Determination of Saxagliptin in Bulk and Pharmaceutical Formulation using Stability indicating RP-HPLC Method with DAD Detector

Pathak Shilpi* and Mishra Pradeep
Institute of Pharmaceutical Research, GLA University, Mathura, INDIA
*Shivi13pharma@gmail.com

Abstract

A strong and approved analytical technique is a key segment at various phases of pharmaceutical product improvement to make certain identity, immaculateness and quality of medicines and its formulations. The separation was done on Eclipse plus C18 (4.6 x 250 mm, 5 µm) column using a mobile phase consisting of a mixture of acetonitrile: water (90:10) at flow rate of 1 mL/min. Saxagliptin was examined using DAD detector at 215nm.

The detection was carried out at 215 nm and retention time of Saxagliptin was found to be 2.42 min. Linearity was observed between 10-40µg/mL. The coefficient of determination (R2) was found to be 0.9998. Saxagliptin was subjected to stress conditions including acidic, basic, oxidative, photolysis, thermal degradation and the results were calculated as %RSD values and were found to be within the limits. The technique was found to be exact, correct, sensitive particularly for the determination Saxagliptin in bulk and formulation.

Keywords: *Saxagliptin*, stress study, DAD detector, validation, HPLC, ICH guidelines.

Introduction

The key contemporary aim of stability indicating methods is to provide data on stress testing condition in order to determine the strength of drug and its dosage form. This research discusses methods for the maintenance of stability. Stress studies are used to differentiate the active pharmaceutical ingredient from its feasible material for decomposition. Method was approved for stability according to ICH Q1A (R2) ICH Q3B (R2) Q6A and FDA 21 CFR guidelines. During the production of stability indicating method, force degradation is required to demonstrate the specificity and for this purpose it must be performed before the stability studies are carried out[8].

Saxagliptin, (1S,3S,5S)-2-[(2S)-2-Amino-2-[(5S,7R)-3-hydroxy-1-adamantyl]acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile[2] , an anti-diabetic drug (Fig. 1) of the novel dipeptidyl peptidase-4 inhibitor class of drugs was approved on July 31, 2009.

Human body has natural hormone called incretins, dipeptidyl peptidase-4 are working on the action of these hormones. Sugar consumption is increasing in the body by incretins with decrease in blood sugar. Formation of insulin also increases by incretins in pancreas.

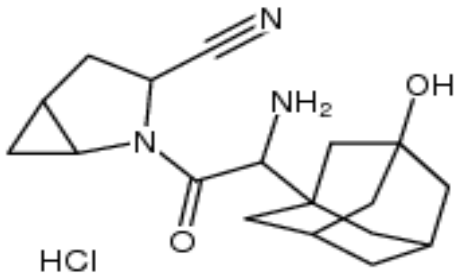


Figure 1: Structure of Saxagliptin

In liver, it decreases formation of sugar. Dipeptidyl peptidase-4 has two mechanism, first is an enzymatic function and other is based on binding of dipeptidyl peptidase-4 with adenosine deaminase. When they are activated, dimerization occurs and gives intracellular signals. A changeable, histidine-aid covalent bond formed in Saxagliptin between its groups on dipeptidyl peptidase-4 Increases the level of glucagon like peptide 1 by inhibition of dipeptidyl peptidase-4, formation of glucagon is inhibited from α cells and increases formation of insulin from β cells of pancreas[3].

According to literature there were only limited methods available, which aimed at the LC-MS method was developed and validated for structure elucidation for the major degradation products[1]. Few other studies discussed about simultaneous study of Dapagliflozin and Saxagliptin in combination[4]. Scheeren et al[9] investigated the RP-HPLC method with DAD for the quantitative analysis of Saxagliptin but this method used buffer phosphoric acid and retention time is high. Tikkeli et al[10] gave a method of HPLC in human plasma with fluorescence detection. In the literature, mostly buffers are used which are decreasing the life of equipment, pump and column of HPLC.

The point of proposed work was to create and approve a basic, delicate and stability indicating assay method for the estimation of Saxagliptin in bulk and formulations for routine investigation with photodiode array detector with applicability of green chemistry.

Material and Methods

Material and Reagents: Saxagliptin drug was obtained from Manus Aktteva Biopharma LLP, India as gift sample.

Marketed formulation containing 5 mg of Saxagliptin (Onglyza, Bristol Myers Squibb and India) was purchased from local pharmacy. Methyl alcohol, water and acetonitrile of HPLC grade were obtained by Spectrochem Pvt. Ltd., Mumbai.

