International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1281-1289 www.ijprajournal.com ISSN: 2456-4494

"Development and Validation of A Reverse Phase-High-Performance Liquid Chromatography Method for the Estimation of Sitagliptin in Bulk and Tablet Dosage Form"

Sunil V. Garad[1] , Priyanka N. Yedle[2], Santosh P. Kumbhar[3], Sidheshwar S. Patil[4], Parvej S. Shaikh[5] .

Date of Submission: 01-04-2025 Date of Acceptance: 10-04-2025

ABSTRACT
A method was developed for the simultaneous estimation of Sitagliptin using a RP-HPLC system. The chromatographic conditions were optimized for Sitagliptin analysis with a COSMICSIL, INERTSIL C18 column (250 x 4.6 mm, 5µm particle size). The flow rate was set at 1.0 ml / min, and the mobile phase consisted of Na_2HPO_4 and Methanol in a 50:50 ratio. The detection wavelength was set at 248.8 nm. Analytical method goals are often defined as method acceptance criteria for peak resolution, precision, specificity and sensitivity. The technique of HPLC has following features: High resolution, Small diameter, Stainless steel Glass column ,Rapid analysis, Relatively higher mobile phase pressure and Controlled flow rate of mobile phase. The primary objective of the HPLC method is to separate and quantify the main active drug, identify any reaction impurities, detect synthetic intermediates, and measure degradants.

Keywords: Sitagliptin, RP-HPLC, Na_2HPO_4, Acetonitrile, KH_2PO_4, Methanol and Water.

I. INTRODUCTION

The development of analytical method typically rely on prior research or existing literature, often using similar or the same instrumentation. It is uncommon for a High-Performance Liquid Chromatography (HPLC)-based method to be created without referencing or comparing it to established techniques. HPLC has become the preferred method in the pharmaceutical industry for analyzing intact drugs and their degradation products. Selecting the right chromatographic conditions is crucial to ensure the method to achieve the required specificity.

UV spectroscopy, a straightforward and commonly used analytical technique, is frequently utilized for routine drug analysis. For certain drugs, HPLC and UV spectroscopy have been chosen as the methods for the proposed procedures. The origins of liquid chromatography date back to the early 1900s when Russian botanist Mikhail Tswett first applied a form of the technique to separate colored plant compounds. The development of modern HPLC began in the late 1960s, aligning with advancements in instrumentation, improvements in separation columns, and the introduction of more reliable and efficient stationary phases and packing materials.

The introduction of high-pressure liquid chromatography led to improvements in column packing materials, boosting its efficiency as a separation technique. When paired with the convenience of online detectors, this method evolved into what we now know as High-Performance Liquid Chromatography (HPLC). The principle of HPLC involves injecting a sample solution into a column packed with a porous material (stationary phase), while a liquid (mobile phase) is pumped through the column under high pressure. Separation occurs based on the solutes' adsorption to the stationary phase, which depends on their affinity for it.

HPLC Method Development

New analytical methods are created when official procedures are not available. Alternative methods for non-pharmacopoeial products are designed to improve cost efficiency, reduce time, and enhance precision and robustness. When suggesting an alternative method to replace an existing one, comparative laboratory data is provided to highlight its advantages and limitations.

The main objective of the HPLC method is to separate and quantify the primary active pharmaceutical ingredient (API), identify reaction-related impurities, detect synthetic intermediates, and measure degradation products. The critical steps in method development include

Gaining an understanding of the physicochemical properties of the drug molecule.

Choosing appropriate chromatographic conditions.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1281-1289 www.ijprajournal.com ISSN: 2456-4494

Developing the analytical technique.
Preparing the sample.
Optimizing the method.
Validating the method.

II. MATERIALS AND METHODS

Instruments used:

HPLC – Waters Model NO. E-2695 series Compact System Consisting of Inertsil-C18 ODS column
UV spectrophotometer (Systronics)
Electronic balance (ASCOSET)
Sonicator (ENERTECH)

Chemicals used:

Methanol HPLC Grade, Buffer HPLC Grade, Sodium hydroxide, Ortho Phosphoric acid, Hydrochloric acid, Peroxide.

