Available online on 15.06.2022 at ijmspr.com

International Journal of Medical Sciences and Pharma Research

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Development and Validation of Simultaneous Equation Method for Estimation of Sitagliptin and Saxagliptin in Combined Pharmaceutical Dosage Form by Using UV Spectrophotometric Method

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Article Info:

Article History:

Received 21 April 2022
Reviewed 29 May 2022
Accepted 07 June 2022
Published 15 June 2022

Cite this article as:

Singh D, Jain DK, Tiwari A, Khan N, Development and Validation of Simultaneous Equation Method for Estimation of Sitagliptin and Saxagliptin in Combined Pharmaceutical Dosage Form by Using UV Spectrophotometric Method, International Journal of Medical Sciences & Pharma Research, 2022; 8(2):83-90

DOI: http://dx.doi.org/10.22270/ijmspr.v8i2.43

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Abstract

Day by day the number of drug groups also the numbers of drugs within these groups for treatment of diabetes are increasing rapidly. The numbers of newer anti-diabetic formulations either in single or in combined dosage forms are marketed and investigated, sitagliptin with saxagliptin are not official in any pharmacopoeia. the aim of the present work was to develop and validate newer analytical methods like UV spectrophotometric methods, which should be applied for the further analysis of anti-diabetic drugs in bulk drugs and its pharmaceutical formulations. To develop and validate simultaneous equation method for simultaneous estimation of sitagliptin and saxagliptin in combined pharmaceutical dosage form by UV spectrophotometric method.

Keywords: Sitagliptin, Saxagliptin, Anti-diabetic, UV spectrophotometric Methods, Method validation

INTRODUCTION:

Diabetes mellitus (DM) [1] consists of a group of syndromes characterized by glycosuria; hyperglycaemia [2]; altered metabolism of lipids, carbohydrates [3], and proteins; and an increased risk of complications from vascular disease [4]. A stability-indicating assay [5] is a validated quantitative analytical procedure that can detect the changes with time in the properties of the drug substance and drug product. The purpose of stability testing is to provide evidence that the quality of a drug substance varies with time due to influence of various environmental factors like humidity, temperature, and light. The objective of validation of analytical procedure [6] is to demonstrate that it is suitable for its intended purpose. Any developed method may be influenced by variables like different elapsed assay times, different days, reagents lots, instruments, equipment, environmental conditions [7] like temperature, humidity, light etc. so it is expected that after the method has been developed and before it is communicated or transferred from one lab to the other, it should be properly validated and the result should be reported.

MATERIALS AND METHODS:

All the chemicals, glassware used in the experimental work were calibrated in the laboratory and out of all only those were in calibration limit were selected for work. Drug samples of Sitagliptin and Saxagliptin were purchased for Gitar Laboratories, Ahmedabad.

Determination of λmax by UVSpectroscopy:

Solution Preparation for UV Spectroscopy:

Accurately weighed SGP (10 mg) standard drug powder. It was transferred into 100 mL volumetric flask and diluted up to the mark with Methanol to give a stock solution concentrating 100 µg/Ml [8, 9]. From that 1 mL of stock solution was further diluted 10 mL with Methanol to get working solution of 10 µg/mL and the working standard was scanned between 200-400 nm to discover λ_{max} of solution.

Characterization by IR Spectroscopy:

Ground a small quantity of SGP working standard powder with a specially purified Potassium Bromide [10] finely. This powder mixture was then pressed in a mechanical die press to form a translucent pellet through which the beam of the spectrometer can pass.

Preparation of Standard Stock Solution of SGP and SAX

Accurately weighed 10 mg of Sitagliptin powder. It was transferred into 100 mL volumetric flask and dissolved into 75 mL of Methanol and the volume was made up to the mark with Methanol to obtained final concentration of 0.1 mg/mL or 100 µg/mL solution of Sitagliptin [11]. Accurately weighed 5 mg Saxagliptin powder. It was transferred into 100 mL volumetric flask and dissolved into 75 mL of Methanol and the volume was made up to the mark with Methanol to obtained final concentration of 0.05 mg/mL or 50 µg/mL solution of Saxagliptin.

