IJBPAS, June, 2022, 11(6): 2715-2724

ISSN: 2277–4998

International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ESTIMATION OF SAXAGLIPTIN IN BULK FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM BY USING RP-UPLC METHOD

SUMA AH, CHERIYAN BV, AANANDHI MV AND RAMACHANDRAN S*

Department of Pharmaceutical Chemistry and Analysis, Vels Institute of Science Technology and Advanced Studies (VISTAS), Chennai-600 117, Tamilnadu, INDIA

***Corresponding Author: S.Ramachandran: ramachandrans.sps@velsuniv.ac.in**

Received 18th June 2021; Revised 19th Aug. 2021; Accepted 6th Oct. 2021; Available online 1st June 2022

https://doi.org/10.31032/IJBPAS/2022/11.6.6165

ABSTRACT

The main aim of the study is to develop a simple, rapid, accurate and precise method for the development and validation for the estimation of saxagliptin in bulk form and marketed pharmaceutical dosage form by using RP-UPLC method. The equipment used for this method is Ultra Performance Liquid Chromatography (UPLC) equipped with auto sample and PDA detector. The software used is Empower version-2. Xterra C_{18} (2.1 X 50 mn, 1.7µm) column is used with the flow rate 3ml/min. Detector is performed at the wavelength of 219nm. The retention time for Saxagliptin samples and standard is found to be 1.301 and 1.303 mins respectively. The calibration plots were linear over the concentration range of 20-100 µg/ml and the corelation co-efficient is found to be 0.999. There is no due to commonly used excipients. The %RSD for intermediate precision is found to be lower than 2.0% which obviously indicates that the present method is said to be highly precise. Regarding accuracy the % Recovery is found to be 99.8% which shows the method is accurate. This method is very sensitive with regard to LOD (0.030) and LOQ (0.090) respectively. The assay of marketed formulation is found to be 98.14463% which is within the limit. Thus, the developed RP-UPLC method was found to be simple, rapid, accurate, precise and highly suitable for routine analysis of drug samples containing Saxagliptin.

Keywords: RP-UPLC, method validation, method development, saxagliptin, ICH guidelines

INTRODUCTION

Saxagliptin is pharmaceutical drug of class of type-2 Diabetes which is Dipeptidyl peptide-4 and shows mechanism of action for the same. It has very fewer systemic side effect because of it is very specific to DPP-4 inhibition. Saxagliptin shows inhibition of enzymatic activity of dipeptidyl peptidase-4 for a period of 24-hours. The working of class of DPP-4 inhibitors is by affecting the action of natural hormones in the body called incretins, which decreases blood sugar from increasing consumption of sugar by the body and reducing production of sugar by the liver, mainly through increasing insulin production in the pancreas **[1-3]**.

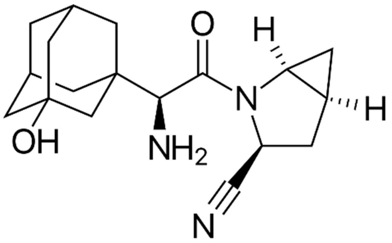

Figure 1: Structure of Saxagliptin

Saxagliptin is (1S,3S,5S)-2-[(2S)-2-amino-2-(3- hydroxyadamantan-1-yl)acetyl]-2-azabicyclo [3.1.0] hexane3-carbonitrile (C18H25N3O2). The structure of Saxagliptin is shown in **Figure 1**. In the patients having severe renal impairment, the dose reduction is required. The bioavailability of Saxagliptin was 67% because it is rapidly absorbed by orally. Saxagliptin is excreted by both renal and hepatic pathways. 75% of Saxagliptin is eliminated in the urine and 22% in the faeces **[4, 5]**.

UPLC is regarded as new invention for liquid chromatography. UPLC brings drastic changes in sensitivity, resolution and speed of analysis can be calculated. It has instrumentation that can perform at higher pressure as compared to that used in HPLC & in this system uses fine particles (less than 2.5 μm) and mobile phases at maximum linear velocities reduces the length of column also reduces solvent consumption and saves time **[6]**.

MATERIALS AND METHODS

INSTRUMENTS

Instrumentation employed in separation was Waters-UPLC, UPLC 2695, PDA detector instrument with UPLC auto sampler. The software used is Empower version-2 (Labs India). Acquity UPLC BEH column is used for the validation method.

REAGENTS AND CHEMICALS

Reagents

0.1% Orthophosphoporic Acid Buffer, 0.01N KH_2PO_4 Buffer, 20% hydrogen peroxide, 2N Hydrochloricacid, 2N sodiumhydroxide, Acetonitrile, distilled water of UHPLC grade supplied by VELS institute of technology and sciences, Chennai.

