IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2019 Vol.:14, Issue:4

UV Spectrophotometric Method Development and Validation for Estimation of Saxagliptin in API and in Pharmaceutical Dosage Form

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Sonali Narsing Koli*[1], Adavirao Venkatrao Belvotagi[2], Rekha Pritishkumar Mudke[3], Somnath Ashok Patil[4]

[*]Department of Quality Assurance, D.S.T.S. Mandal's College of Pharmacy, Solapur, Maharashtra, India.
[2]Department of Pharmaceutical Chemistry, D.S.T.S. Mandal's College of Pharmacy, Solapur, Maharashtra, India.
[3]Department of Quality Assurance, D.S.T.S. Mandal's College of Pharmacy, Solapur, Maharashtra, India.
[4]Department of Pharmaceutical Analysis, D.S.T.S. Mandal's College of Pharmacy, Solapur, Maharashtra, India

Submission: 23 February 2019
Accepted: 28 February 2019
Published: 30 March 2019

Keywords: Saxagliptin, UV Spectrophotometer, Acetonitrile, method validation

ABSTRACT

Simple, rapid, sensitive, precise and specific UV Spectrophotometric method for the estimation of saxagliptin in API and pharmaceutical dosage form was developed and validated. In this method, the solution of saxagliptin was prepared in acetonitrile. Saxagliptin standard solution was scanned in the UV range (200-400nm) in1cm quartz cell in a double beam UV Spectrophotometer. The standard solution of Saxagliptin showed maximum absorption at wavelength 212nm. The sample obeys Beer's law in the concentration range from 10-50µg /mL. The correlation coefficient was 0.999 and the equation for the regression curve was found to be the *y=0.013x+0.004* with excellent recovery of 94-104%. Limit of detection and limit of quantification were found to be 0.191µg/mL and 0.579 µg/ml respectively. The evaluation for ruggedness and robustness was performed. The method was validated for several parameters like accuracy, precision as per ICH guidelines. Statistical analysis proved that the methods are repeatable and specific for the determination of the said drug. These methods can be adopted in the routine assay analysis of saxagliptin in API and pharmaceutical dosage form.

INTRODUCTION

Saxagliptin is an oral hypoglycemic (antidiabetic drug) of the dipeptidyl peptidase-4 inhibitor class of drugs. Saxagliptin is part of a class of diabetes medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 is an enzyme that breaks down incretin hormones. As a DPP-4 inhibitor, Saxagliptin slows down the breakdown of incretin hormones, increasing the level of these hormones in the body. This increase in incretin hormones that is responsible for the beneficial actions of Saxagliptin, including increasing insulin production in response to meals and decreasing the amount of glucose that the liver produces. Because incretin hormones are more active in response to higher blood sugar levels, the risk of dangerously low blood sugar (hypoglycemia) is low with Saxagliptin. Saxagliptin is available as tablets (2.5 mg) in the market under the brand name of *Riax* by Dr. Reddy's laboratories. Saxagliptin is chemically(1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl)acetyl]-2-azabicyclo[3.1.0] hexane-3-carbonitrile.An orally bio-available, potent, selective and competitive, cyanopyrrolidine-based inhibitor of dipeptidyl peptidase-4 (DPP-4), with hypoglycemic activity.[2] With empirical formula $C_{18}H_{25}N_3O_2$ and molecular weight 315.43 g/mol. The structural formula is:

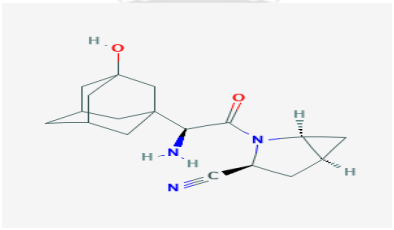

Figure 1: Chemical structures of Saxagliptin

Saxagliptin it is a white fine powder which is freely soluble in methanol, acetonitrile, acetone, polyethylene glycol, ethanol, Isopropyl Alcohol and sparingly soluble in water and slightly soluble in ethyl acetate.

