Larix Publications
Journal of Pharma Research
http://www.jprinfo.com/
Vol. 12, Issue 02, 2023 ISSN: 2319-5622

Research Article

METHOD DEVELOPMENT AND VALIDATION OF SAXAGLIPTIN BY USING UV SPECTROPHOTOMETRIC AND RP-HPLC TECHNIQUES IN BULK AND TABLET DOSAGE FORM

Dr. A. Madhukar[1] *, Shradha Rajesh Mahajan[2], Vaishnavi Rameshrao Siral[2], Ravindranath Bhagwan Rathod[2], Bhagwan Shesharao Ingole[2]

[1]Professor, Department of Pharmaceutical Chemistry, Dnyansadhana College of Pharmacy, Dharmapuri, Parbhani, Maharashtra, INDIA.
[2]B. Pharm 4th Year, Department of Pharmacy, Dnyansadhana College of Pharmacy, Dharmapuri, Parbhani, Maharashtra, INDIA.

Received on: 08-03-2023; Revised and Accepted on: 14-04-2023

ABSTRACT

Simple, precise, economical, fast and reliable UV and RP- HPLC Techniques have been developed for the estimation of Saxagliptin in bulk and pharmaceutical dosage form. UV method is based on measurement of absorption at maximum wavelength of 281 nm for Saxagliptin. Linearity for detector response was observed in the concentration range of 1 - 18µg/ml. The accuracy of the methods was assessed by recovery studies and was found to be 98.26 to 101.143 %. Separation was achieved with an Inertsil - Extend - C18 (250 × 4.6 mm, 5 µm) HPLC column. A mobile phase comprising Methanol and Water (40:60) was developed. The detection was carried out by using a UV detector set at a wavelength of 281nm. Validation experiments were performed to demonstrate linear over the concentration range of 10 - 125µg/ml and get the correlation Regression (r2) 0.999, showed good recoveries (98.95 – 101.22 %), the % relative standard deviations of Injection Precision and Method Precision were 0.044 and 0.0807 % respectively. The method can be used for quality control assay of Saxagliptin.

KEYWORDS: *UV-Spectrophotometry, RP-HPLC, Saxagliptin, Validation, and ICH guidelines.*

INTRODUCTION:

Saxagliptin is chemically 2-[2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile [1]. Is an oral hypoglycemic (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. Early development was solely by Bristol-Myers Squibb; in 2007 AstraZeneca joined with Bristol-Myers Squibb to co-develop the final compound and collaborate on the marketing of the drug [2-4].

Hence the method was developed and validated as per ICH guidelines [5, 6]. The literature reveals that various methods for the determination of Saxagliptin and pharmaceutical validations among these methods are UV - Spectrophotometric [7-13], HPLC & & UPLC method for Saxagliptin was reported [14-16].

MATERIALS AND METHODS

Equipment and Reagent:

A Shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Younglin RP-HPLC, Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

METHOD - A: UV Spectroscopy

Preparation of standard stock solution:

Stock solution was prepared by diluting 10 mg of drug in sufficient quantity of methanol in separate volumetric flask and volume was made up to 100 ml to get the concentrations 100 µg/ml for each drug. Dilutions from stock solution were prepared in the range of 1-18 µg/ml. Methanol: Water: 0.1N NaOH (40:30:30) was used as a blank solution.

***Corresponding Author:**

Dr. A. Madhukar
Professor,
Department of Pharmaceutical Chemistry,
Dnyansadhana College of Pharmacy,
Dharmapuri, Parbhani, Maharashtra, INDIA.
E-mail: *dr.amk2014@gmail.com*
DOI: https://doi.org/10.5281/zenodo.8094571

Absorption Maxima Method (λmax):

For the selection of analytical wavelength, standard solution of Saxagliptin was scanned in the spectrum mode from 400 nm to 200 nm separately. From the spectra of drug λmax of Saxagliptin 281 nm was selected for the analysis. Aliquots of standard stock solution were made and calibration curve was plotted.

Application of the proposed methods for the determination of Saxagliptin in tablet dosage form:

For the estimation of drugs in the tablet formulation, 20 tablets were weighed and weight equivalent to 30 mg was transferred to 100 ml volumetric flask and ultrasonicated for 20 minutes and volume was made up to the mark with methanol. The solution was then filtered through a Whatmann filter paper (No.42). The filtrate was appropriately diluted further.

The concentration of Saxagliptin was determined by measuring the absorbance of the sample at 281 nm in zero order spectrum modes. By using the calibration curve, the concentration of the sample solution was determined.

Method Validation:

The methods were validated with respect to accuracy, linearity, precision and selectivity.

Accuracy: Accuracy of an analysis was determined by systemic error involved. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. Recovery studies carried out for both the methods by spiking standard drug in the powdered formulations 80%, 100%, 120% amount of each dosage content as per ICH guidelines.

Linearity: The linearity of measurement was evaluated by analyzing different concentration of the standard solution of Saxagliptin. Result should be expressed in terms of correlation co-efficient (r2).

Precision: The reproducibility of the proposed method was determined by performing tablet assay at different time intervals (morning, afternoon and evening) on same day (Intra-day assay precision) and on three different days (Inter-day precision). Result of intra-day and inter-day precision is expressed in % RSD.