III. EXPERIMENTAL WORK:

Stock and Standard Working Solution:

Sitagliptin serves as the working standard in method development.

Preparation of Stock Solution:

Weigh 50 mg of Sitagliptin working standard and dissolve it in 100 ml of volumetric flask (V.F) with methanol. Sonicate the solution for 30 minutes to obtain a 1000 ppm solution.

Further Dilution or Trial Solutions:

Take 10 ml of the above stock solution and transfer it to a 100 ml V.F. Add methanol up to the mark and sonicate for 10 minutes. This results in a 100 ppm solution.

Selection of Wavelength:

Scan the standard solution using a UV spectrophotometer over the range of 200 nm to 400 nm in spectrum mode, with the diluent as the blank. Sitagliptin exhibits a λmax at 248.8 nm..

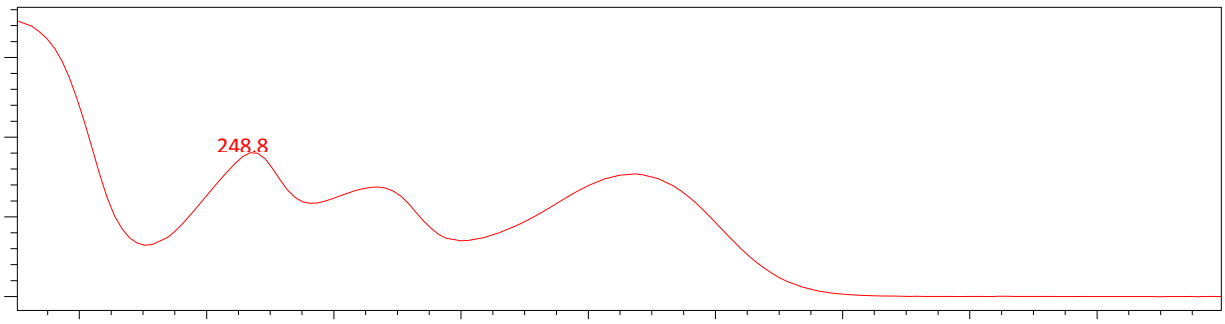

Fig 1:- Absorbance

IV. DEVELOPMENT OF HPLC METHOD:

This study aimed to improve the assay method for the simultaneous quantification of Sitagliptin, guided by findings from literature reviews. The following trials illustrate the optimization process that was carried out.

Table 1 :- Trials

Sr. No.	Trial	Mobile Phase	Name of the peak	Retention time (min)	Flow Rate (ml/ min)	Time to run (min)	Temp. in the column
1.	1	KH_2PO_4:Methanol (60:40)	Sitagliptin	13.833	1.0	8	25^0C
2.	2	NaH_2PO_4:Methanol (70:30)	Sitagliptin	4.649	1.0	5	25^0C
3.	3	NaH_2PO_4:Methanol	Sitagliptin	2.250	1.0	5	25^0C

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1281-1289 www.ijprajournal.com ISSN: 2456-4494

		(50:50)					