Reference standard used: Saxagliptin drug sample is gifted by S.R chemicals &

Pharmaceuticals, Surat and Dr Reddy's laboratories, Hyderabad respectively.

Internal standard used: Onglyza branded tablet purchased from local dispensary.

CHROMATOGRAPHIC CONDITIONS:

Xterra C_{18} (2.1 X 50mm, 1.7µm) column is used for the separation technique at the separation wavelength of 219nm. The flow rate is set to be 0.3ml/min. The run time and injection volume was set to be 4 mins and 4µl. The optimised chromatographic conditions is shown in **Table 1**.

Preparation of 0.1% Octa sulphonic acid (buffer) :

To prepare 0.1% Octasulphonic acid, pippet out 1ml Octasulphonic acid in 1000ml water.Adjust the pH upto 3.5 with sodium hydroxide solution.

Preparation of mobile phase

Mix a mixture of buffer 300 ml (30%) and 700 ml Acetonitrile UPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µm filter under vacuum filtration.

Diluents Preparation:

Mobile phase is used as a diluent

Preparation of the Saxagliptin Standard & Sample Solution:

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Saxagliptin working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. **(Stock solution)**. Further pipette 0.6 ml of Daclatasvir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents (60 ppm).

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 10 mg of Saxagliptin sample (appoximately 70mg of Tablet powder) into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. **(Stock solution)**. Further pipette 0.6 ml of Saxagliptin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

Inject 10 µL of the standard, sample into the chromatographic system and measure the areas for the Saxagliptin peaks. Chromatogram for saxagliptin standard and sample is shown in **Figure 2** and **Figure 3**.

Table 1: Optimised chromatographic conditions

PARAMETERS	SAXAGLIPTIN
COLUNM	Xterra C_{18} (2.1 x 50mm, 1.7 µm)
MOBILE PHASE	0.1% Octa sulphonic acid : acetonitrile (30:70)
FLOW RATE	0.3 ml per min
INJECTION VOLUME	4 µl
DETECTOR	PDA detector(Acquity Model)
RUN TIME	4 min
WAVELENGTH	219nm
COLUMN TEMPERATURE	Ambient

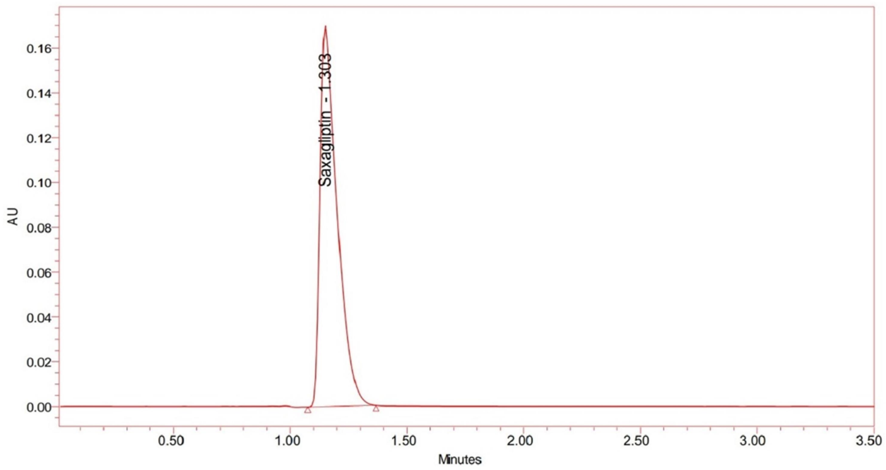

Figure 2: Chromatogram for standard

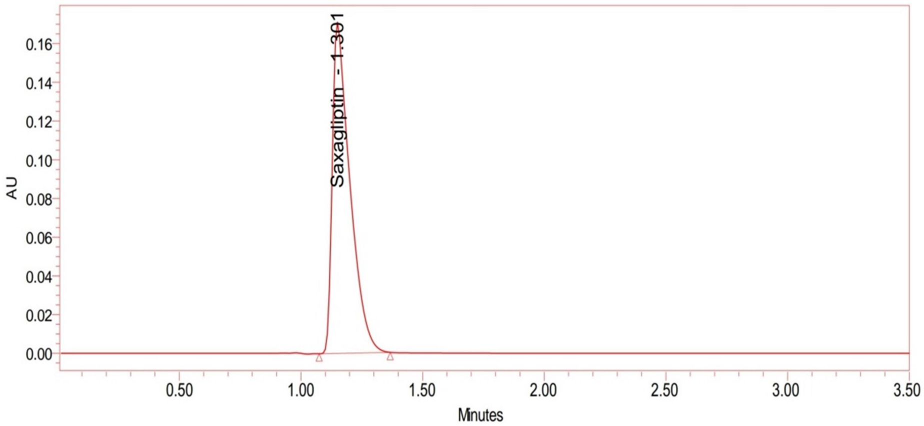

Figure 3: Chromatogram for sample

METHOD VALIDATION:

PRECISION:

Procedure:

The standard solution was injected for five times and measured the area for all five injections in UPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%.

