"Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Saxagliptin and Sitagliptin in Bulk and Pharmaceutical Dosage Forms"

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ABSTRACT

An accurate and precise method was developed for the simultaneous estimation of Saxagliptin and Sitagliptin in bulk dosage form. The chromatographic analysis was carried out using a Discovery C18 column (4.6 x 150 mm, 5 µm). The mobile phase consisted of acetonitrile and 0.01N KH2PO4 in a 65:35 ratio, with a flow rate of 1.0 ml/min through the column. The buffer used was OPA with a pH of 4.4, and the temperature was maintained at 30°C. The wavelength optimized for detection was 210 nm. The retention times for Saxagliptin and Sitagliptin were determined to be 2.216 minutes and 2.650 minutes, respectively. The %RSD for Saxagliptin and Sitagliptin were found to be 0.5 and 1.1, respectively. The %recovery for Saxagliptin was 99.61%, and for Sitagliptin, it was 100.20%. The LOD and LOQ values, derived from the regression equations, were 0.10 and 0.31 for Saxagliptin, and 1.69 and 5.13 for Sitagliptin. The regression equation for Saxagliptin was y = 12784x + 1088.7, and for Sitagliptin, it was y = 12398x + 11053. The reduced retention times and run time make this method simple and cost- effective, suitable for routine quality control testing in industrial settings.{1}

Key Words: Saxagliptin, Sitagliptin, RP-HPLC, spectrophotometry, Estimation

INTRODUCTION

The quality of a drug is crucial for guaranteeing its safety and efficacy. Ensuring the quality assurance and control of pharmaceutical and chemical formulations is essential to providing consumers with safe and effective drug products. Consequently, the analysis of pure drug substances and their pharmaceutical dosage forms is vital in determining their suitability for patient use. The reliability of analytical data is directly related to the quality of the methods used to generate this data. Therefore, developing robust and reliable analytical methods is essential for obtaining regulatory certification for drugs and their formulations. {2}

Ensuring the quality and safety of a drug involves effectively monitoring and controlling its assay and impurities. The assay measures the drug's potency, while impurities impact its safety profile. Conducting accurate assays of pharmaceutical products is crucial for determining the drug's efficacy in patients. Developing methods for various drugs presents a wide range of challenges, largely dependent on the drugs' distinct nature and properties. Researchers must address the critical aspects of selectivity, speed, cost, simplicity, sensitivity, reproducibility, and accuracy to develop new analytical methods that can be adopted by the pharmaceutical industry and chemical laboratories. {3}

Among these, optical methods (such as refractometry, polarimetry, emission, and fluorescence analysis), photometric methods (including photocolorimetry and spectrophotometry across UV-Visible, IR spectroscopy, and nepheloturbidimetry), and chromatographic techniques (like column, paper, thin-layer, gas- liquid, and high-performance liquid chromatography) are particularly important. Additionally, methods like nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are gaining popularity. Combining mass spectrometry

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(MS) with gas chromatography is one of the most powerful analytical tools available. Chemical methods, including gravimetric and volumetric techniques based on complex formation, acid- base, precipitation, and redox reactions, are also utilized. Titrations in non-aqueous media and complexometry have found applications in pharmaceutical analysis as well. With the continuous emergence of new drugs, there is an ongoing need for innovative methods to control their quality, meeting the demands of modern pharmaceutical analysis. {4}

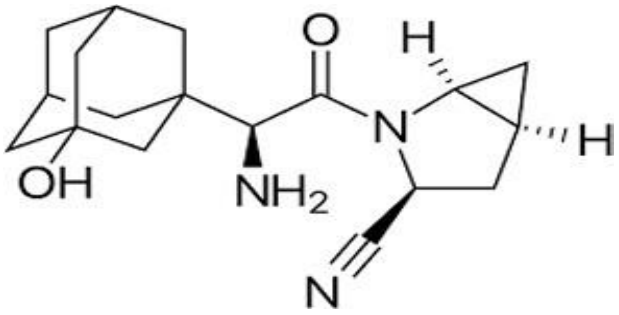

Fig. 1 Structure of Saxagliptin

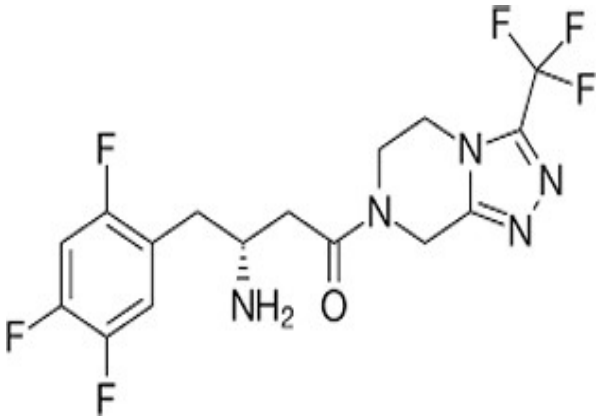

Fig. 2 Structure of Sitagliptin

METHODS

Instruments:

- Electronics Balance-Denver
- pH meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Saxagliptin and Sitagliptin solutions.