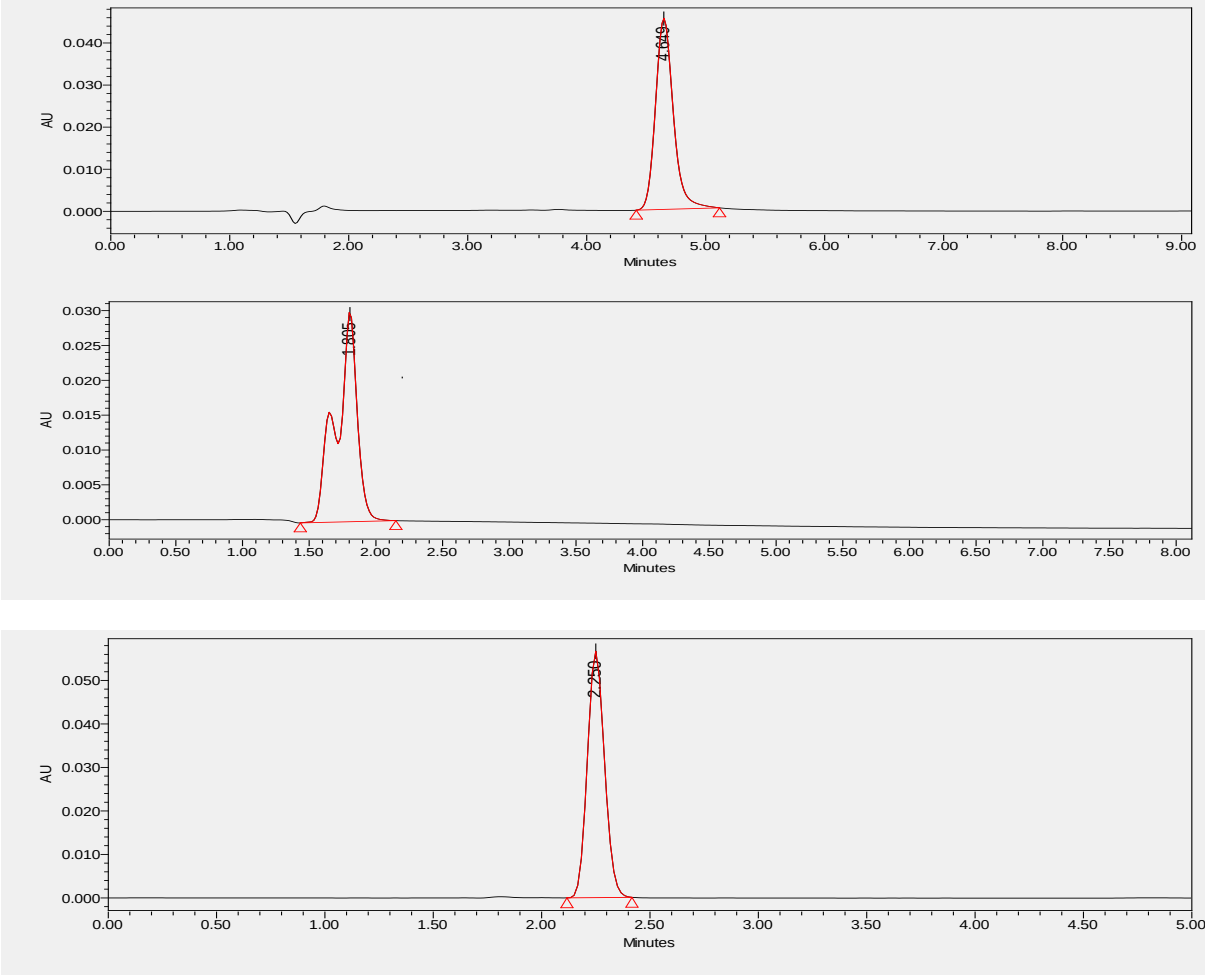

Fig. 2:- Trials

V. METHOD VALIDATION:

System Suitability:

A standard solution was prepared using the Sitagliptin working standard following the test method and injected into the HPLC system five times. System suitability was evaluated by calculating the % RSD from five replicate injections, focusing on the retention times and peak areas of Sitagliptin. The tailing factor for the Sitagliptin peaks in the standard solution should be ≤ 2.0, and the theoretical plates for these peaks should be ≥ 2000.

SPECIFICITY:

The standard, sample, blank, and placebo solutions were prepared following the test procedure and then injected into the HPLC system.

Preparation of sample:

Weigh 50 mg of Sitagliptin sample and place it in a 100 ml volumetric flask. Add 10 ml of water and 10 ml of methanol then sonicate for 20 minutes. Complete the volume with water. Next, transfer 1 ml of this solution into a 10 ml volumetric flask and dilute to the mark with water.

The method precision was determined by calculating the % RSD of the peak areas from six replicate injections of the sample chromatograms.