Preparation of Sample Solution

Separately weighed 20 tablets and average weight of individual tablets were found out and weight equivalent to SGP (10 mg) and SAX (5 mg) was taken into 100 mL volumetric flask and dissolved into 60 mL of Methanol with sonication for 20 minutes. The solution was filtered through 0.45 µ Millipore nylon filter and the residues were washed thoroughly with Methanol. The filtrate and washings were combined in a 100 mL volumetric flask and diluted up to the mark with Methanol to get a final concentration of 100 µg/mL of SGP and 50 µg/mL of SAX [12,13]. Then 1 mL of this solution was pipetted out into 10 mL volumetric flask and diluted up to the mark with Methanol to produce final concentration of 10 µg/mL of SGP and 5 µg/mL of SAX.

Method Validation:

Linearity and Range:

Calibration curves were plotted over concentration range of 5 – 15 µg/mL for Sitagliptin and of 2.5 – 7.5 µg/mL for Saxagliptin. Mixed standard stock solution which contains Sitagliptin (100 µg/mL) and Saxagliptin [14] (50 µg/mL) were diluted as mentioned in the following Table 1. Volume was made up to the mark with Methanol and mixed. Absorbances [15] of solutions were measured at λ_{max} of Sitagliptin 256 nm (λ1) and at λ_{max} of Saxagliptin 270 nm (λ2) against Methanol as a blank. The calibration curve was constructed by plotting the graph of absorbance Vs. concentration.

Table 1: Preparation of linearity solutions of SGP and SAX for method

Linearity Level	% With respect to sample concentration	Volume of mix standard preparation (mL)	Diluted with Methanol up to (mL)	Final concentration (µg/mL)	
				SGP	SAX
Level 1	50	0.5	10	5	2.5
Level 2	75	0.75	10	7.5	3.75
Level 3	100	1	10	10	5
Level 4	125	1.25	10	12.5	6.25
Level 5	150	1.5	10	15	7.5

Accuracy (% Recovery):

Accuracy was determined in terms of % recovery. Recovery [9, 16] experiments were carried out in triplicate by spiking standard drug to previously analyzed samples of the tablets (5 µg/mL and 2.5 µg/mL for SGP and SAX) with three level of standards (80, 100 and120 % for both). Amounts of SGP and SAX were determined using corresponding regression equations of calibration curves.

Precision:

Method Precision (Repeatability):

Method precision of the instrument was established by repeatedly injecting six standard solutions of SGP (10 µg/mL) and SAX (5 µg/mL) under same conditions on the same day. Results were reported in term of % RSD [17] which should not be more than 2 %.

Intermediate Precision (Reproducibility):

Intermediate precision was evaluated in terms of intra - day and inter - day precision by analysing 3 concentrated solutions 3 times on the same day and on different days over entire concentration range for both drugs. Results were reported in terms of % RSD.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ [18] of the drugs were derived by visual detection or calculating using these equations as per International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3\ d / S \quad LOQ = 10\ d / S$$

Where, d = the standard deviation of the response S = slope of calibration curve.

Estimation of Sitagliptin and Saxagliptin in Formulation:

The absorbance of sample solution was measured against Methanol as blank at 256 nm and at 270 nm for estimation of SGP and SAX, respectively. The amount of SGP and of SAX present in the sample solutions were determined by solving the simultaneous equations.

RESULT AND DISCUSSION:

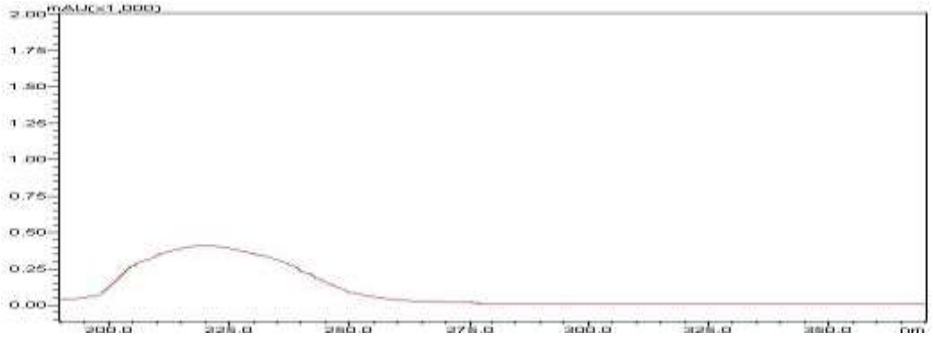

Figure 1: UV absorption maxima of sitagliptin

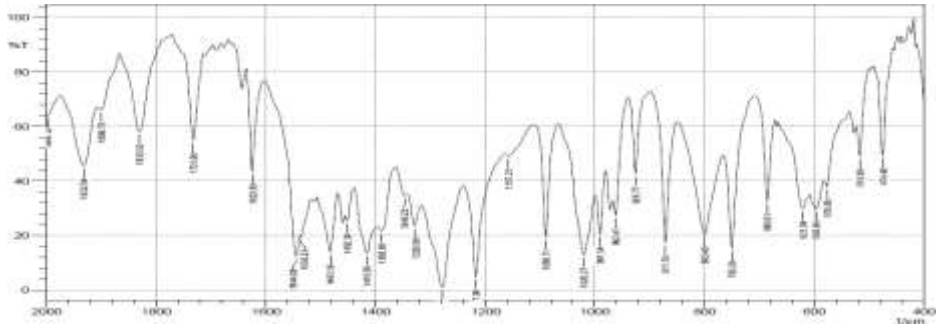

Figure 2: FTIR spectrum of sitagliptin

Table 2: IR spectrum interpretation for sitagliptin

Sr. No.	Functional group	Standard frequency (cm^{-1})	Observed frequency (cm^{-1})
1.	C=O Stretching	1775 – 1720	1731
2.	O–H Bending	1420 – 1330	1415
3.	C–FStretching	850 – 550	750
4.	C–H Bending	810 ± 20	800

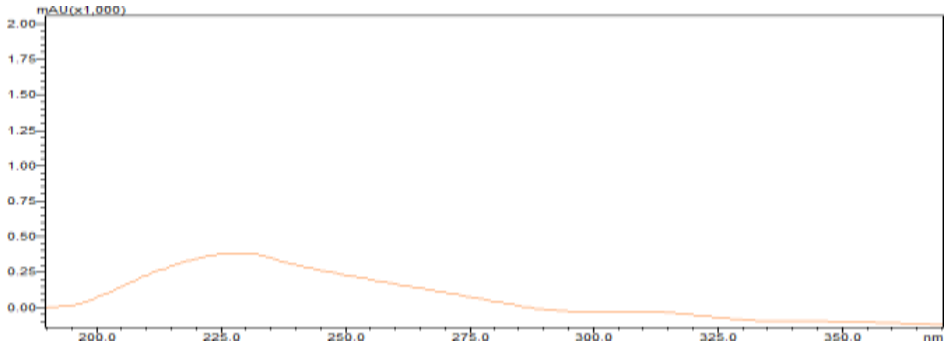

Figure 3: UV absorption maxima of Saxagliptin

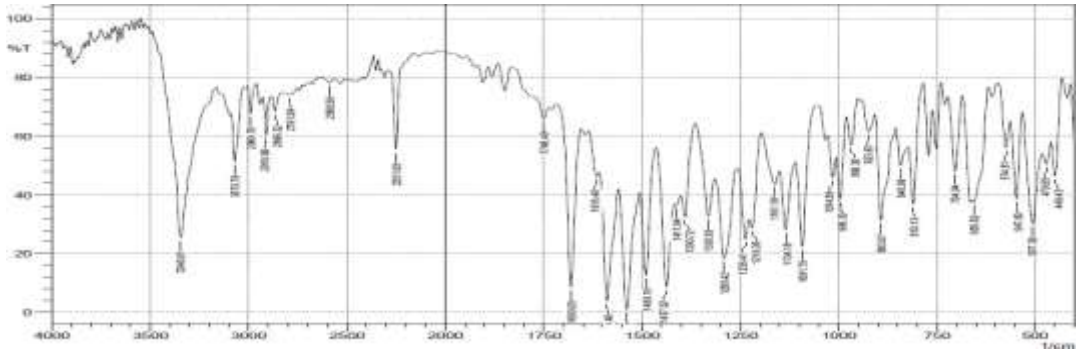

Figure 4: FTIR spectrum of Saxagliptin

Table 3: IR spectrum interpretation for Saxagliptin

Sr. No.	Functional group	Standard frequency (cm-1)	Observed frequency (cm-1)
1.	N–H Stretching	3400 – 3250	3346
2.	O–H Bending	1420 – 1330	1411
3.	C–N Stretching	1250 – 1020	1236
4.	C–H Stretching	3000 – 2840	2910
5.	C=O Stretching	1775 – 1720	1749