Sensitivity: The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3xσ/ S and LOQ = 10xσ/S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.075µg/ml and 0.25µg/ml respectively.

METHOD - B: RP-HPLC

Preparation of standard stock solution:

Stock solution was prepared by diluting 10 mg of drug in sufficient quantity of methanol in separate volumetric flask and volume was made up to 10 ml to get the concentrations 1000 µg/ml for each drug. Dilutions from stock solution were prepared in the range of 10-125 µg/ml. This solution (0.5ml) was further diluted to 10 ml with mobile phase to obtain a working standard stock solution of 50µg/ml for the RP- HPLC method.

Method development:

Optimization of the chromatographic condition:

Several mobile phases were tried to resolve Saxagliptin but the resolution was not satisfactory. So modification was made in the above mobile phase. Finally the system containing methanol: Water (40:60 v/v) as the mobile phase at a flow rate of 1.2ml/min was found to be satisfactory and gave well resolved peak for Saxagliptin. The retention time for Saxagliptin was 6.733 min. For the selection of detection wavelength, the spectrum of 10 ppm Saxagliptin revealed that, at 281 nm the drug possesses significant absorbance. So considering above fact, 281 nm was selected as a detection wavelength for estimation of Saxagliptin using HPLC. Complete resolution of the peaks with clear baseline separation was obtained.

Method Validation:

Linearity and range: Linearity was demonstrated by analyzing six different concentrations of active compound. Accurately measured standard working solutions of Saxagliptin 10, 25, 50, 75, 100, 125µg/ml were prepared in 10ml volumetric flasks and diluted to the mark with mobile phase. Aliquots (20µl) of each solution were injected under the operating chromatographic conditions described above. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs. concentrations Saxagliptin. Coefficient of correlation was 0.999.

Accuracy (recovery): The accuracy of the method was determined by calculating recoveries of Saxagliptin by the standard addition method. Known amounts of standard solutions of Saxagliptin (50%) were added to pre quantified sample solutions of tablets. The amounts of Saxagliptin were determined by applying these values to the regression equation of the calibration curve.

Method precision (repeatability): The precision of the method was checked by repeatedly injecting (n = 6) solutions of Saxagliptin (50µg/ml) for the RP-HPLC method. The accuracy of the method was evaluated by determination of the recovery of Saxagliptin on two days at six levels concentration. Tablets sample solutions were spiked with Saxagliptin standard solution,

corresponding to 50 to 150% of the nominal analytical concentration (25µg/ml). The results showed good recoveries ranging from 98.95 to 101.22%. The mean recovery data obtained for each level as well as for all levels combined were within 2.0% of the label claim for the active substance with an R.S.D. < 2.0%, which satisfied the acceptance criteria set for the study.

Limit of Detection (LOD) & Limit of Quantification (LOQ): The sample was dissolved by using Mobile Phase and injected until peak was diapered. After 0.053µg/ml dilution Peak was not clearly observed. So it confirms that 0.03µg/ml limit of Detection (LOD) & 0.1µg/ml Limit of Quantification (LOQ).

Robustness: The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ± 0.1 ml/min) and mobile phase composition (methanol ± 5%).

RESULT AND DISCUSSION

METHOD - A: Results of UV - Spectroscopy

The methods discussed in the present work provide a convenient and accurate way for analysis of Saxagliptin in its bulk and pharmaceutical dosage form. An absorbance maximum of Saxagliptin at 281 nm was selected for the analysis. Linearity for detector response was observed in the concentration range of 1-18 µg/ml.

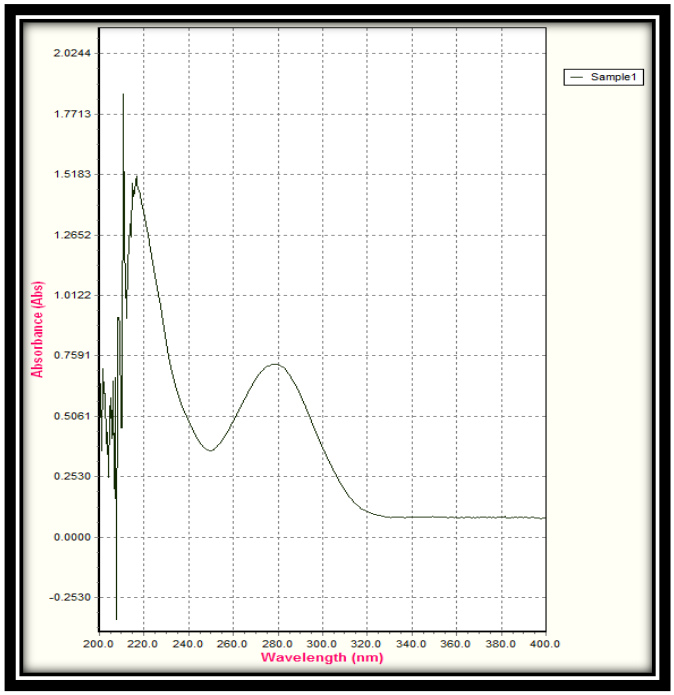

Fig. 2: It shows λmax of Saxagliptin

Standard deviation and coefficient of variance for three determinations of tablet formulation, was found to be less than ± 2.0 indicating the precision of the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. % recovery of Saxagliptin was found in the range of 98.267 % - 101.143 % and coefficient of variation was satisfactorily low indicating the accuracy of all the methods. % RSD for Intra-day & Inter-day assay precision for Saxagliptin was found to be 0.923 and 1.325. The LOD and LOQ were found to be 0.075µg/ml and 0.25µg/ml respectively. Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible & economical and can be employed for routine quality control of Saxagliptin in bulk drug and its pharmaceutical dosage form.