Acceptance criteria: The injection reproducibility is deemed acceptable if the % RSD for peak areas is below 2.0 and for retention times, it is also below 2.0.F

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1281-1289 www.ijprajournal.com ISSN: 2456-4494

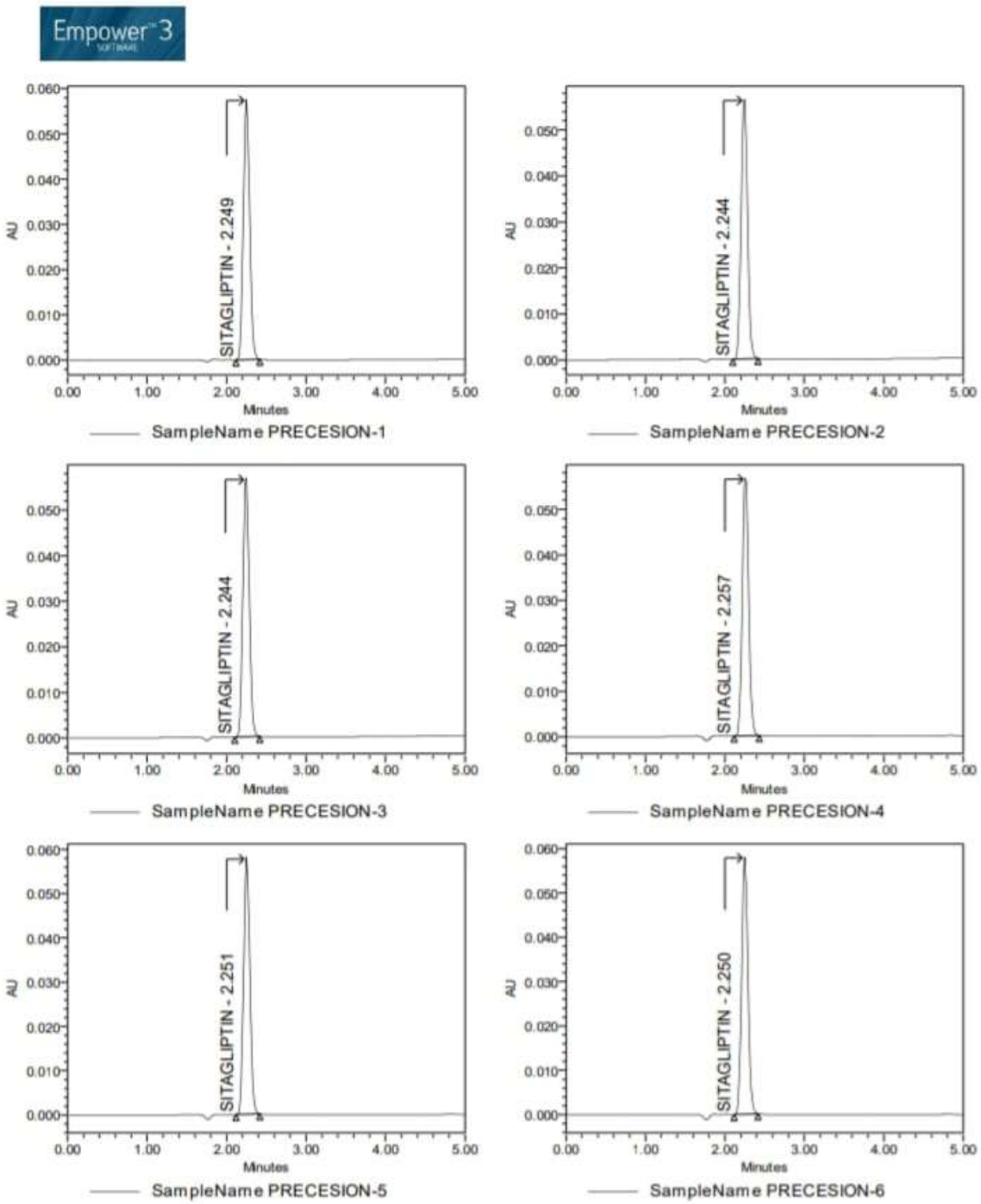

Fig. 3:- Precision

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1281-1289 www.ijprajournal.com ISSN: 2456-4494