Method Development:

The working standard solution of SGP and of SAX were prepared separately in Methanol. They were scanned in the wavelength range of 200 nm - 400 nm against Methanol as blank, using medium scan speed. Maximum absorbance was obtained at 256 nm for SGP and 270 nm for SAX. These two wavelengths were AGPloyed for the determining SGP and SAX. Overlain spectra of both the drugs are shown in Figure 5.

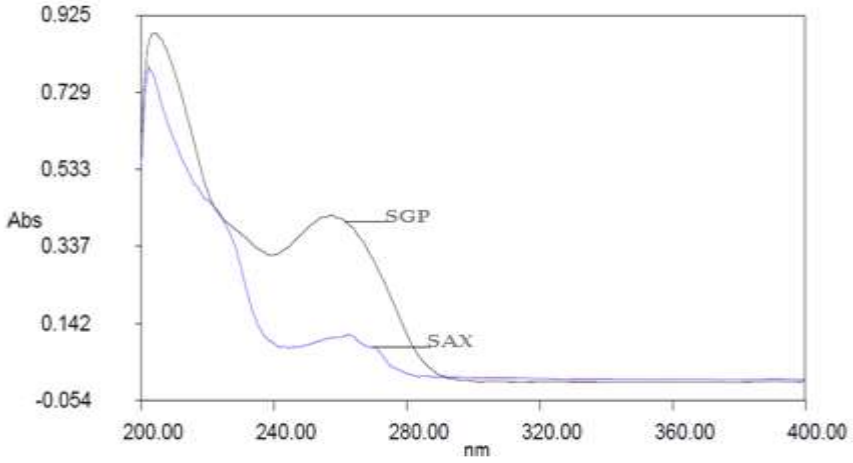

Figure 5: Overlain absorption spectra of SGP and of SAX in Methanol

Validation of Proposed Method:

Linearity and Range

Linearity of the method was evaluated at five concentration levels by diluting the standard stock solution to give solutions of SGP and of SAX in the concentration range of 5 – 15 µg/mL and of 2.5 – 7.5 µg/mL. Results show good correlation between absorbance and concentration of analyte. The calibration curves were prepared by plotting absorbance versus concentration of standard drugs (Figures 6 and 9).