INTERMEDIATE PRECISION/RUGGEDNESS:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in UPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%.

ACCURACY:

Accurately weigh and transfer 5 mg, 10mg, 15mg of Saxagliptin working standards into a 10ml clean dry volumetric flask separately, add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. (Stock solution)

Further pipette 0.6 ml of Saxagliptin of the above stock solutions into a 10ml volumetric flask separately and dilute up to the mark with diluent.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Saxagliptin and calculate the individual recovery and mean recovery values.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0%

LINEARITY:

Preparation of Level – I (20ppm of Saxagliptin):

0.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (40ppm of Saxagliptin):

0.4 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (60ppm of Saxagliptin):

0.6 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (80ppm of Saxagliptin):

0.8 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (100ppm of Saxagliptin):

1.0 ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Acceptance Criteria:

Correlation coefficient should not be less than 0.999.

ROBUSTNESS:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a). The flow rate was varied at 0.9 ml/min to 1.1ml/min.

b). The Organic composition in the Mobile phase was varied ±10%

LIMIT OF DETECTION:

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\mathbf{LOD = 3.3\ X\ \frac{\sigma}{S}}$$

Where

σ - Standard deviation (SD)

S - Slope

LIMIT OF QUANTIFICATION

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$\mathbf{LOQ = 10\ X\ \frac{\sigma}{S}}$$

Where

σ - Standard deviation

S - Slope

RESULTS AND DISCUSSION

PRECISION:

Precision is the closeness of results obtained from multiple sampling of the same homogeneous sample under the prescribed conditions and it is expressed in the form of Relative standard deviation. The precision was not more than 2.0% RSD. The results of precision are shown in **Table 2**.

INTERMEDIATE PRECISION/RUGGEDNESS:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The %RSD was found to be less than 2.0. The results of intermediate precision/ruggedness are shown in **Table 3**.

ACCURACY:

Accuracy of the method determines the closeness of the results obtained by the method to the true value. From the results of accuracy testing, it was showed that the method is accurate within the acceptable limits. The results of accuracy are shown in **Table 4**.

LINEARITY:

The corelation coefficient was found to be 0.999 which is within the limits. The results and calibration curve of linearity are shown in **Table 5** and **Figure 4**.

ROBUSTNESS:

a). The flow rate was varied at 0.9 ml/min to 1.1ml/min.

The system suitability results for varied flow rate are shown in **Table 6**.

On evaluation of the results (**Table 6**), it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%. The method is robust only in less flow condition.

b). The Organic composition in the Mobile phase was varied ±10%

The system suitability results for varied mobile phase composition are shown in **Table 7**.

On evaluation of the results (**Table 7**), it can be concluded that the variation in 10% Organic composition in the mobile phase does not affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

LIMIT OF DETECTION:

The sensitivity of LOD is found to be 0.030. The results LOD is shown in **Table 8**.

LIMIT OF QUANTIFICATION

The sensitivity ofLOQ is found to be 0.090. The results LOQ is shown in **Table 9**.

ASSAY OF MARKETED FORMULATIONS:

The % purity obtained from the formulations were found and the assay results are within the limits. The assay results of Saxagliptin is shown in **Table 10**.

System Suitability:

- Tailing factor for the Saxagliptin peaks in Standard solution should not be more than 2.0
- Theoretical plates for the Saxagliptin peaks in Standard solution should not be less than 2000

System Suitability Results:

1) Tailing factor Obtained from the standard injection is 1.15

2) Theoretical Plates Obtained from the standard injection is 9559.08.

Table 2: Results for precision

S. No.	PEAK NAME	RT	AREA	HEIGHT	USP PLATE COUNT	USP TAILING
1	Saxagliptin	1.299	1693252	166957	9900.24	1.10
2	Saxagliptin	1.300	1718953	168910	9983.91	1.19
3	Saxagliptin	1.301	1721186	168754	9982.30	1.19
4	Saxagliptin	1.301	1725670	170257	9989.45	1.18
5	Saxagliptin	1.301	1733700	170430	9989.85	1.19
Mean			1718552.4			
Std. dev			15226.1			
%RSD			0.9			