Table 2:- Data of Precision

Sr. No	Sample Weight	RT(min)	Area(µV*sec)	%Assay
injection1	76	2.249	311864	99
injection2	76	2.244	311965	99
injection3	76	2.244	312131	99
injection4	76	2.257	311273	99
injection5	76	2.251	312024	99
injection6	76	2.250	312111	99
Mean	76	2.249167	311894.67	99
Std. Dev.	0	0.004875	319.9179	0.10
% RSD	0%	0.217%	0.103%	0.10%

I. ACCURACY (RECOVERY):

In the validation study, accuracy is determined by calculating concentrations of 50%, 100%, and 150%. The assay of Sitagliptin should fall between 98% and 102%. The results obtained are presented in the table.

Table-3: Data of Accuracy

Sr. No	Accuracy level	Injection	Sample area (µV*sec)	RT (min)	Sample weight	µg/ml added	µg/ml found	% Recovery	%Mean
1	50%	1	154421	2.232	38	24.750	24.57	99	99
		2	154101	2.236	38	24.750	24.52	99	
		3	154120	2.246	38	24.750	24.52	100	
2	100%	1	311424	2.253	76	49.500	49.55	100	100
		2	311451	2.250	76	49.500	49.56	100	
		3	311669	2.249	76	49.500	49.59	100	
3	150%	1	467440	2.260	114	74.250	74.38	100	100
		2	467402	2.247	114	74.250	74.37	100	
		3	467541	2.250	114	74.250	74.39	100	

II. LINEARITY:

The limit of detection (LOD) is the smallest concentration that can be detected, though it may not be quantified with precision. The limit of quantification (LOQ) refers to the lowest concentration of analyte in a sample that can be accurately and precisely quantified. To determine these limits, prepare a series of standard solutions and inject them into the HPLC system. Plot a graph of the standard concentration versus the actual concentration in µg/ml, and calculate the coefficient of correlation, using 100% response as the basis for the analysis.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1281-1289 www.ijprajournal.com ISSN: 2456-4494

