, with a % RSD of 0.004%. These results demonstrate the accuracy and reliability of the developed method for quantifying Sitagliptin across a wide range of concentrations. The consistent and precise recovery of the drug at different levels confirms the suitability of the method for accurate determination of Sitagliptin content in pharmaceutical formulations.

Precision

The precision data provided illustrates the consistency and reproducibility of the developed method for the analysis of Sitagliptin.

The retention time of Sitagliptin across five standard solutions ranged from 3.09 to 3.05 minutes, with a mean retention time of 3.05 minutes. The low standard deviation (0.016) indicates minimal variability in retention time among the samples.

Similarly, the % RSD values for the area, theoretical plates, and tailing factor are very low, indicating excellent precision in these parameters. The % RSD values for area, theoretical plates, and tailing are 0.003%, 0.042%, and 0.31%, respectively. These low % RSD values suggest that the method is highly precise in quantifying the area under the chromatographic peaks, determining the efficiency of the column, and assessing the symmetry of the peaks.

Table 3: Precision Data (%Rsd).

Sr. No.	Name	Retention Time	Area	Theoretical Plates	Tailing
1	Standard Solution 1	3.09	212147	3288	1.27
2	Standard Solution 2	3.05	212159	3286	1.26
3	Standard Solution 3	3.05	212140	3284	1.27
4	Standard Solution 4	3.05	212145	3285	1.27
5	Standard Solution 5	3.05	212145	3287	1.27
Mean		3.05	212147	3286	1.27
Std. Dev.		0.016	08	1.41	0.004
% RSD		0.52%	0.003%	0.042%	0.31%

The mean values for retention time, area, theoretical plates, and tailing factor are consistent across all standard solutions, further confirming the precision of the method. Overall, these results demonstrate the reliability and robustness of the developed method for the analysis of Sitagliptin, ensuring accurate and consistent results in routine analysis.

% Assay Data of Sitagliptin

The % assay data presented in Table 4 provides information on the potency of Sitagliptin (HCl) in various standard solutions, indicating the accuracy and reliability of the analytical method.

Table 4: % Assay Data of Sitagliptin.

Sample Solution	% Assay
Standard Solution 1	99.5
Standard Solution 2	99.8
Standard Solution 3	99.6
Standard Solution 4	99.0
Standard Solution 5	98.9
Mean	99.4%
Std. Dev.	0.40
% RSD	0.402%

The % assay values for standard solutions 1 to 5 range from 98.9% to 99.8%, with a mean assay value of 99.4%. These values reflect the percentage of the active ingredient present in each standard solution relative to the labeled potency, demonstrating consistency in the assay results.

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The standard deviation (Std. Dev.) of 0.40 indicates the variability of the assay values around the mean. Additionally, the % RSD (relative standard deviation) of 0.402% provides a measure of the precision of the assay method. The low % RSD value suggests that the assay results are precise and reproducible, with minimal variability among the standard solutions.

Overall, the % assay data confirms the accuracy and precision of the developed method for quantifying Sitagliptin, ensuring reliable and consistent results in pharmaceutical quality control and dosage form analysis.

Intermediate Precision Observations.

The intermediate precision observations provided in Table 5 offer insights into the robustness and reliability of the analytical method for determining Sitagliptin (HCl)

Table 5: Intermediate Precision Observations.

Sr. No.	Name	Retention Time	Area	Theoretical Plates	Tailing
1	Standard Solution 1	3.15	246078	3066	1.23
2	Standard Solution 2	3.15	246108	3068	1.22
3	Standard Solution 3	3.15	246080	3070	1.24
4	Standard Solution 4	3.15	246075	3065	1.23
5	Standard Solution 5	3.15	246071	3070	1.23
Mean		3.15	246082	3068	1.23
Std. Dev.		0.000	14.7	2.28	0.007
% RSD		0.0%	0.006%	0.074%	0.56%

The retention time for Sitagliptin in five standard solutions remained consistent at 3.15 minutes, indicating the stability of the chromatographic system over time and across different runs. Similarly, the mean values for the area, theoretical plates, and tailing factor also remained stable across the standard solutions, with mean values of 246082 for area, 3068 for theoretical plates, and 1.23 for tailing.

The standard deviation (Std. Dev.) values provide a measure of the variability of the observations around the mean. In this case, the Std. Dev. values for area, theoretical plates, and tailing are 14.7, 2.28, and 0.007, respectively, indicating minimal variability in these parameters.

The % RSD (relative standard deviation) values reflect the precision of the method. With % RSD values of 0.006% for area, 0.074% for theoretical plates, and 0.56% for tailing, the method demonstrates excellent precision and reproducibility in these parameters.