Table 3: Results for intermediate precision/ruggedness

S. No.	PEAK NAME	RT	AREA	HEIGHT	USP PLATE COUNT	USP TAILING
1	Saxagliptin	1.295	1707855	170404	9907.40	1.08
2	Saxagliptin	1.298	1722965	167425	9960.05	1.00
3	Saxagliptin	1.299	17815296	168241	9992.37	1.00
4	Saxagliptin	1.300	1729185	169719	9984.86	1.00
5	Saxagliptin	1.300	1718725	168403	9982.34	1.00
Mean			1718805.3			
Std. dev			8010.9			
%RSD			0.5			

Table 4: Results of accuracy

	Area mean	Std. deviation	%RSD	Recovery
50% accuracy	801981.5	10566.1	1.3	96.5276%
100% accuracy	16016720	10563.5	0.6	99.8%
150% accuracy	2573164.2	13290	0.5	103.23%
				99.8 (mean recovery)

Table 5: Results for linearity

S. No.	PEAK NAME	RT	AREA	HEIGHT	SAMPLE NAME
1	Saxagliptin	1.311	298643	27055	Linearity 4
2	Saxagliptin	1.311	364351	29789	Linearity 5
3	Saxagliptin	1.314	218536	22804	Linearity 3
4	Saxagliptin	1.320	152413	20139	Linearity 2
5	Saxagliptin	1.331	76965	17656	Linearity 1

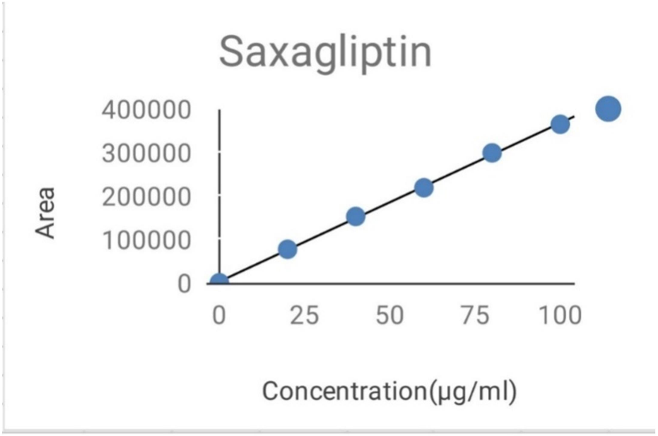

Figure 4: Calibration curve for saxagliptin

Table 6: System suitability results for varied flow rate:

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.27ml/min	9140	1.19
2	0.3ml/min	9997	1.14
3	0.33ml/min	9115	1.18

Table 7: System suitability results forvaried mobile phase composition:

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	9321	1.19
2	*Actual	9997	1.14

3	10% more	9315	1.18

Table 8: Results for LOD

PEAK NAME	RT	AREA	HEIGHT
Saxagliptin	1.065	495	148

Table 9: Results for LOQ

PEAK NAME	RT	AREA	HEIGHT
Saxagliptin	0.952	1021	490

Table 10: Assay results

	% purity
saxagliptin	98.14463

CONCLUSION

The proposed method was found to be simple, accurate, precise and rapid for the method validation for the estimation of Saxagliptin in pharmaceutical dosage form and could be used for routine analysis. All the parameters meet the criteria of ICH guidelines for method validation and found to be simple, sensitive, accurate and precise. It can therefore be concluded that the reported method is more economical and can find practical application for the method validation for the estimation of Saxagliptin in pharmaceutical dosage form both in research and quality control laboratories.

ACKNOLEDGEMENT

The authors are immensely thankful to the organization Vels Institute of Science, Technology and Advanced Studies (VISTAS) for providing helpful support for the study.

REFERENCE

[1] P. B. N. Prasad, *et al.*, Development and Validation of a Method for Simultaneous Determination of Metformin and Saxagliptin in a Formulation by RP-HPLC American Journal of Analytical Chemistry, 6, 2019, 841-850.

[2] Sarath Nalla *et al.*, A review on analysis of Dapagliflozin, Journal of current pharma research, 8 (2), 2018, 2321-2335.

[3] R. Advaita *et al.*, Estimation of saxagliptin hydrochloride and dapagliflozinpropendiol monohydrate in combined dosage form, Journal of applied pharmaceutical science, vol 3, 2018, 01-07.

[4] Ketan P. Dadhania, Parthika A. Nadpara, Yadvendra K. Agrawal: Development and validation of spectrophotometric method for simultaneous estimation of saxagliptin and metformin hydrochloride in bulk and tablets dosage form by simultaneous equation method. International Journal of Pharmaceutical Sciences and Research, 2(6), 2011, 1559-1563.

[5] Pawanjeet. J. Chhabda, *et al.*, 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, International journal of research and development in pharmacy and life science, 3(3), 2019, 993-1003.

[6] S. V. Chopade, Dr. V. R. Patil, Introduction to new chromatography technique - UPLC, pharmatutor-art-1206, 2011.