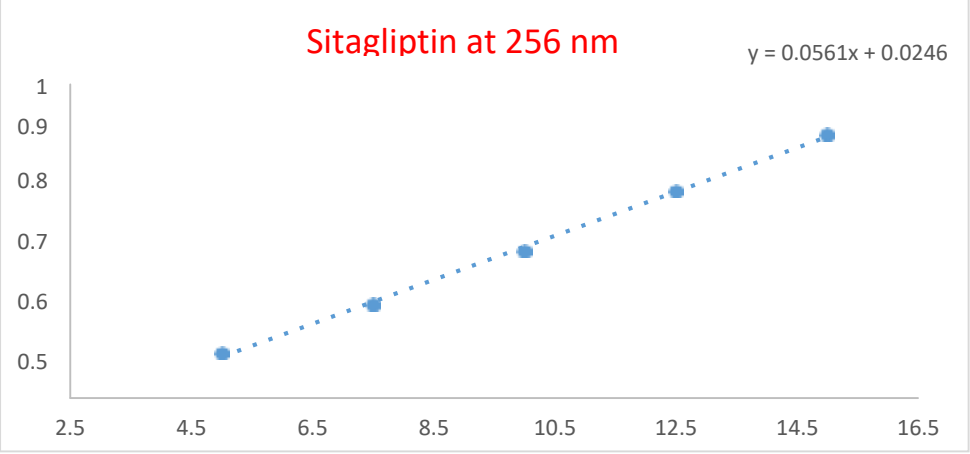

Figure 6: Calibration curve of SGP at 256 nm

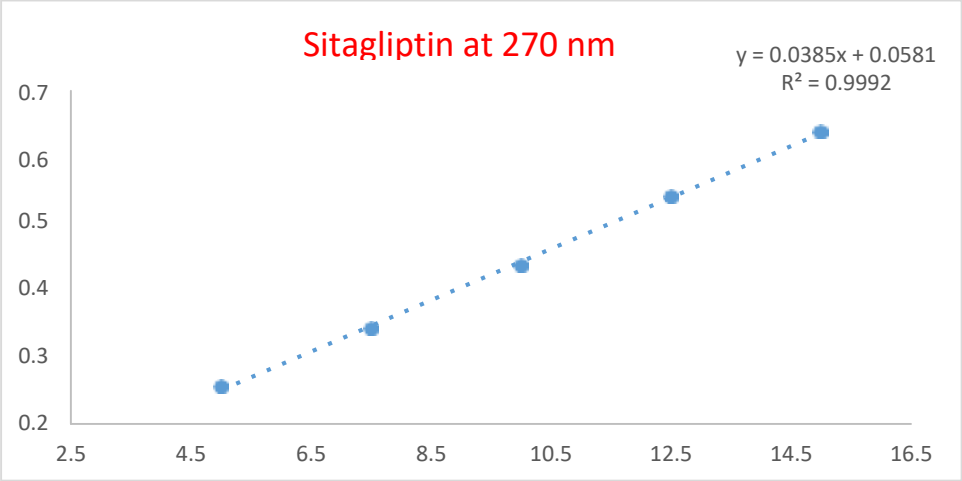

Figure 7: Calibration curve of SGP at 270 nm

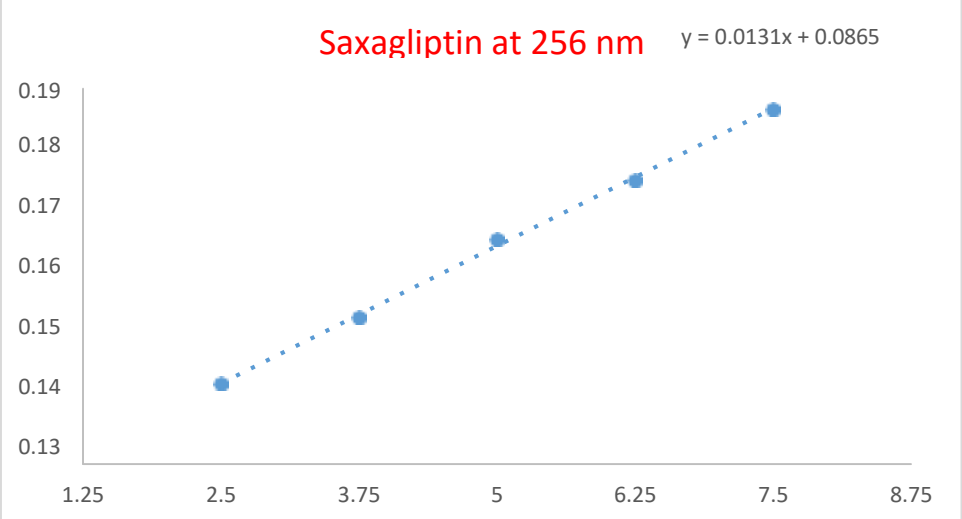

Figure 8: Calibration curve of SAX at 256 nm

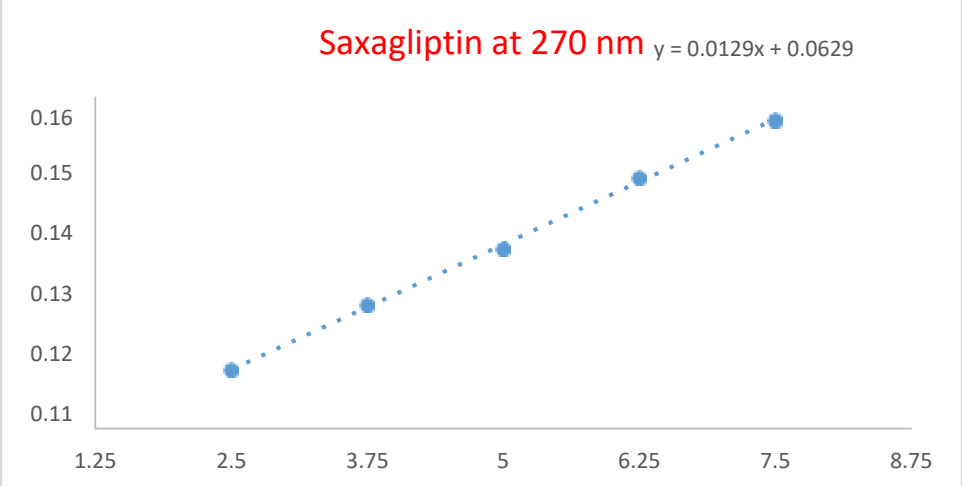

Figure 9: Calibration curve of SAX at 270 nm

Table 4: Linearity data of SGP and SAX

Parameters	Sitagliptin		Saxagliptin	
Wavelength (nm)	**256**	**270**	**256**	**270**
Beer's Law Limit (µg/mL)	5 – 15 µg/mL	5 – 15 µg/mL	2.5 – 7.5 µg/mL	2.5 – 7.5 µg/mL
Sandell's Sensitivity (µg/mL/cm2)	0.0172	0.0221	0.0318	0.0380
Regression Equation (y = mx + c) Slop (m) Intercept (c)	y = 0.0561x + 0.0246 0.0561 0.0246	y = 0.0385x + 0.0581 0.0385 0.0581	y = 0.0131x + 0.0865 0.0131 0.0865	y = 0.0129x + 0.0629 0.0129 0.0629
Correlation Coefficient (r2)	0.9985	0.9992	0.9984	0.9988

Accuracy (% Recovery):

Accuracy of the method was assured by the standard spiking method involving analysis of formulation samples to which standard drugs were added in 80, 100 and 120 % proportions. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. Good recoveries with the standard addition method (Table 5) prove the accuracy of the proposed method.

Table 5: % Recovery data of SGP and SAX (n = 3)

Drug	Amount of sample (µg/mL)	Amount of standard added (µg/mL)	Amount recovered (µg/mL)	% Recovery ± SD (n = 3)	% RSD