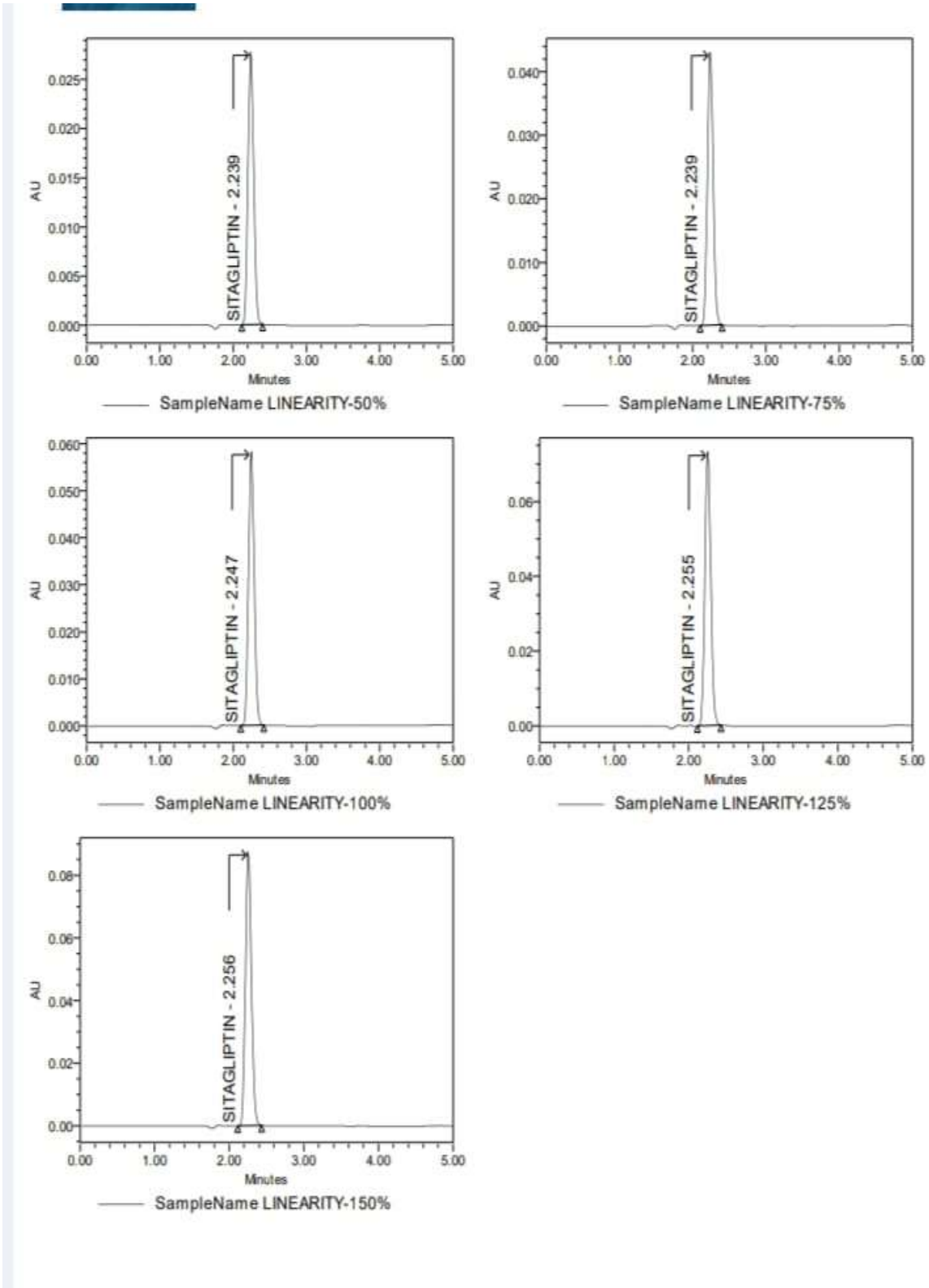

Fig. no .4:- Linearity

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1281-1289 www.ijprajournal.com ISSN: 2456-4494

Table4 : Data of Linearity

Sr. No	Conc (µg/ml)	µg/ml	RT(min)	Area(µV*sec)
1.	50	25	2.239	154112
2.	75	37.50	2.239	232902
3.	100	50	2.247	311922
4.	125	62.5	2.255	390050
5.	150	75	2.256	467803
Correlation coefficient (r^2)				0.9997