Literature survey reveals that the Saxagliptin has been estimated: in human plasma by LCMS/MS[3]; by UV Spectroscopic method[4-5]; by HPLC[6-7]; stability indicating the method by LC-MS[7]. The present work is a simple, sensitive, accurate and precise Spectrophotometric Method for the estimation of Saxagliptin in API and its Pharmaceutical Dosage Forms with the help of Acetonitrile solvent.

MATERIALS AND METHODS

Instruments-

For weighing, a calibrated weighing balance (Shimadzu) of 1mg sensitivity was used. A Systronic UV-visible double beam spectrophotometer- 2201 was used. All the glass wares and were made of borosilicate and were calibrated.

Chemicals

API- Saxagliptin pure drug was gifted by Torrent Pharmaceutical Ltd, Ahmadabad, Gujarat.

Tablets of 2.5 mg strength were purchased from the local pharmacy in Solapur under commercially available brand name Riax (Dr. Reddy's), Acetonitrile LR was used in this study.

UV Spectroscopic Method

Solvent Selection

Saxagliptin is soluble in acetonitrile (ACN) so, ACN is used as the solvent.

Preparation of Standard Stock Solution

The standard stock solution Saxagliptin (SXG) was prepared by transferring, accurately weighed 10 mg of Saxagliptin into 10 mL volumetric flask containing 5mL Acetonitrile, dissolved properly. The volume was made up to the mark by using Acetonitrile to give a concentration of 1000 µg/mL. From this, 2.5 mL of the solution was transferred to a 25 mL volumetric flask and make up the volume with Acetonitrile to give a concentration of 100 µg/mL which is a standard stock solution and it is further diluted with ACN to get concentration range of 10-50 µg/mL.

Determination of Absorption Maxima

The standard stock solution of 100µg/mL was scanned in the range of 400-200 nm to determine the wavelength of Maximum Absorption. The drug showed Absorption maxima at 212 nm.

Preparation of Calibration Curve

For the preparation of calibration curve, the concentration of 10-50µg/mL was prepared by pipetting out 1, 2, 3, 4 and 5mL of the 100 µg/mL solution into 10 mL volumetric flasks and made up the volume with Acetonitrile.

The absorbance of each solution was measured at 212 nm against Acetonitrile as blank. Calibration curve of the Saxagliptin was plotted by taking the absorbance obtained on the y-axis and concentration of the solution on the x-axis. The curve showed linearity in the range of 10-50µg/mL with correlation coefficient 0.999.

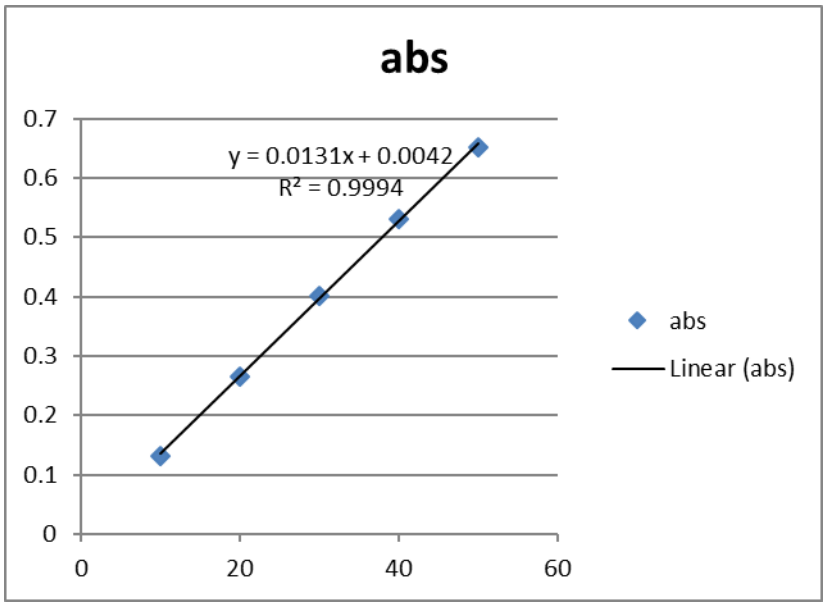

Figure 2: Calibration curve of Saxagliptin (SXG)