SGP	5	4	3.99	99.85 ± 0.68	0.69
		5	5.09	101.87 ± 0.74	0.73
		6	6.04	100.73 ± 0.75	0.75
SAX	2.5	2	2.01	100.52 ± 1.12	1.13
		2.5	2.49	99.54 ± 0.62	0.62
		3	3.01	100.17 ± 1.49	1.52

Precision:

Method Precision (Repeatability):

The % RSD values of SGP were 0.58 and 0.44 % at 256 nm and at 270 nm. The % RSD value of SAX were 0.97 % and 0.95 % at 256 nm and at 270 nm (Table 6). Relative standard deviation were less than 2 %, which indicates that proposed method is repeatable.

Table 6: Repeatability data for SGP and SAX (n = 6)

Concentration (µg/mL)		SGP		SAX	
SGP	**SAX**	**256 nm**	**270 nm**	**256 nm**	**270 nm**
10	5	0.583	0.443	0.153	0.129
10	5	0.590	0.447	0.151	0.128
10	5	0.589	0.444	0.154	0.126
10	5	0.588	0.442	0.152	0.127
10	5	0.584	0.445	0.150	0.129
10	5	0.582	0.442	0.151	0.127
Mean		0.586	0.444	0.152	0.128
SD		0.003	0.002	0.002	0.001
% RSD		0.58	0.95	0.97	0.95

Intermediate Precision:

The low % RSD values of intra - day precision (0.36 – 0.58 % and 0.24 – 0.96 % for SGP at 256 nm and at 270 nm and 0.98 – 1.88 % and 0.75 – 1.58 % for SAX at 256 nm and at 270 nm) and inter - day precision (0.29 – 0.64 % and 0.35 – 0.79 % for SGP at 256 nm and at 270 nm and 1.41 – 1.94 % and 1.11 – 1.57 % for SAX at 256 nm and at 270 nm) for SGP and for SAX show that the proposed method is precise (Table 7-8)