Table No. 1: Optical Characteristics and Precision of UV – Spectroscopy

S. No.	Parameter	Saxagliptin
1	**λ range**	**200-400 nm**
2	**Regression Equation (y= mx+c)**	**Y=0.068x+0.097**
3	**Measured wavelength**	**281 nm**
4	**Linearity range**	**1 - 18µg/ml**
5	**Slope**	**0.068x**
6	**Intercept**	**0.097**
7	**Correlation coefficient (R2)**	**0.998**
8	**Limit of Detection (LOD) µg/ml**	**0.075**
9	**Limit of Quantitation (LOQ) µg/ml**	**0.25**

Table No. 2: Linearity study of Saxagliptin by UV - Spectroscopy

S. No.	Concentration (µg/ml)	Saxagliptin
1	**1.00**	**0.1601**
2	**2.00**	**0.2457**
3	**5.00**	**0.4546**
4	**8.00**	**0.6441**
5	**10.00**	**0.7582**
6	**12.00**	**0.9243**
Correlation Coefficient (r2)		**0.998**

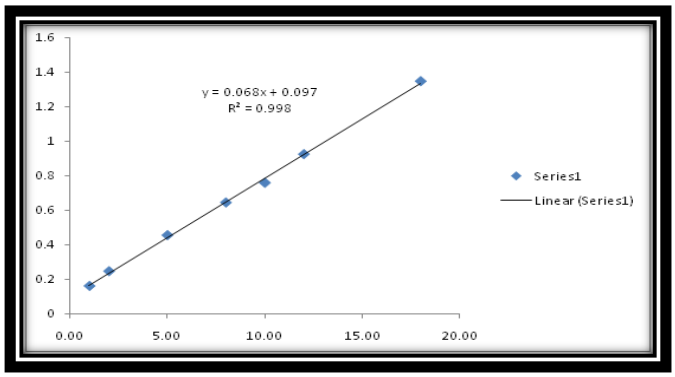

Fig. 3: Calibration curve for Saxagliptin at 281 nm

Table No. 3: UV - Spectroscopic Results of drug content and % Recovery of Saxagliptin

Excess drug added to the analyte (%)	Drug	% Recovery	% RSD
80	**Saxagliptin**	**98.26783**	**0.752249**
100		**99.69665**	**0.47312**
120		**101.1431**	**0.231805**

Table No. 4: UV - Spectroscopic Results of Intra-day and Inter-day Precision

S. No.	Intra-day	Inter-day
1	**0.7341**	**0.8015**
2	**0.7453**	**0.8245**
3	**0.7357**	**0.8057**
4	**0.7298**	**0.8298**
5	**0.7258**	**0.8158**
6	**0.7384**	**0.8184**
Mean	**0.73485**	**0.81595**
SD	**0.00679**	**0.010815**
% RSD	**0.923947**	**1.325442**

METHOD - B: Results of RP-HPLC

Saxagliptin standard having concentration 50µg/ml was scanned in UV- region between 200- 400 nm. λ max of Saxagliptin was found to be at 281nm. The Saxagliptin peak in the sample was identified by comparing with the Saxagliptin standard and the Retention time was found to be around 6.7333 minutes. The estimation Saxagliptin was carried out by RP-HPLC using Mobile phase having a composition of Methanol and Water (40:60). Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes. The column used was Inertsil - Extend C18 (250 × 4.6 mm, 5 µm). Flow rate of Mobile phase was 1.2 ml/min.

System suitability parameters such as %RSD for six replicate injections were found to be 0.166 %, Theoretical Plates – 5617.7, and Tailing Factor – 1.2917. The acceptance criteria of System Suitability is RSD should be not more than 2.0% and the method show Method Repeatability 0.0807 % which shows that the method is precise. The validation of developed method shows that the drug stability is well within the limits. System suitability parameters are listed in table.2.