Quantitative Analysis of Tablet Dosage Form

Twenty tablets were weighed accurately and powdered. Powder equivalent to 10 mg Saxagliptin (SXG) was weighed and transferred to a 10 mL volumetric flask. It was dissolved in 10 ml acetonitrile and sonicated for 15 minutes to get a homogeneous solution. Then it was filtered through a 0.45 µ Whatman filter paper. A final concentration of 100 µg/mL of SXG was prepared. This solution was filtered through filter paper to remove some undissolved excipients. After filtration, from this 5 mL was taken and diluted to 10 mL with ACN which gives 50 µg/mL solution and the absorbance of the solution was measured at 212 nm.

Table 1: Results obtained in the determination of SXG in tablet dosage form

Tablet formulation	Label claim	Amount is taken	Amount found	Assay %
Riax 2.5 mg	2.5mg	50µg/mL	49µg/mL	98%

Method Validation

The developed method was validated as per ICH guidelines for the following parameters:

1. **Linearity:** 1, 2, 3, 4, 5mL of standard SXG solution was transferred into a series of 10 mL volumetric flasks. The volume was made up to the mark with ACN to obtain the concentration of 10, 20, 30 40, 50µg/mL. The absorption of these solutions was recorded and the graph was plotted of absorption against concentration. The correlation coefficient (r^2) of the least squares linear regression of SXG was calculated.

2. **Range:** The Range of the analytical method was decided from the interval between the upper and lower level of the calibration curve by plotting curve.

3. **Accuracy:** Recovery study was carried out by the standard addition method by adding a known amount of SXG to the pre-analyzed sample at three different concentration levels that are 80%, 100%, 120% of assay concentration and percent recovery were calculated. 1mL of tablet solution was transferred to 4 different 10 mL volumetric flasks (labeled as blank, 80%, 100%, 120%) separately and 0, 1.6, 2, 2.4 mL of 100 µg/mL standard solution was added respectively and the volume was made up to the mark with ACN. Absorbances were noted for these samples. Standard deviation and %RSD was calculated. Accuracy is reported as % recovery, which was calculated from the expression as equation given below,

$$\% \text{ Recovery} = \text{Observed value / True value} \times 100$$

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same sample under the prescribed conditions. The precision of the method was determined in terms of repeatability and intra-day and inter-day precisions. Intra-day and inter-day precision (Intermediate Precision).

Intraday precision was determined by analyzing the drugs at concentrations (30µg/mL) and each concentration for three times, on the same day. Inter-day precision was determined similarly, but the analysis is carried out daily, for two consecutive days.

Repeatability

Repeatability of the method was determined by analyzing six samples of the same concentrations of the drug (30µg/mL). The absorbance of each was measured.

4. **Robustness:** The robustness of the developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberate and the assay was evaluated. The effect of detection wavelength was studied at ±5 nm.

5. **Ruggedness:** Ruggedness was determined by carrying out the analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

6. **Limit of Detection:** Detection limit was determined based on the standard deviation of absorbance of same concentration that is a standard solution of SXG (30µg/mL) and LOD calculated by LOD = 3.3(SD/S) Where, SD- standard deviation; S= slope of the curve.

7. **Limit of Quantification:** Quantification limit was determined based on the standard deviation of the peak area of same concentration that is standard solution SXG (30µg/mL) prepared six times and LOQ calculated by LOD = 10(SD/S) Where SD= standard deviation; S= slope of Curve.

RESULTS

Determination of wavelength of maximum absorption the wavelength of maximum absorption was found to be 212 nm.