Chromatographic condition: Agilent HPLC 1260 Infinity II Quaternary Pump VL system with DAD Detector was utilized. Separation was carried out on a particle size Poroshell 120 EC-C18, 4.6 x 100 mm, 4 µm column (at ambient Temperature), and isocratic run under reverse phase partition chromatographic condition. The equipment was controlled by a PC with properly installed chromatographic software.

Different mobile phase compositions were tried for the optimization of the method. A satisfactory result was obtained by using acetonitrile: water (90:10) mobile phase at 215 nm UV detection reverse phase separation method by HPLC.

Preparation of mobile phase: Tried different ratio of selected mobile phases consisting of water and acetonitrile at different flow rates. In acetonitrile: water (90:10), good symmetrical peak was obtained. Water was sonicated for 20 min and membrane filtration was performed.

Preparation of standard stock solutions: Accurately weighed 20mg of drug dissolved in 100 mL volumetric flask and then suitably diluted to give 200 µg/mL was stored under refrigerator. Stock was used for further dilutions and filtered through 0.2-µm nylon filter.

Selection of wavelength: Drug concentration 10 µg/mL was made from 200 µg/mL stock solution and further diluted using methanol. The standard drug solution was scanned between 200 to 400 nm and result showed that the maximum absorbance was recorded at 215 nm by spectrophotometer.

Calibration curve construction: Solutions with various concentration of the drug made by suitable dilutions of the stock solutions with methanol and their chromatogram were recorded at the optimized condition. The linearity was found in the range of 10-50 µg/mL concentration. Peak area was recorded for the calibration curve construction. The value of coefficient of determination (R^2) was evaluated by the calibration curve.

Method validation: The developed method has been validated in compliance with the ICH Guidelines[6,7] (ICH Q2A 1994; ICH Q2B 1996) to ensure the positive results of the study for various variables like linearity, precision, repeatability, accuracy, robustness, ruggedness, limit of quantization, limit of detection and specificity. Linearity was calculated in triplicate from the drug concentration (10-40µg /mL) in methanol. Accuracy was checked by recovery method by the pre-analyzed samples with the standard solution. This was repeated six times. The precision of the method was determined by the peak area obtained from the different replication of the drug concentration (10µg / mL).

It is calculated in terms of inter-day and intra-day accuracy. Serial drug dilutions were chromatographed from minimum to maximum concentration, repeated three times to determine the LOD and LOQ. In these studies we can interpret the acid, base, oxidation, thermal, photolytic degradation in the sample.

Tablet dosage form analysis: Powdered tablet 5 mg of Saxagliptin was weighed and dissolved in methanol with the help of sonication. Further filtration of solution was done with 0.2µm nylon membrane filter paper. Chromatogram obtained by dilution analysis and drug content percent were determined by corresponding regression equation.

Results and Discussion

Optimization of method: Chromatography method was optimized with the aim of developing a fast, robust, rugged and specific assay method for Saxagliptin. We tried many mobile phases according to solubility and chemistry of drug Saxagliptin.

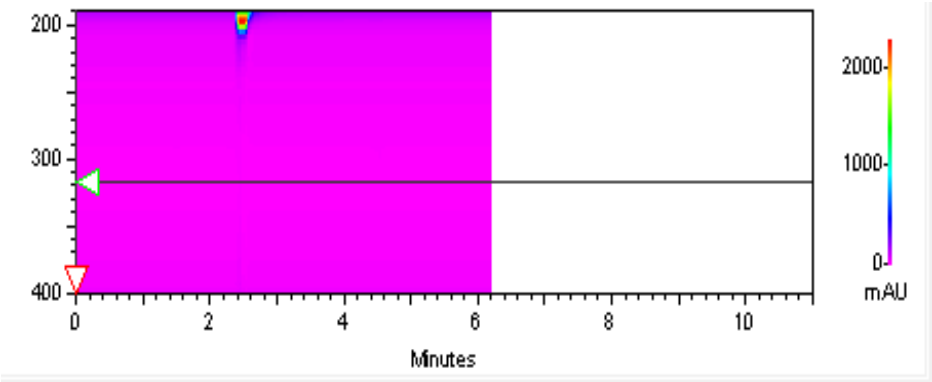
In different ratios of methanol and water, peaks was observed which show that drug ionizes in this separation phase. In ratio of acetonitrile and methanol tailing value was high and negative peak was observed. According to the Pka values, acetonitrile and water ratio was suitable for analysis.

A satisfactory result was obtained by using acetonitrile: water (90:10) mobile phase separation method and it was performed at 215 nm UV detection. Chromatogram was clearly shown in figure 2A, B. View of three dimensional chromatograph was shown in figure 3. Spectra are shown in figure 4. Optimized chromatographic conditions are shown in table 1.