Table 7: Intermediate precision data for SGP and SAX at 256 nm

Drug	% Concentration	Intra – day precision Mean absorbance ± %RSD (n=3)	Inter – day precision Mean absorbance ± %RSD (n=3)
SGP	50	0.346 ± 0.58	0.337 ± 0.62
	100	0.576 ± 0.36	0.561 ± 0.64
	150	0.879 ± 0.50	0.875 ± 0.29
SAX	50	0.110 ± 1.88	0.107 ± 1.94
	100	0.156 ± 0.98	0.148 ± 1.41
	150	0.193 ± 1.30	0.182 ± 1.68

Table 8: Intermediate precision data for SGP and SAX at 270 nm

Drug	% Concentration	Intra – day precision Mean absorbance ± %RSD (n=3)	Inter – day precision Mean absorbance ± %RSD (n=3)
SGP	50	0.263 ± 0.96	0.265 ± 0.79
	100	0.442 ± 0.47	0.439 ± 0.35
	150	0.639 ± 0.24	0.625 ± 0.40
SAX	50	0.097 ± 1.58	0.097 ± 1.57
	100	0.134 ± 0.75	0.138 ± 1.11
	150	0.162 ± 1.24	0.161 ± 1.57

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ for both the drugs were calculated theoretically as given in Table 9. Low value of LOD & of LOQ indicates that the method is sensitive.

Table 9: LOD and LOQ data for SGP and SAX

Wavelength (nm)	LOD (µg/mL)		LOQ (µg/mL)	
	SGP	SAX	SGP	SAX
256	0.58	0.30	1.77	0.90
270	0.43	0.26	1.29	0.77

Estimation of Sitagliptin and Saxagliptin in Formulation:

The proposed validated method was successfully applied to determine SGP and SAX in tablet dosage forms. Results are given in Table 10. Assay results were obtained in the range of 98 - 102 % of label claim for SGP and SAX. No interference of the excipients with the absorbance of interest appeared; hence the proposed applies for the routine simultaneous estimation of Sitagliptin and Saxagliptin in tablet dosage forms.

Table 10: Analysis of formulation of SGP and SAX by proposed method (n = 3)

Sample no.	Label claim (mg/tab)		Amount found (mg/tab)		% Label claim	
	SGP	SAX	SGP	SAX	SGP	SAX
1	10	5	10.02	5.01	100.28	100.22
2	10	5	9.92	4.95	99.21	98.97
3	10	5	10.03	5.02	100.36	100.34
Mean			9.99	4.99	99.95	99.84
SD			0.06	0.04	0.65	0.76
% RSD			0.65	0.76	0.65	0.76

Summary of Validation Parameters:

Table 11: Regression data analysis and summary of validation parameters of SGP and SAX for proposed method

Parameters	Sitagliptin		Saxagliptin	
Wavelength (nm)	256	270	256	270
Beer's Law Limit (µg/mL)	5 – 15 µg/mL	5 – 15 µg/mL	2.5 – 7.5 µg/mL	2.5 – 7.5 µg/mL
Sandell's Sensitivity (µg/mL/cm^2)	0.0172	0.0221	0.0318	0.0380
Regression Equation (y = mx + c)	y = 0.0561x + 0.0246	y = 0.0385x + 0.0581	y = 0.0131x + 0.0865	y = 0.0129x + 0.0629
Slop (m)	0.0561	0.0385	0.0131	0.0129
Intercept (c)	0.0246	0.0581	0.0865	0.0629
Correlation Coefficient (r^2)	0.9985	0.9992	0.9984	0.9988
Repeatability (% RSD, n = 6)	0.58	0.95	0.97	0.95
Precision (% RSD) Intra - day (n = 3)				
Inter - day (n = 3)	0.36 – 0.58 0.29 – 0.64	0.24 – 0.96 0.35 – 0.79	0.98 – 1.88 1.41 – 1.94	0.75 – 1.58 1.11 – 1.57
LOD (µg/mL)	0.58	0.43	0.30	0.26
LOQ (µg /mL)	1.77	1.29	0.90	0.77

CONCLUSION:

Based on the results obtained from the analysis using described method, it can be concluded that the method has linear response in the range of 5 – 15 µg/mL and of 2.5 – 7.5 µg/mL for SGP and for SAX, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and agrees with label claim of the drugs. The additives usually present in the pharmaceutical formulations of the assayed samples were not interfering with Sitagliptin and with Saxagliptin. The method can be used for the routine analysis of Sitagliptin and of Saxagliptin in combined dosage form.

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