Table No. 5: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	**Inertsil-Extend C18 (250 × 4.6 mm, 5 µm)**
Mobile Phase	**Methanol and Water (40:60)**
Flow rate (ml/min)	**1.2**
Run time (minutes)	**10.0**
Column temperature (°C)	**Ambient**
Volume of injection loop (µl)	**20**
Detection wavelength (nm)	**281**
Drugs RT (min)	**6.733**

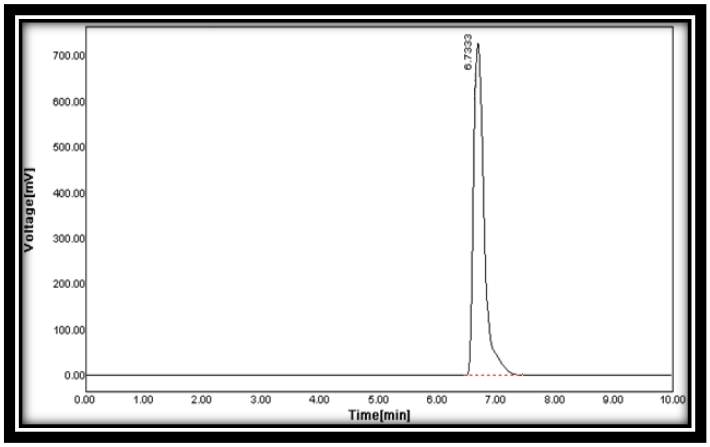

Fig. 4: Standard Chromatogram of Saxagliptin

Table No. 6: System suitability parameters

Parameter	Saxagliptin
Calibration range (µg/ml)	**10 – 125**
Theoretical plates	**5617.7**
Tailing factor	**1.2917**
Correlation Coefficient (r2)	**0.999**
% Recovery	**98.95 – 101.22 %**
System Suitability % RSD	**0.166**
Method Repeatability %RSD	**0.0807**

Table No. 7: Summary of results of Injection Precision parameter for Saxagliptin

S. No.	Saxagliptin
I.P-1	**2410835**
I.P-2	**2409735**
I.P-3	**2411035**
I.P-4	**2410063**
I.P-5	**2409025**
I.P-6	**2412072**
Mean	**2410460.833**
SD	**1078.169637**
% RSD	**0.044728776**

Table No. 8: Summary of results of Method Precision parameter for Saxagliptin

S. No.	inj-1	inj-2	Avg	MEAN	SD	% RSD
MP-1	**2409365**	**2408724**	**2409044.5**	**2408826.4**	**1944.4211**	**0.08072068**
MP-2	**2410654**	**2410936**	**2410795**			
MP-3	**2407394**	**2406363**	**2406878.5**			
MP-4	**2410047**	**2409154**	**2409600.5**			
MP-5	**2409853**	**2410631**	**2410242**			
MP-6	**2404439**	**2408357**	**2406398**			

Table No. 9: Summary results of Accuracy for Saxagliptin

Conc.		inj-1	inj-2	inj-3	Mean	% Recovery	STD	% RSD
25ppm	**50%**	**1182464**	**1196743**	**1192567**	**1190591**	**98.95359**	**7341.655**	**0.616639**
50ppm	**100%**	**2420762**	**2422832**	**2422663**	**2422086**	**100.6534**	**1149.439**	**0.047457**
75ppm	**150%**	**3654063**	**3658946**	**3647835**	**3653615**	**101.2209**	**5569.051**	**0.152426**

The linearity of the detector response was found to be linear from 10 to 125µg/ml of target concentration for Saxagliptin standard with a correlation coefficient value is greater than 0.999.

The correlation coefficient of (r2) = 0.999, which shows that the method **is capable of producing good response in UV-detector.**

Table No. 10: Summary of results of Linearity parameter for Saxagliptin

Conc. (µg/ml)	Average Area
10	**705560**
25	**1288524**
50	**2455048**
75	**3541523**
100	**4602442**
125	**5568099**