METHODS

Preparation of buffer:

- **N KH2PO4 Buffer**: Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5.4 with dil. Formic acid .

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue I January 2026

Validation:

Preparation of Standard stock solutions: Accurately weighed 2.5mg of Saxagliptin, 50mg of Sitagliptin and transferred to 50ml volumetric flask. 3/4 Th of diluents was added to the flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution 1. (50µg/ml of Saxagliptin and 1000µg/ml of Sitagliptin). {5}

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (5µg/ml of Saxagliptin and 100µg/ml of Sitagliptin).

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 100ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (50µg/ml of Saxagliptin and 1000µg/ml of Sitagliptin).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (5µg/ml of Saxagliptin and 100µg/ml of Sitagliptin). .{6}

Precision:

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 100ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (50µg/ml of Saxagliptin and 1000µg/ml of Sitagliptin).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (5µg/ml of Saxagliptin and 100µg/ml of Sitagliptin).

The precision were determined by preparing sample solutions of Saxagliptin (5ppm) and Sitagliptin(100ppm) and the solutions were injected six times and The % RSD for the area of six standard injections results should not be more than 2%..{7}

Linearity:

Preparation of Standard stock solutions: Accurately weighed 2.5mg of Saxagliptin, 50mg of Sitagliptin and transferred to 50ml volumetric flask. 3/4 Th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution 1. (50µg/ml of Saxagliptin and 1000µg/ml of Sitagliptin).

25% Standard solution: 0.5ml from standard stock solution was pipetted out and made up to 10ml. (1.25µg/ml of Saxagliptin, and 25µg/ml of Sitagliptin).

50% Standard solution: 0.5ml from standard stock solution was pipetted out and made up to 10ml. (2.5µg/ml of Saxagliptin, and 50µg/ml of Sitagliptin)

75% Standard solution: 0.75ml from standard stock solution was pipetted out and made up to 10ml. (3.75µg/ml of Saxagliptin, and 75µg/ml of Sitagliptin)

100% Standard solution: 1.0ml from standard stock solution was pipetted out and made up to 10ml. (5µg/ml of Saxagliptin, and 100µg/ml of Sitagliptin)

125% Standard solution: 1.25ml from two standard stock solution was pipetted out and made up to 10ml. (6.25µg/ml of Saxagliptin and 125µg/ml of Sitagliptin)

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue I January 2026

150% Standard solution: 1.5ml from two standard stock solution was pipetted out and made up to 10ml. (7.5µg/ml of Saxagliptin and 150µg/ml of Sitagliptin) .{8}

Accuracy:

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 100ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (50µg/ml of Saxagliptin and 1000µg/ml of Sitagliptin).

Preparation of Standard working solutions (100% solution): 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (5µg/ml of Saxagliptin and 100µg/ml of Sitagliptin).

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.{9}

Degradation studies:

Oxidation: To 1 ml of stock solution of Saxagliptin and Sitagliptin, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 5µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. **Acid Degradation Studies:** To 1 ml of stock ssolution Saxagliptin and Sitagliptin, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c .The resultant solution was diluted to obtain 5µg/ml&100µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample. .{10}

Alkali Degradation Studies: To 1 ml of stock solution Saxagliptin and Sitagliptin, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 100µg/ml&10µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 5µg/ml&100µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 50µg/ml&1000µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or CMR COLLEGE OF PHARMACY Department of Pharmaceutical Analysis 55 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 5µg/ml&100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. .{11}

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6hr s at a temperature of 60º. For HPLC study, the resultant solution was diluted to 5µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue I January 2026

Method development: Method development was done by changing various, mobile phase ratios, buffers etc. .{12}

Optimized method:

Chromatographic conditions:

Mobile phase **:** MeCN [Acetonitrile] and 0.01N KH2Po4(35:65)

Flow rate **:** 1 ml/min

Column **:** Discovery 150(C18) (4.6 x 150mm, 5µm)

Detector wave length **:** 228nm

Column temperature **:** 30°C

Injection volume **:** 10□L

Run time **:** **6.0**min

Results **:** Both peaks have good resolution, tailing factor, Theoretical plate count and resolution. The total runtime for each validation parameter was set to 6mins.