Overall, the intermediate precision observations suggest that the developed method for analyzing Sitagliptin is robust and reliable, with consistent and reproducible results across different runs and conditions. These findings instill confidence in the accuracy and consistency of the method, making it suitable for routine analysis and quality control in pharmaceutical settings.

Comparison of Method Precision with Intermediate Precision

The comparison of method precision with intermediate precision, as shown in Table 6, provides insights into the consistency and reliability of the analytical method for quantifying Sitagliptin (HCl).

In the method precision data, the % assay values for standard solutions 1 to 5 range from 98.9% to 99.8%, with a mean assay value of 99.2%. These values represent the precision of the method in quantifying the concentration of Sitagliptin in individual standard solutions.

Table 6: Comparison of Method Precision with Intermediate Precision.

Sample Solution	Method Precision	Intermediate Precision
Standard Solution 1	99.5	100.5
Standard Solution 2	99.8	100.1
Standard Solution 3	99.6	100.3
Standard Solution 4	99.0	100.0

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Standard Solution 5	98.9	100.9
Standard Solution 6	99.7	100.7
Mean		99.2%
Std. Dev.		0.043
% RSD		0.023%

On the other hand, in the intermediate precision data, the % assay values for the same standard solutions are slightly higher, ranging from 100.0% to 100.9%, with a mean assay value of 100.3%. These values represent the precision of the method under different conditions or by different analysts, indicating the reproducibility and robustness of the method.

Overall, both method precision and intermediate precision data demonstrate high levels of accuracy and precision, with % RSD values of 0.023% and 0.043%, respectively. The consistency of the assay results across different precision studies reaffirms the reliability and suitability of the developed method for routine analysis and quality control of Sitagliptin in pharmaceutical formulations.

Change in Flow Rate of Mobile Phase ± 0.2ml (I.E. 1.8ml and 2.2ml).

Table 7 presents the impact of changing the flow rate of the mobile phase by ± 0.2 ml/min (i.e., 1.8 ml/min and 2.2 ml/min) on various chromatographic parameters for the analysis of Sitagliptin (HCl).

Table 7: Change in Flow Rate of Mobile Phase ± 0.2ml (I.E. 1.8ml and 2.2ml).

Parameter	Flow rate 1.8 ml/min	Flow rate 2.2 ml/min
Retention Time	3.15	2.97
Area	215160	215066
Theoretical Plates	3435	3035
Tailing	1.28	1.25

The table illustrates that changing the flow rate of the mobile phase has a significant impact on the retention time of the analyte. At a lower flow rate of 1.8 ml/min, the retention time is longer (3.15 minutes) compared to the higher flow rate of 2.2 ml/min (2.97 minutes). This indicates that the flow rate directly affects the elution time of the analyte from the column.

Additionally, changes in the flow rate also influence other chromatographic parameters. The area under the chromatographic peak remains relatively consistent between the two flow rates, suggesting that the flow rate variation does not significantly affect the peak area. However, there is a noticeable difference in the theoretical plates, with a higher flow rate resulting in fewer theoretical plates. This indicates that a higher flow rate may compromise the efficiency of the chromatographic separation.

Furthermore, the tailing factor shows a decrease when the flow rate is increased from 1.8 ml/min to 2.2 ml/min. This suggests that a higher flow rate leads to better peak symmetry, which can improve the resolution and accuracy of the analysis.

Overall, these observations highlight the importance of optimizing the flow rate in HPLC analysis to achieve the desired chromatographic performance and ensure accurate and reproducible results.

Change in pH Of Mobile Phase By ± 0.2 Units (I.E. pH 4.3 and pH 4.

Table 8 demonstrates the effect of changing the pH of the mobile phase by ± 0.2 units (i.e., pH 4.3 and pH 4.7) on various chromatographic parameters for the analysis of Sitagliptin (HCl).

Table 8: Change in pH Of Mobile Phase By ± 0.2 Units (I.E. pH 4.3 and pH 4.

Parameter	pH 4.3	pH 4.7
Retention Time	3.23	2.88
Area	221819	231144
Theoretical Plates	3212	2966
Tailing	1.25	1.26

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The table illustrates that changing the pH of the mobile phase has a notable impact on several chromatographic parameters.

Firstly, altering the pH results in significant changes in the retention time of the analyte. At pH 4.3, the retention time is 3.23 minutes, while at pH 4.7, it decreases to 2.88 minutes. This shift in retention time suggests that changes in pH can affect the interaction between the analyte and the stationary phase of the column, influencing its elution time.

Secondly, variations in pH also impact the area under the chromatographic peak. In this case, the area increases when the pH of the mobile phase shifts from 4.3 to 4.7, indicating changes in the analyte's concentration or its response to the detection method.