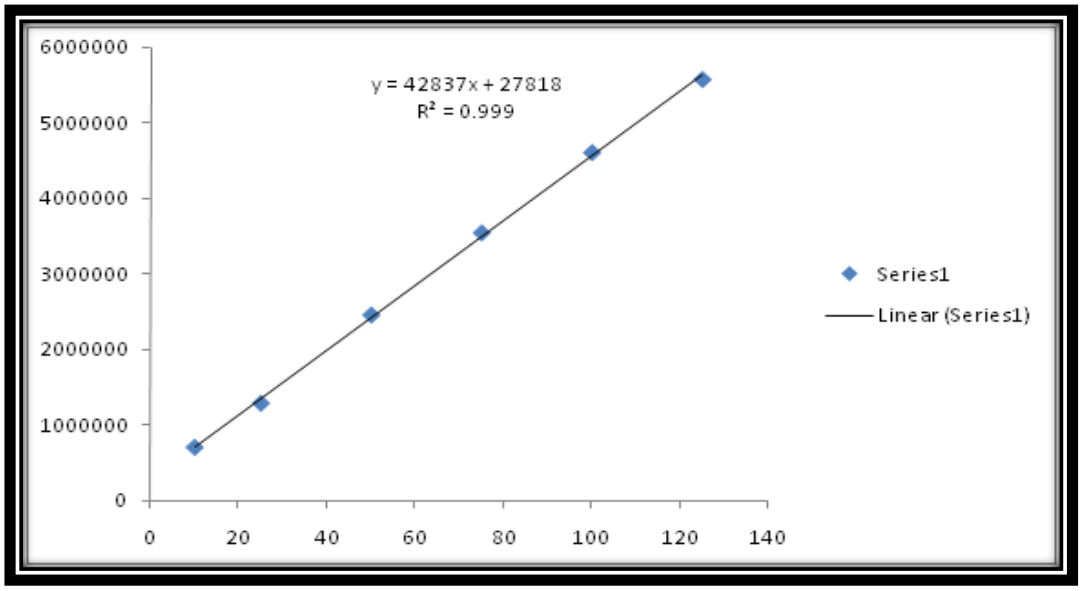

Fig. 5: Linearity Curve of Saxagliptin

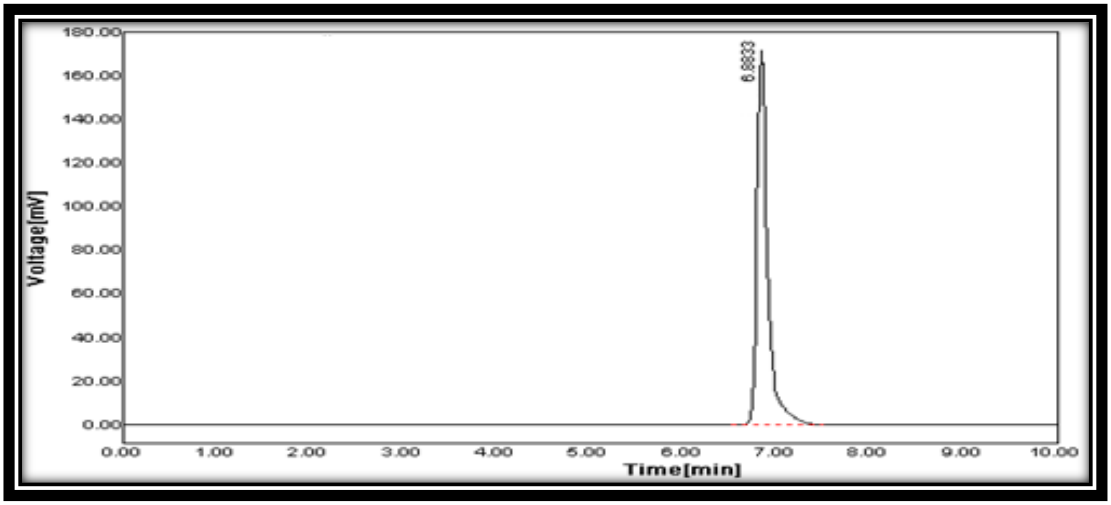

Fig. 6: Chromatogram of Saxagliptin (10 µg/ml)

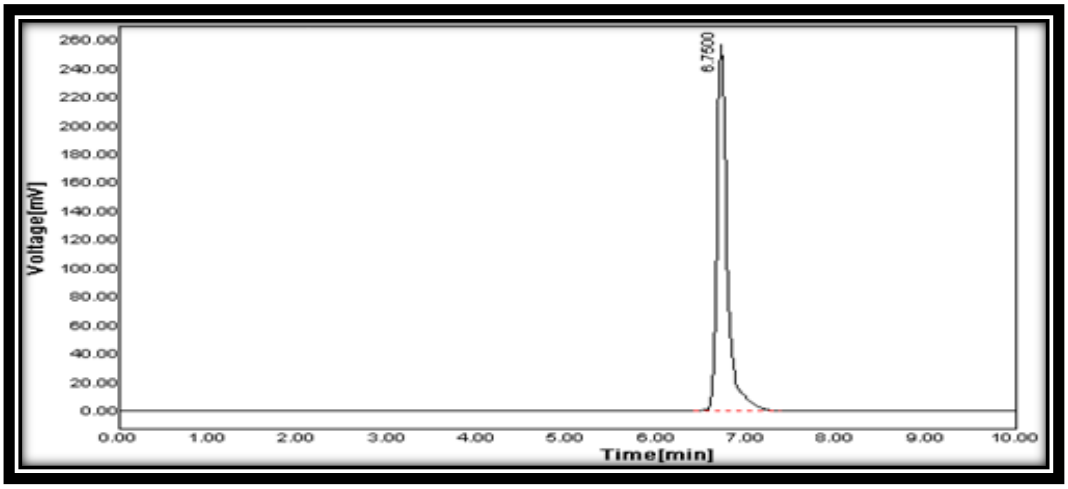

Fig. 7: Chromatogram of Saxagliptin (25 µg/ml)