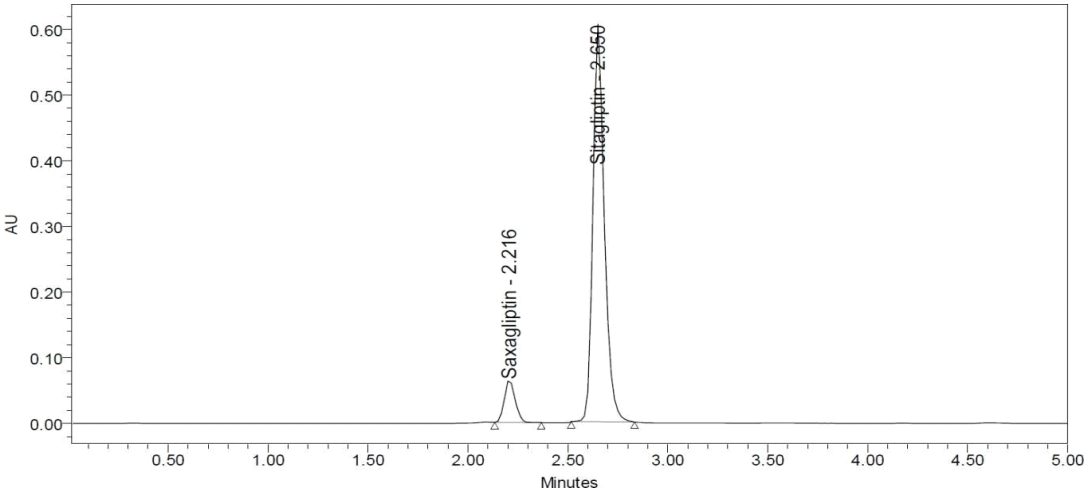

Fig.3. Optimized Chromatogram

Observation: Saxagliptin and Sitagliptin were eluted at 2.216min and 2.650 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated. .{13}

RESULTS

Linearity:

Table 1 Linearity table for Saxagliptin and Sitagliptin.

Saxagliptin		**Sitagliptin**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue I January 2026

1.25	16385	25	316459
2.5	32829	50	627160
3.75	49996	75	943651
5	65454	100	1264105
6.25	81490	125	1563560
7.5	95954	150	1860100