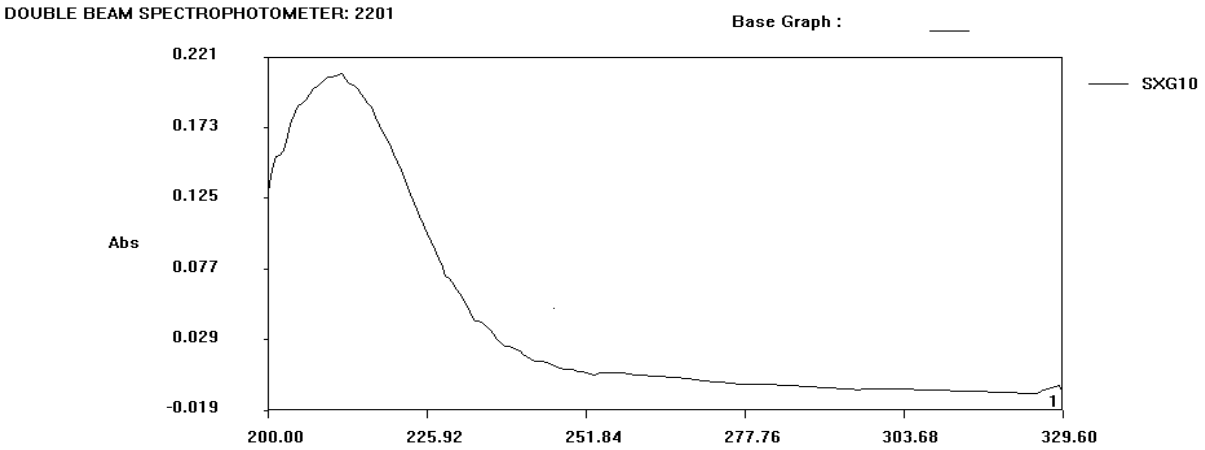

Figure 3: Wavelength of maximum absorption of Saxagliptin

Linearity: The linearity of this method was determined at ranges from 10-50 µg/mL for Saxagliptin. The regression equation was found to be *Y=0.013x +0.0004*, R^2=0.999.

Table 2: Linearity table

Sr. No.	Conc.	Absorbance
1.	10	0.131
2.	20	0.265
3.	30	0.402
4.	40	0.531
5.	50	0.651

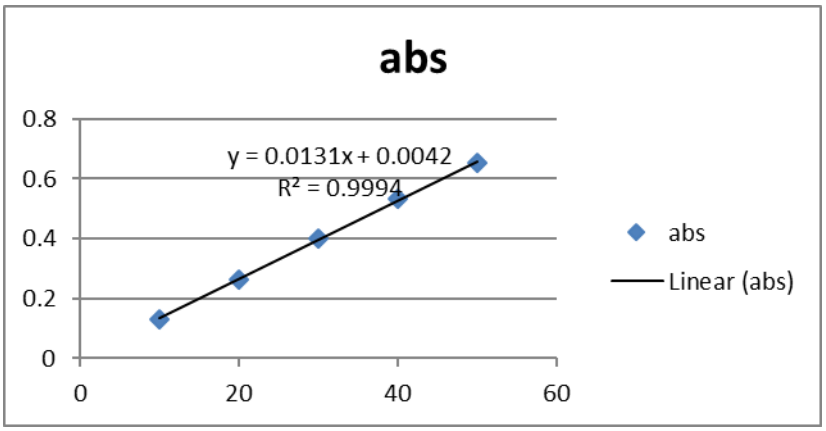

Figure 4: Linearity graph of Saxagliptin.

The linearity for Saxagliptin was found to be linear in the range of 10-50µg/mL with R^2= 0.999 and the straight line equation as *y= 0.0013x+0.004*

Accuracy

The accuracy of the analytical method for Saxagliptin was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 212 nm and results were expressed in terms of % recoveries.

Table 3: Table for accuracy

Sr.no.	Level of % Recovery	Amount of tablet sample (mL)	Amount of standard drug added (µg/mL)	Amount added (µg)	Amount found (µg/mL)	% Recovery
1	0	1	0	0	0	0
2	80	1	0.8	18	17	94.44%
3	100	1	1	20	20	100%
4	120	1	1.2	22	23	104%

Precision

The precision (measurement of intra-day, inter-day, repeatability) results showed good reproducibility with the relative standard deviation (% RSD) below 2.0 %. This indicated that the method was highly precise.