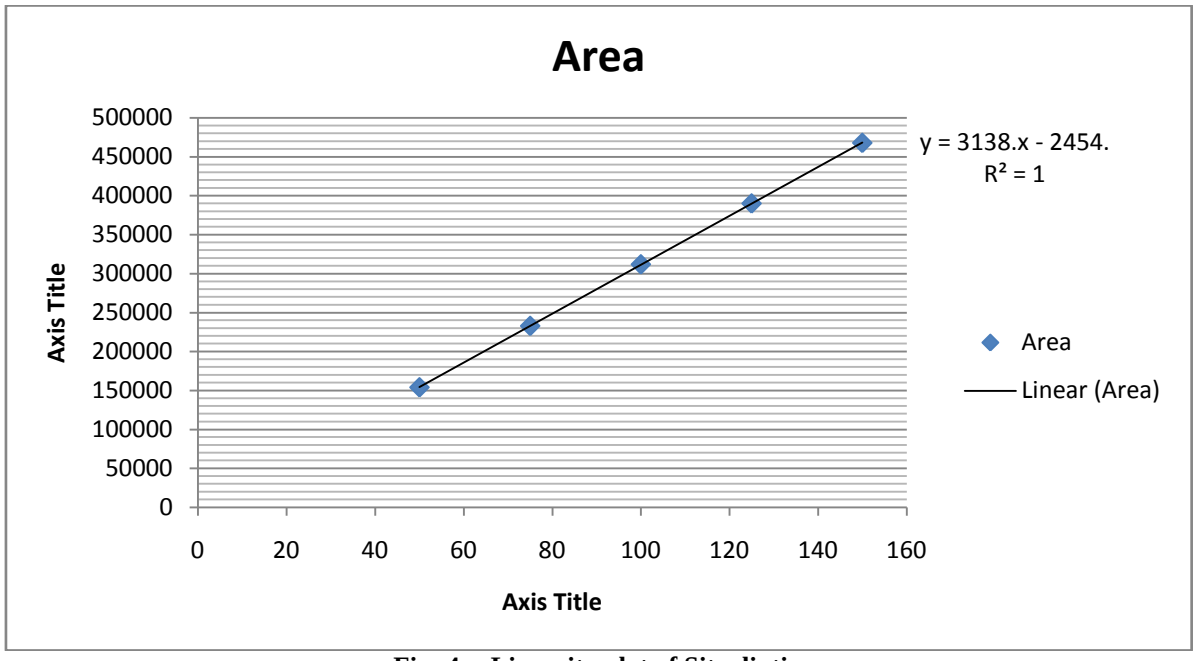

Fig: 4 :- Linearity plot of Sitagliptin

III. Ruggedness:

System variability:

A system variability study was performed on the HPLC system under consistent conditions at different times. Five samples were prepared and each analyzed according to the test method. The results from the HPLC system indicate that the assay test method is rugged in terms of system variability. The % relative standard deviation of Sitagliptin from the five sample preparations should not exceed 2.0%, and the % assay of Sitagliptin should range between 98.0% and 102.0%.

IV. Robustness:

I. Effect of variation in flow rate:

Prepare the system suitability solution according to the test method and inject it into the HPLC system, maintaining a flow rate within ±0.2 ml of the method's specified flow rate. Evaluate the system suitability parameters for both flow rates as outlined in the test method. The actual flow rate was 1.0 ml/min, which was adjusted to 0.8 ml/min and 1.2 ml/min, and the solution was injected into the HPLC system for suitability testing.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 2 Mar–Apr 2025, pp: 1281-1289 www.ijprajournal.com ISSN: 2456-4494

II. Effect of variation in wavelength:

Prepare the system suitability solution according to the test method and inject it into the HPLC system with a ±2 nm variation in wavelength. Evaluate the system suitability parameters for both wavelengths as specified in the test method..

III. Forced Degradation Evaluation:

Acidic Stress: Add 1 ml of 0.1N hydrochloric acid and sonicate for 30 minutes at room temperature.

Basic Stress: Add 1 ml of sodium hydroxide and sonicate for 30 minutes at room temperature.

Oxidative Stress: Add 1 ml of 30% hydrogen peroxide and sonicate for 30 minutes at room temperature.

Neutral Stress: Add 1 ml of distilled water and sonicate for 30 minutes at room temperature.

Photo Stress: Expose 1 ml of the tablet stock solution to direct sunlight for 24 hours.

Dry Heat Stress: Expose 1 ml of the tablet stock solution to 105°C for 30 minutes in a hot air oven**.**

Table 5:- Forced degradation study

Sr.no	Name	Sample Weight	Sample area(µV*sec)	%Assay
1	Acid (0.1N HCl)	76	281432	89.56
2	Base(0.1N NAOH)	76	297905	94.81
3	Peroxide (30% H_2O_2)	76	291468	92.76
4	Heat	76	286013	91.02
5	Sunlight	76	299991	95.47
6	Water	76	320713	98.88
	Mean	76	296253.67	93.75
	StdDev	0	13873.78	3.358214
	%RSD	0%	4.68%	3.58%

TABLE 6 : Data for Effect of variation in flow rate:

Parameter	RT(min)	USP Plate Count	Area(µV*sec)	USP Tailing
Decreased flow rate	1.829	3911	313365	1.04
Increased flow rate	2.984	3587	521337	1.19
Decreased temp.	2.030	3086	349526	1.04
Increased temp.	2.568	3760	449601	1.09
Decreased comp rate	1.829	3911	313365	1.04
Increased comp rate	2.568	3760	449601	1.09
Decreased pH	2.244	3793	313965	1.08
Increased pH	2.249	3695	313864	1.09

V. LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD AND LOQ):

From the linearity plot the LOD and LOQ are calculated:

LOD = 3.3 σ/S
LOD = 3.3x (0.111/3.1)
LOD = 0.3663/3.1
LOD= 0.118

LOQ = 10 σ/S
LOQ = 10X (0.401/10.1)
LOQ = 40.1/10.1
LOQ=3.97

VI. SUMMARY AND CONCLUSION:

Several parameters were assessed to develop the analytical method. The maximum absorbance of Sitagliptin was determined to be at 248.8 nm. A 10 µL injection volume was selected,

which resulted in a well-defined peak area. A Cosmicsil C18 column was employed, producing sharp peak shapes, and ambient temperature was found to be optimal for the drug solution. Based on favorable peak areas, appropriate retention times, and resolution, the flow rate was optimized to 1.0 mL/min. Various mobile phase ratios were tested, and a 50:50 mixture of Na2HPO4 and methanol was chosen due to its symmetrical peaks and excellent resolution. This mobile phase was therefore adopted for the study.

The accuracy and precision of the system and method were confirmed to be within acceptable limits. The correlation coefficient and curve fitting were established during the linearity assessment. The method demonstrated linearity within a concentration range of 20–70 ppm for both analytes. The developed method was shown to be rapid, accurate, precise, specific and robust. Robustness and ruggedness tests were successfully passed, with an excellent relative standard deviation observed in both cases.

REFERENCES:

[1]. Day RA, Underwood AL. Quantitative analysis, 5th ed. New Delhi: Prentice- Hall India Pvt Ltd; 1986. p. 1-6.
[2]. Basett J, Denney RC, Jerrery GH, Mendham J. Vogel's text book of quantitative inorganic analysis. 4th ed. England: Longman Group; 1986. p. 2
[3]. Willard HH, Merrit LL (Jr), Dean JA, Settle FA (Jr). Instrumental methods of analysis. 6th ed. New Delhi: CBS Publishers and Distributors; 1986. p. 1-15.
[4]. Kirkbright GF. Development and publication of new spectrophotometric methods of analysis.Talanta 1966:1-14.
[5]. www.pubmed.com.
[6]. Pattengill MD, Sands DE. Statistical significance of linear least-squares parameters. J ChemEduc 1979;56:244.
[7]. Sandell EB. Colorimetric determination of traces of metals. New York: Wiley InterScience; 1950. p. 2-10.
[8]. Guide for the use of terms in reporting data. Anal Chem 1982;54:157-59.
[9]. Massart DL, Vandeginste BGM, Deming SN, Michotte Y, Kaufman L. Chemometrics: A text book. Amsterdam: Elsevier Science; 1988. p. 1-50.
[10]. Gennaro AR, Remington: The science and practice of pharmacy. Volume-II 21st ed. Lippincott Williams & Wilkins; 1997. p. 1680-82.
[11]. Munson JW. Pharmaceutical analysis: modern methods. Part B. New York: Marcel Dekker Inc; 2001. p. 38-42.
[12]. Macek T, Karel D. Pharmaceutical applications of thin-layer and paper chromatography. Amsterdam: Elsevier Publishing Company; 1972. p. 1-25.
[13]. RamanaRao G, Murthy SSN, Khadgapathi P. Gas chromatography to pharmaceutical analysis. Eastern Pharmacist 1987; 30(353): 35.
[14]. Ramana Rao G, Murthy SSN, Khadgapathi P. High performance liquid chromatography and its role in pharmaceutical analysis. Eastern Pharmacist 1986; 29(346): 53.
[15]. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. 2nd ed. New York: Wiley InterScience; 1997. p. 1-57.
[16]. Sharma BK. Instrumental methods of chemical analysis. 23rd ed. Meerut: Goel Publishing House; 2004. p. 1-16.
[17]. Kasture AV, Mahadik KR, Wadodker SG, More HN. Instrumental methods of Pharmaceutical analysis.Vol-II, 14th ed. Pune: NiraliPrakashan; 2006. p. 1-30.
[18]. DevalaRao G. Text book of pharmaceutical analysis. Vol-I. 2nd ed. New Delhi: Birla Publications; 2005. p. 1-2.