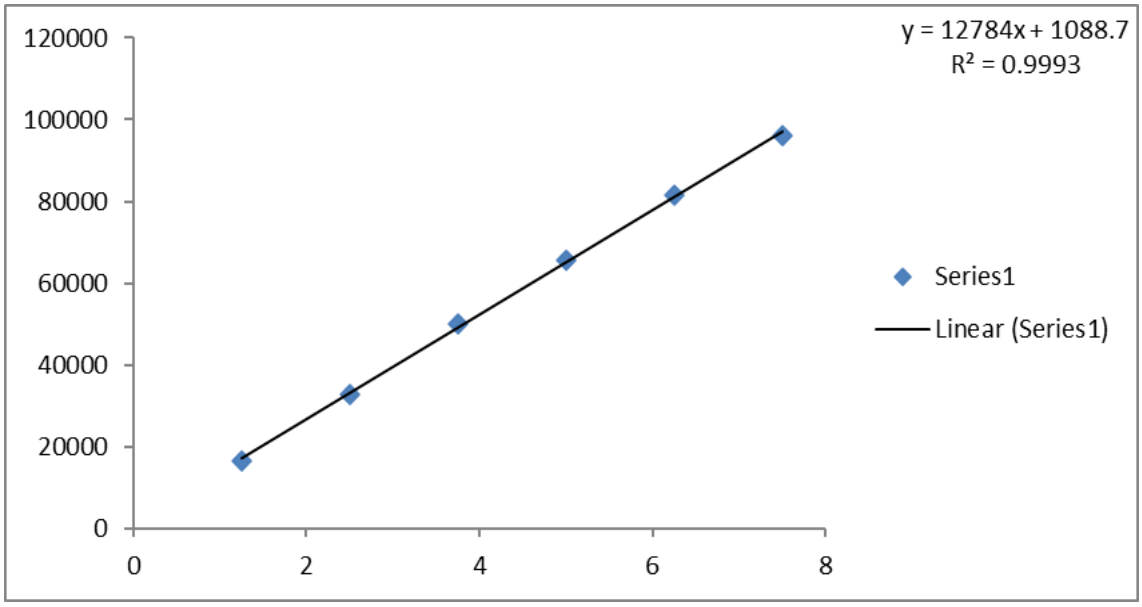

Fig 4. Calibration curve of Saxagliptin

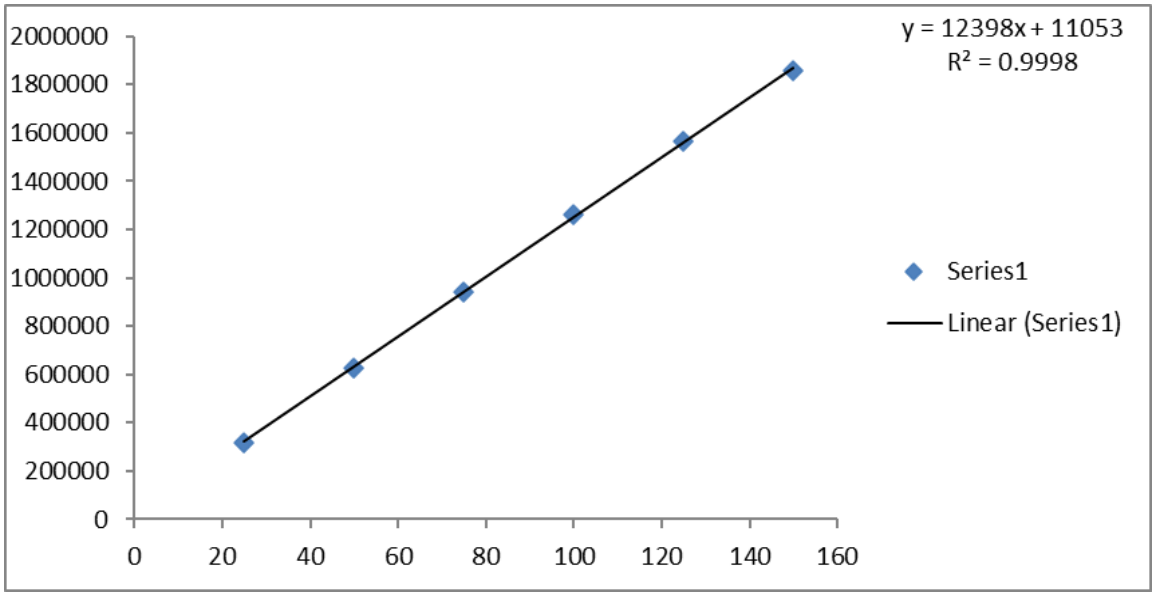

Fig 5. Calibration curve of Sitaliptin

Discussion: Six linear concentrations of Saxagliptin (1.25-7.5µg/ml) and Sitagliptin (25- 150µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Saxagliptin was y = 12784x + 1088.7 And of Sitagliptin was y= 12398x + 11053. Correlation coefficient obtained was 0.999 for the two drugs. .{14}

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue I January 2026

Precision:

Repetability:

Table 2. System precision table of Saxagliptin and Sitagliptin

S. No	Area of Saxagliptin	Area of Sitagliptin
1.	65454	1266565
2.	65355	1244556
3.	65454	1235455
4.	64954	1257564
5.	65065	1254544
6.	65946	1274545
Mean	65371	1255538
S.D	349.9	14225.9
%RSD	0.5	1.1

Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.5% and 1.1% respectively for Saxagliptin and Sitagliptin. As the limit of Precision was less than "2" the system precision was passed in this method. .{15}

Accuracy:

Table 3. Accuracy table of Saxagliptin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	2.5	2.51	100.24	99.61%
	2.5	2.51	100.58	
	2.5	2.49	99.61	
100%	5	4.97	99.42	
	5	4.96	99.27	
	5	4.96	99.26	
150%	7.5	7.40	98.62	
	7.5	7.50	99.95	
	7.5	7.47	99.55	

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue I January 2026

Table 4. Accuracy table of Sitagliptin

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	49.50	99.00	100.20%
	50	50.31	100.63	
	50	49.50	99.00	
100%	100	100.39	100.39	
	100	100.56	100.56	
	100	99.76	99.76	
150%	150	151.22	100.81	
	150	151.28	100.86	
	150	151.20	100.80	

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean

%Recovery was obtained as 99.61% and 100.20% for Saxagliptin and Sitagliptin respectively. {16}

Robustness:

Table 5. Robustness data for Saxagliptin and Sitagliptin.