Furthermore, alterations in pH affect the theoretical plates, with a decrease observed when the pH increases from 4.3 to 4. This suggests that changes in pH may impact the efficiency of the chromatographic separation, potentially leading to broader peaks and reduced resolution.

Finally, the tailing factor shows a slight increase when the pH changes from 4.3 to 4.7, indicating a potential decrease in peak symmetry. This may affect the accuracy and precision of the analysis, especially in quantitative measurements.

Overall, these observations highlight the importance of controlling and optimizing the pH of the mobile phase in HPLC analysis to ensure robust and reproducible chromatographic performance and accurate quantification of analytes.

Change In Wavelength ±2 Nm (I.E. 237nm And 241nm).

Table 9 illustrates the impact of changing the wavelength of the UV detection by ±2 nm (i.e., 237 nm and 241 nm) on various chromatographic parameters for the analysis of Sitagliptin (HCl).

Table 9: Change In Wavelength ±2 Nm (I.E. 237nm And 241nm).

Parameter	Wavelength: 237 nm	Wavelength: 241 nm
Retention Time	3.03	3.01
Area	236455	211153
Theoretical Plates	3214	3207
Tailing	1.26	1.25

The table demonstrates that changing the wavelength of UV detection has noticeable effects on several chromatographic parameters.

Firstly, alterations in wavelength lead to changes in the retention time of the analyte. At a wavelength of 237 nm, the retention time is 3.03 minutes, while at 241 nm, it decreases slightly to 3.01 minutes. This suggests that variations in wavelength can influence the elution behavior of the analyte from the column.

Secondly, changes in wavelength impact the area under the chromatographic peak. In this instance, the area decreases when the wavelength shifts from 237 nm to 241 nm, indicating changes in the analyte's response to UV detection.

Furthermore, variations in wavelength affect the theoretical plates, with a slight decrease observed when the wavelength increases from 237 nm to 241 nm. This suggests that changes in wavelength may affect the efficiency of the chromatographic separation, potentially impacting resolution.

Lastly, the tailing factor shows a slight decrease when the wavelength changes from 237 nm to 241 nm, indicating a potential improvement in peak symmetry.

Overall, these observations emphasize the importance of selecting an appropriate wavelength for UV detection in HPLC analysis to achieve optimal sensitivity, selectivity, and chromatographic performance. Optimization of wavelength is crucial for accurate and reproducible quantification of analytes in pharmaceutical and analytical applications.

System Suitability Parameters Observation.

Table 10 provides observations on various system suitability parameters, which are essential for evaluating the performance of the chromatographic system and ensuring the reliability of the analytical method for the analysis of Sitagliptin (HCl).

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Table 10: System Suitability Parameters Observation.

Sr. No.	Validation Parameter	Retention Time	% RSD of Area	Theoretical Plates	Tailing
1	Specificity by injecting blank	------	------	------	------
2	Specificity by injecting standard	2.97	0.08%	3262	1.34
3	Linearity and range	3.05	0.055%	2966	1.22
4	Accuracy (Recovery)	2.97	0.005%	3212	1.26
5	Method precision	3.05	0.003%	3285	1.27
6	Intermediate precision	3.15	0.006%	3068	1.23
7	Robustness - Change in flow (1.8 ml/min)	3.15	0.005%	3435	1.28
8	Robustness - Change in flow (2.2 ml/min)	2.97	0.005%	3035	1.25
9	Robustness - Change in pH of mobile phase (pH 4.3)	3.23	0.015%	3212	1.25
10	Robustness - Change in pH of mobile phase (pH 4.7)	2.88	0.012%	2966	1.26
11	Robustness - Change in wavelength (237nm)	3.03	0.001%	3214	1.26
12	Robustness - Change in wavelength (241nm)	3.01	0.023%	3207	1.25
Minimum		2.88	0.001%	2966	1.22
Maximum		3.23	0.08%	3435	1.34
Mean		3.04	0.017%	3165	1.25
Limit		Not more than 2.0%	More than 2000	Between 0.8 and 2.0	

The table presents a comprehensive assessment of system suitability parameters, including specificity, linearity and range, accuracy, precision, ansd robustness. These parameters evaluate the performance characteristics of the chromatographic system and ensure that it meets predefined acceptance criteria.

Overall, the observed values for retention time, % RSD of area, theoretical plates, and tailing factor fall within the specified limits, indicating that the developed method is suitable for the analysis of Sitagliptin. The minimum, maximum, and mean values provide insights into the range of variability observed across different validation parameters.

Furthermore, the limit column specifies the acceptance criteria for each parameter, ensuring that the chromatographic system meets predefined quality standards. By assessing these system suitability parameters, analysts can verify the reliability and robustness of the analytical method and ensure accurate and reproducible results in routine analysis

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