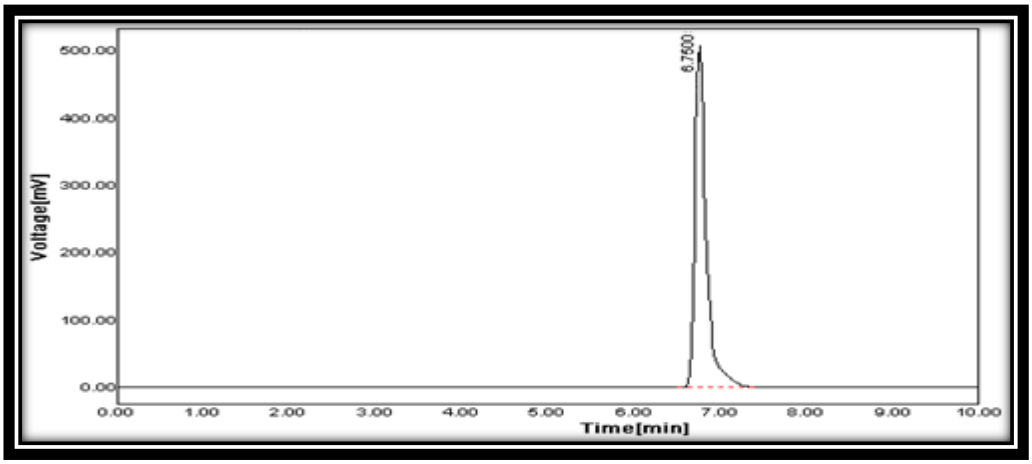

Fig. 8: Chromatogram of Saxagliptin (50 µg/ml)

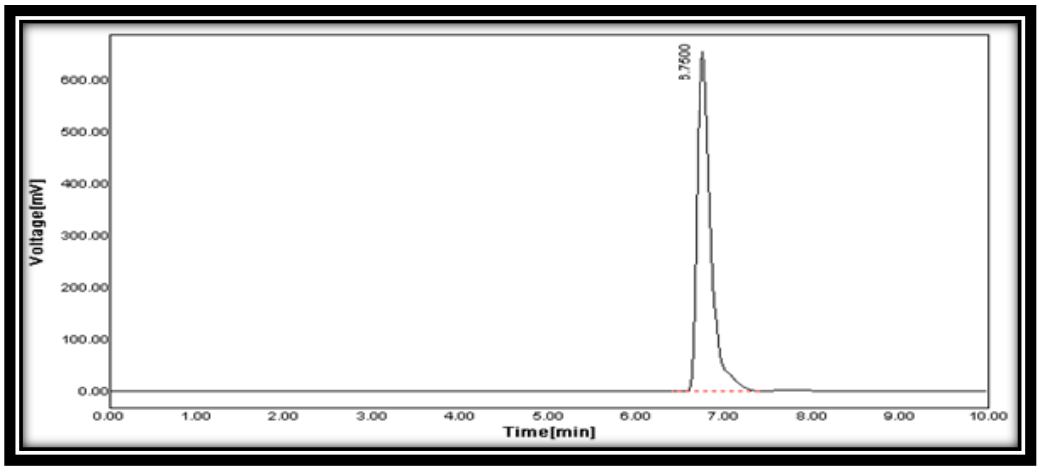

Fig. 9: Chromatogram of Saxagliptin (75 µg/ml)

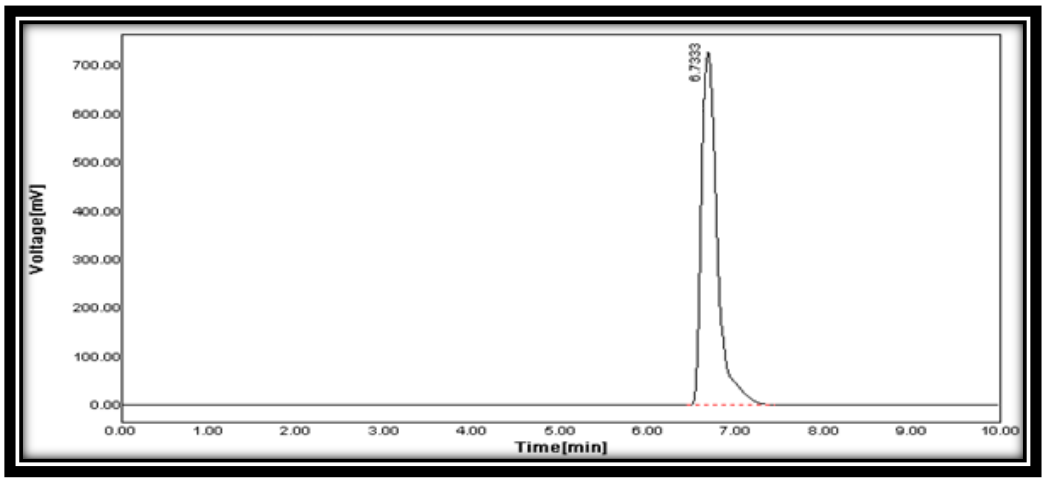

Fig. 10: Chromatogram of Saxagliptin (100 µg/ml)