S.no	Condition	%RSD of Saxagliptin	%RSD of Sitagliptin
1	Flow rate (-) 0.9ml/min	0.5	1
2	Flow rate (+) 1.1ml/min	1.1	1.5
3	Mobile phase (-) 60B:40A	0.7	0.5
4	Mobile phase (+) 70B:30A	0.7	0.9
5	Temperature (-) 27°C	1	0.8
6	Temperature (+) 33°C	1.1	0.6

Discussion: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (60B:40A), mobile phase plus (70B:30A), temperature minus (27°C) and temperature plus(33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. .{17}

Table. 6. Degradation data

Type of degradation	Saxagliptin			Sitagliptin		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	60987	93.11	6.89	1186676	94.33	5.67

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue I January 2026

Base	64354	98.25	1.75	1256767	99.90	0.10
Peroxide	61787	94.33	5.67	1186566	94.32	5.68
Thermal	64342	98.23	1.77	1247677	99.18	0.82
Uv	64786	98.91	1.09	1237677	98.38	1.62
Water	65078	99.35	0.65	1237565	98.37	1.63

Assay: - synthetic formulation was prepared which are equivalent to 5mg and 100mg of Saxagliptin and sitagliptin, Assay was performed with the above formulation. Average % Assay for Saxagliptin and sitagliptin. Obtained was 100.11%and 99.68% respectively.The Assay were determined by preparing sample solutions of Saxagliptin (5ppm) and Sitagliptin(100ppm) and the solutions were injected six times and calculated by assay formula and the % RSD for the area of six standard injections results should not be more than 2%..{18}

Table 7 Assay Data of Saxagliptin

S.no	Standard Area	Sample area	% Assay
1	65454	65454	99.93
2	65355	65866	100.56
3	65454	65354	99.77
4	64954	65464	99.94
5	65065	65467	99.95
6	65946	65856	100.54
Avg	65371	65577	100.11
Stdev	349.9	224.1	0.342
%RSD	0.5	0.3	0.3

Table 8 Assay Data of Sitagliptin

S.no	Standard Area	Sample area	% Assay
1	1266565	1245655	99.01
2	1244556	1245454	99.00
3	1235455	1244544	98.93
4	1257564	1268434	100.83
5	1254544	1255455	99.79
6	1274545	1264544	100.52
Avg	1255538	1254014	99.68

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue I January 2026

Stdev	14225.9	10523.5	0.84
%RSD	1.1	0.8	0.8

Table 9.Summary and Conclusion

Parameters		**Saxagliptin**	**Sitagliptin**	**LIMIT**
Linearity Range (µg/ml)		1.25-7.5µg/ml	25-150 µg/ml	
Regression coefficient		0.999	0.999	
Slope(m)		12784	12398	
Intercept(c)		1088.7	11053	
Regression equation (Y=mx+c)		y = 12784x + 1088.7	y = 12398x + 11053	R< 1
Assay (% mean assay)		100.11%	99.68%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.5	1.1	NMT 2.0%
Method precision %RSD		0.3	0.8	NMT 2.0%
Accuracy %recovery		99.61%	100.20%	98-102%
LOD		0.10	1.69	NMT 3
LOQ		0.31	5.13	NMT 10
Robustness	**FM**	0.5	1	%RSD NMT 2.0
	FP	1.1	1.5	
	MM	0.7	0.5	
	MP	0.7	0.9	
	TM	1	0.8	
	TP	1.1	0.6	

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Saxagliptin and Sitagliptin in tablet dosage form. Retention time of Saxagliptin and Sitagliptin were found to be 2.216 min and 2.650 min. %RSD of the Saxagliptin and Sitagliptin were and found to be 0.5 and 1.1 respectively. %Recovery was obtained as 99.61% and 100.20% for Saxagliptin and Sitagliptin respectively. LOD, LOQ values obtained from regression equations of Saxagliptin and Sitagliptin were 0.10, 0.31 and 1.69, 5.13 respectively. Regression equation of Saxagliptin is y = 12784x + 1088.7. And Y = 12398x + 11053 of Sitagliptin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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Conflicts Of Interest

The authors declare that they have no conflicts of interest related to this work.

Author Contribution

All authors contributed significantly to the conception, design, execution, and interpretation of the study. All authors reviewed and approved the final manuscript.

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