Intra-day Precision

Table 4: Intra-day morning precision

Sr. No.	Concentration (µg/mL)	Absorbance	SD	% RSD
1	30	0.452		
2	30	0.454		
3	30	0.452	0.00816	1.80
4	30	0.453		
5	30	0.453		
6	30	0.452		
		$\bar{y} = 0.452$		

Table 5: Intra-day afternoon precision

Sr. No.	Concentration (µg/mL)	Absorbance	SD	%RSD
1	30	0.513		
2	30	0.514		
3	30	0.514	0.000753	0.14
4	30	0.513		
5	30	0.512		
6	30	0.513		
		$\bar{y} = 0.513$		

Table 6: Intra-day evening precision

Sr. No.	Concentration (µg/mL)	Absorbance	SD	%RSD
1	30	0.505		
2	30	0.506		
3	30	0.505	0.000753	0.14
4	30	0.504		
5	30	0.504		
6	30	0.505		
		$\bar{y} = 0.504$		

Inter-day Precision

Table 7: Inter-day morning precision study

Sr.No.	Concentration (µg/mL)	Absorbance	SD	%RSD
1	30	0.53		
2	30	0.530		
3	30	0.531	0.000753	0.14
4	30	0.531		
5	30	0.532		
6	30	0.530		
		$\bar{y} = 0.530$		

Table 8: Inter-day afternoon precision study

Sr.No.	Concentration (µg/mL)	Absorbance	SD	%RSD
1	30	0.544		
2	30	0.542		
3	30	0.547	0.00292	0.53
4	30	0.547		
5	30	0.549		
6	30	0.542		
		$\bar{y}$ = 0.545		

Table 9: Interday evening precision study

Sr. No.	Concentration (µg/mL)	Absorbance	SD	%RSD
1	30	0.554		
2	30	0.553		
3	30	0.550	0.00154	0.27
4	30	0.554		
5	30	0.554		
6	30	0.553		
		$\bar{y}$ = 0.553		

Repeatability

Table 10: Repeatability study

Sr.No.	Concentration (µg/mL)	Absorbance	SD	%RSD
1	30	0.551		
2	30	0.551		
3	30	0.552	0.00147	0.26
4	30	0.554		
5	30	0.553		
6	30	0.550		
		$\bar{y}$ = 0.551		

Limit of Detection

Table 11: For Limit of Detection

LOD (µg/mL)	0.191 µg/mL

Limit of Quantification

Table 12: For Limit of Quantification

LOQ (µg/mL)	0.579 µg/mL

Robustness (30µg)

Table 13: Robustness study

Sr.No.	Wavelength (nm)	Absorbance	SD	%RSD
1	212	0.513		
2	213	0.515		
3	214	0.517		
4	215	0.515	0.0270	5.4
5	216	0.508		
6	217	0.493		
7	218	0.476		
8	219	0.463		
9	220	0.443		
		$\bar{y}$ = 0.493		

Ruggedness (30µg)

Ruggedness was determined by carrying out the analysis by two different analysts and the respective absorbance was noted and the results were indicated as % RSD.

Table no 14. For Ruggedness

Analyst-1		
Concentration (µg/mL)	**Absorbance**	**Statistical analysis**
30	0.526	
30	0.527	Mean =0.527
30	0.529	SD=0.0012
30	0.527	%RSD=0.22
30	0.527	
30	0.529	
Analyst-2		
30	0.512	
30	0.515	Mean = 0.514
30	0.513	SD=0.00126
30	0.515	%RSD=0.24
30	0.514	
30	0.515	

DISCUSSION

Preliminary Analysis of Saxagliptin

Preliminary analysis of Saxagliptin such as description, solubility was performed.