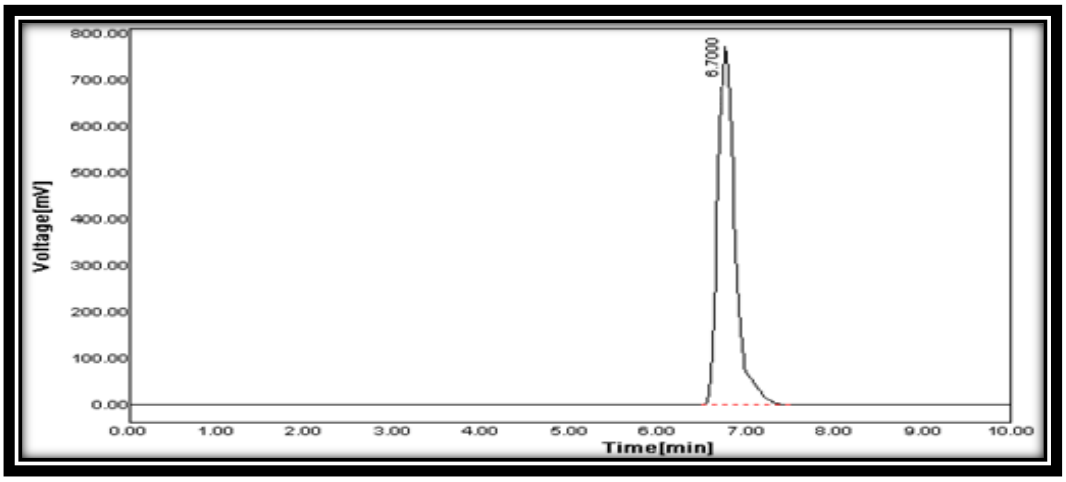

Fig. 11: Chromatogram of Saxagliptin (125 µg/ml)

Table No. 11: Summary of results of LOQ for Saxagliptin

Injection	Area of Saxagliptin (0.1 µg/ml)
Inj-1	4994
Inj-2	4897
Inj-3	4871
Inj-4	4998
Inj-5	4873
Inj-6	4937
Mean	4928.333
SD	57.56967
% RSD	1.168137

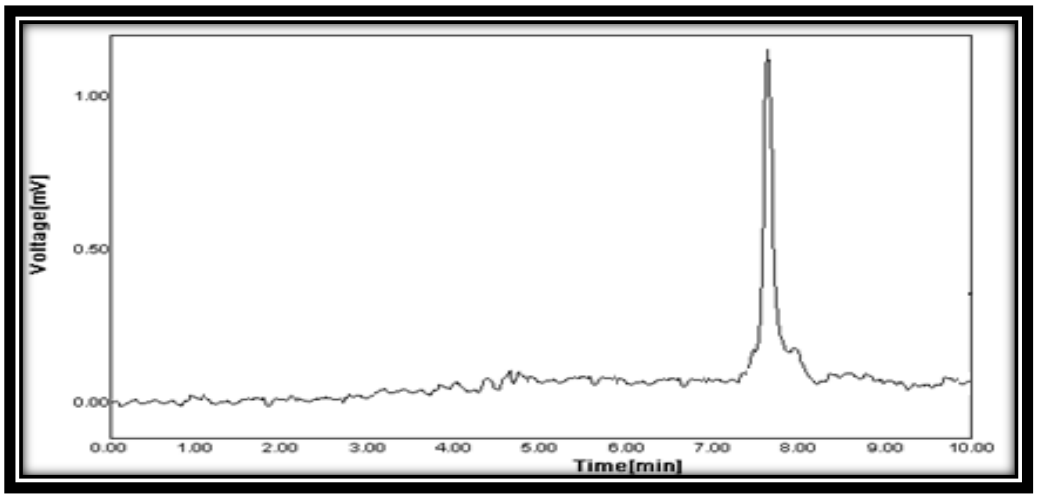

Fig. 12: LOD Chromatogram of Saxagliptin (0.03 µg/ml)

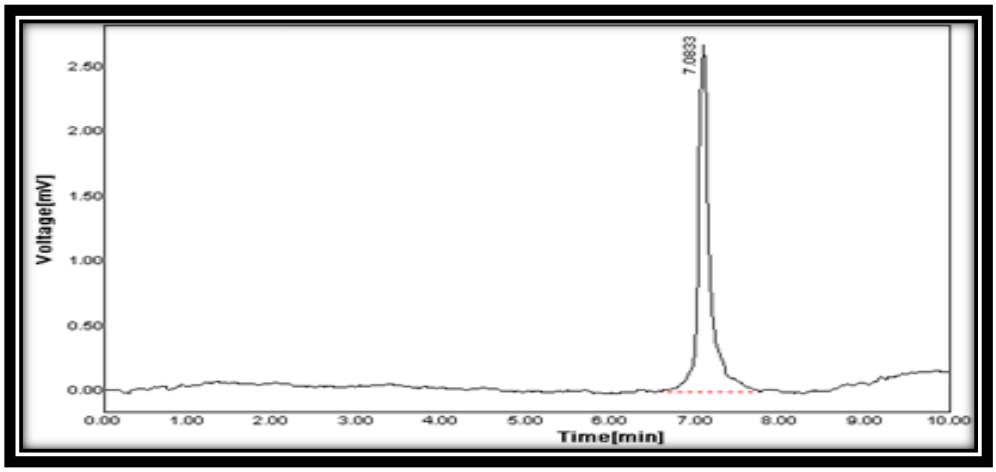

Fig. 13: LOQ Chromatogram of Saxagliptin (0.1 µg/ml)

Table No. 12: Summary of results of Robustness parameter for Saxagliptin

Parameters	Adjusted to	Avg. Areaa	RT	SD	% RSD
Flow Rate As per method 1.2 ml/min	**1.1 ml/min**	**5270501.7**	**7.21**	**12826.87**	**0.24**
	As it is	**4609032.2**	**6.71**	**4614.40**	**0.10**
	1.3ml/min	**4179819.7**	**6.22**	**11283.46**	**0.27**
Mobilephase compn (40:60v/v, Methanol:Water)	**45:55**	**4847538.83**	**6.36**	**15528.59**	**0.32**
	As it is	**4609032.2**	**6.71**	**4614.40**	**0.10**
	35:65	**4375673.5**	**7.15**	**10866.32**	**0.25**

aAvg. Area = Six Repeatable injections.