UV-spectrophotometry for Saxagliptin

Saxagliptin being UV absorbing has been successfully employed for its quantitative determination by UV Spectrophotometric method. Being soluble in Acetonitrile, stock solutions and working standards were prepared in Acetonitrile. The maximum wavelength of absorption of a drug was determined by taking a scan of the drug solution in the UV region (200-400 nm). The correlation of the standard curve for the drug was 0.999. The accuracy was from 88-104% at 212nm. The proposed method showed absorption maxima at 212nm and obeyed Beer's law in the concentration range of 10-50µg/mL. The limit of detection (LOD) was found to be 0.191 µg/mL and the limit of quantification (LOQ) to be 0.579 µg/ml respectively. All statistical data prove the validity of the proposed method, which can be applied for the routine analysis of saxagliptin.

Assay of the tablet formulation

Amount of drug present in tablet formulation was calculated using equation at 212 nm, and *y=0.013 xs+0.004* and amount of Saxagliptin were found to be 98% of label claim respectively. This method can be employed for the routine analysis of Saxagliptin.

Summary and conclusion

Summary of UV Spectrophotometric Method of Saxagliptin.

Table 15: For Summary

Sr.no.	Parameters	Values
1	Beer's Law limit (µg/mL)	10-50
2	Absorption maxima (nm)	212
3	Standard regression equation	0.013x+0.004
4	The correlation coefficient (R^2)	0.999
5	Accuracy	94.44-104%
6	Precision (%RSD) Repeatability	0.26
7	LOD (µg/mL)	0.191
8	LOQ (µg/mL)	0.579
9	Robustness (%RSD)	5.4
10	Ruggedness	0.22 and 0.24
11	Assay (%)	98%

CONCLUSION

The UV-Spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Saxagliptin in API and its tablet dosage form without any interference from the excipients. This method can be effectively applied for the routine analysis of Saxagliptin in API. Its advantages are the low cost of reagents, speed, and simplicity of sample treatment, satisfactory precision, and accuracy.

Abbreviations

UV-Ultra Violet

API- Active Pharmaceutical Ingredient

ACKNOWLEDGMENT

The authors are very thankful to the Principal of D.S.T.S. Mandal's College of Pharmacy, Solapur, Maharashtra, India and cooperative staff for providing the required facilities and guidance to carry out this research work.

REFERENCES

1. International Conference on Harmonization. ICH Q2 (R1), Validation of analytical procedures: text and methodology, 2005.
2. National Centre for Biotechnology Information. PubChem Compound Database; CID11243969. Available from: https://pubchem.ncib,nlm.nih.gov/compound/11243969[accessed 24th Jan 2018].
3. Hess C, Musshoff F, Madea B. Simultaneous identification and validated quantification of oral hypoglycaemic drugs in plasma by electrospray ionization liquid chromatography-mass spectrometry. *Anal. Bioanal. Chem*. 2011; 400: p.33-41.
4. Kalaichevi R, Jayachandra E. Validated spectroscopic method for the estimation of Saxagliptin in pure and from tablet formulation. *Int. J. Pharmacy. Pharm. Sci.* 2011; 3(3): p.179-180.
5. Sanjeev D, Madhumita R, Shubhangi D. Development and Validation of UV Spectrophotometric Method for Estimation of Saxagliptin in Bulk and Pharmaceutical Dosage Form. *Int. J. Pharm.* 2016; 4, (1): p. 30-34.
6. Shaban A. Validation of stability indicating PR-HPLC method for the estimation for Saxagliptin in tablet formulations, *Indo. Am. J. Pharm. Res.* 2014; 4, (08): p.3550-3558.
7. Chhabda P, Balaji M, Srinivasarao V, Ramakrishna K, Apparao K. Development and validation of simple stability indicating RP-HPLC method for analysis of Saxagliptin and its forced degradation impurities in bulk drug and pharmaceutical dosage form, *Int. J. Res. Dev. Pharm L. Sci.* 2014,3(3): p.993-1003.
8. Maha F, Abdel-Ghany, Omar A, Miroam F, Mariam M, et al. Stability indicating the liquid chromatographic method for determination of Saxagliptin and structure elucidation of the major degradation products using LC-MS. *J. Chromatogr. Sci*. 2015; 53: p.554-564.