CONCLUSION

UV Spectrophotometric and RP-HPLC methods for Saxagliptin was developed separately in bulk and tablet dosage form by, Absorbance maxima method. Further, a UV Spectrophotometric and RP-HPLC method for the estimation of Saxagliptin was in bulk and combined dosage form. The methods were validated as per ICH guidelines. The standard deviation and % RSD calculated for these methods are < 2 %, indicating high degree of precision of the methods. The results of the recovery studies showed the high degree of accuracy of these methods. In conclusion, the developed methods are accurate, precise, robust and selective and can be employed successfully for the estimation of Saxagliptin in bulk and pharmaceutical dosage form. Because of cost-effective and minimal maintenance, the present UV spectrophotometric and RP-HPLC methods can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of Saxagliptin in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

Conflict of Interest: The authors declare no conflict of interest

REFERENCES:

1. https://go.drugbank.com/drugs/DB06335.

2. https://en.wikipedia.org/wiki/Saxagliptin.

3. Augeri D, et al. Discovery and preclinical profile of Saxagliptin (BMS-477118): a highly potent, long-acting, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. J Med Chem. 2005; 48(15): 5025-5037. doi:10.1021/jm050261p. PMID 16033281.

4. Bristol, Takeda Drugs Offer Alternatives to Januvia (Update2). Bloomberg. 2008-06-07.

5. International conference on Harmonization, Guidance for industry In; Q2A Text on validation of analytical method, Switzerland; IFPMA 1994; 1-4.

6. International conference on Harmonization, Guidance for industry In; Q2B Validation analytical procedures, Methodology, Switzerland; IFPMA 1996; 1-8.

7. Bhadauria RS, Agarwal V, Development and Validation of UV Spectroscopic Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin in marketed formulation. J Drug Del and Therap, 2019; 9(4-s):1160-1164 DOI: http://dx.doi.org/10.22270/jddt.v9i4-s.3828.

8. Shinde, et al. UV-Spectrophotometric Method Development and Validation for estimation of Saxagliptin in API and Pharmaceutical dosage form. Int J Curr Pharm Res, 2020; 12(5): 63-66. DOI: http://dx.doi.org/10.22159/ijcpr.2020v12i5.39768.

9. Kalaichelvi, et al. Validated Spectroscopic Method for Estimation of Saxagliptin in Pure and from Tablet formulation. Int J Pharm Pharm Sci, 2011; 3(Suppl 3): 179-180.

10. Sonali Narsing Koli, et al. UV Spectrophotometric Method Development and Validation for Estimation of Saxagliptin in API and in Pharmaceutical dosage form. Ijppr. Human, 2019; 14(4): 166-179.

11. Pravin Cholke, et al. Development and validation of UV-Visible spectroscopy method for simultaneous estimation of Saxagliptin hydrochloride and metformin hydrochloride in tablet dosage form. Int J Res in Pharm Pharm Sci, 2018; 3(4): 31-34.

12. Anusha. G, et al. Determination of Saxagliptin Monohydrate by Derivatization UV-Vis Spectroscopy. Int J Sci & Res, 2021; 10(11): 353-355. DOI: 10.21275/SR20610163533.

13. Narendra Nyola, et al. Development and validation of uv-vis specctroscopy method for simultaneous estimation of saxagliptin hydrochloride and Metformin hydrochloride in active pharmaceutical ingrident. J Pharm Educ Res, 2012; 3(2): 19-23.

14. Md. Rageeb Md. Usman, et al. Stability Indicating RP-HPLC Method for Determination of Saxagliptin and Dapagliflozin in Bulk and Tablet Dosage Forms. J Pharm Sci and Res, 2020; 12(4): 499-506.

15. Magharla Dasaratha Dhanaraju, et al. Stability indicating HPLC method for the simultaneous determination of dapagliflozin and saxagliptin in bulk and tablet dosage form. Curr Issues Pharm Med Sci, 2018; 31(1): 39-43. DOI: 10.1515/cipms-2018-0009.

16. S. Madhavi, et al. Development and Validation of a Method for Simultaneous Determination of Dapagliflozin and Saxagliptin in a Formulation by RP-UPLC. World J Pharm Res, 2017; 6(12): 904-916. DOI: 10.20959/wjpr201712-9703.

How to cite this article:

Authors Name. Dr. A. Madhukar. METHOD DEVELOPMENT AND VALIDATION OF SAXAGLIPTIN BY USING UV SPECTROPHOTOMETRIC AND RP-HPLC TECHNIQUES IN BULK AND TABLET DOSAGE FORM. J Pharm Res 2023; 12(02): 15-24 DOI: https://doi.org/10.5281/zenodo.8094571

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nils