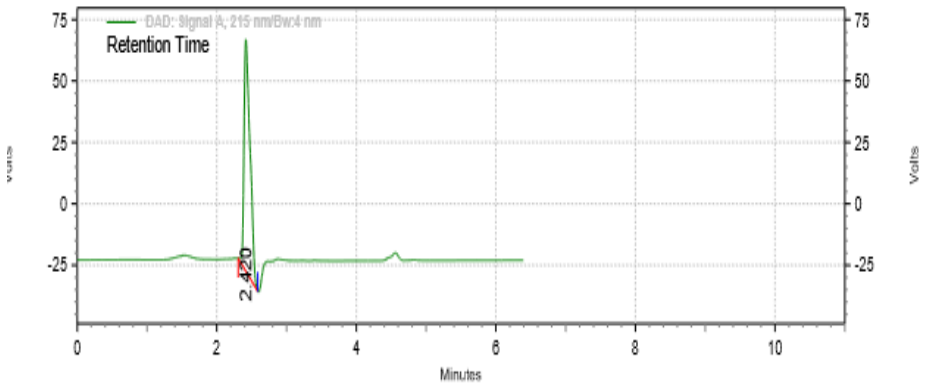
Linearity: Prepare seven samples of concentration range from 10,15,20,25,30,35,40 µg/mL from stock solution and inject into system. The calibration curve was generated by repeat analysis (n=3) at different concentration and the coefficient of determination was calculated. The curve was shown in figure 5.

Table 1
Developed separation conditions of Saxagliptin

Parameters	Conditions
Stationary phase	Eclipse plus C_{18} (4.6 x 250 mm, 5 µm)
Mobile phase	Acetonitrile: Water (90:10)
Flow rate (mL/min)	1
Run time (min)	10
Detection wavelength (nm)	215
Injection Volume (µL)	20
Retention time (min)	2.42



(A)



(B)

Figure 2: Counter plot (A) and developed chromatogram (B) Saxagliptin is in Acetonitrile and water (90:10) at 2.42 min

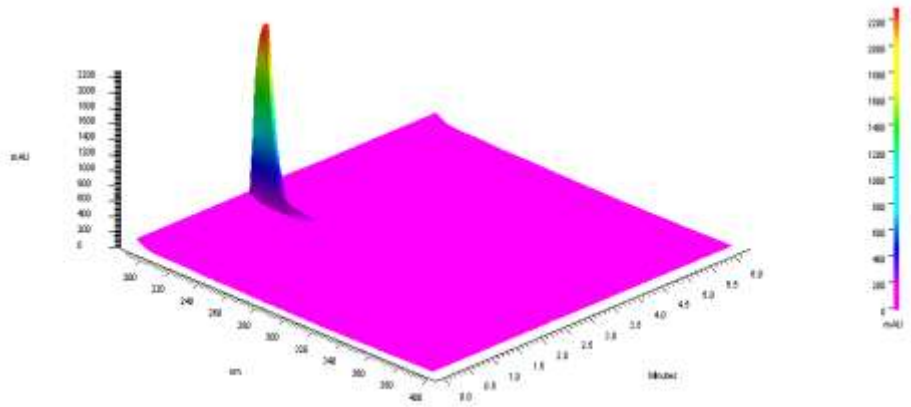


Figure 3: Three dimensional chromatogram of Saxagliptin in Acetonitrile and water (90:10) at 2.42 min

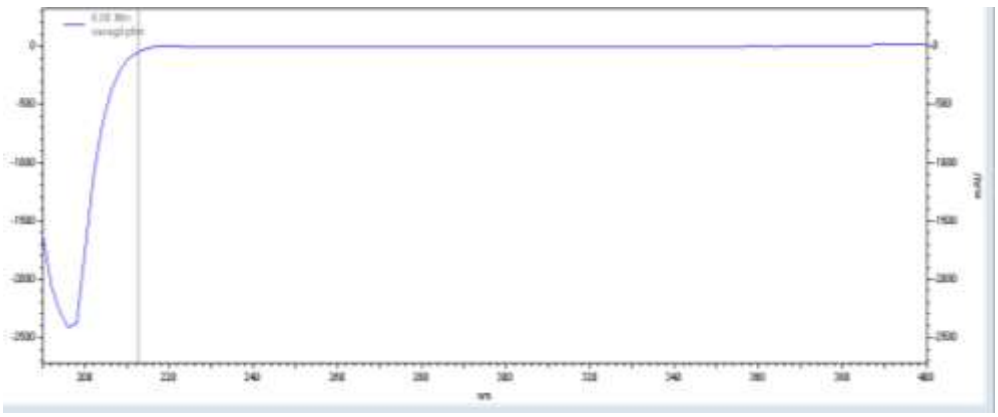


Figure 4: Spectrum of Saxagliptin at 215nm

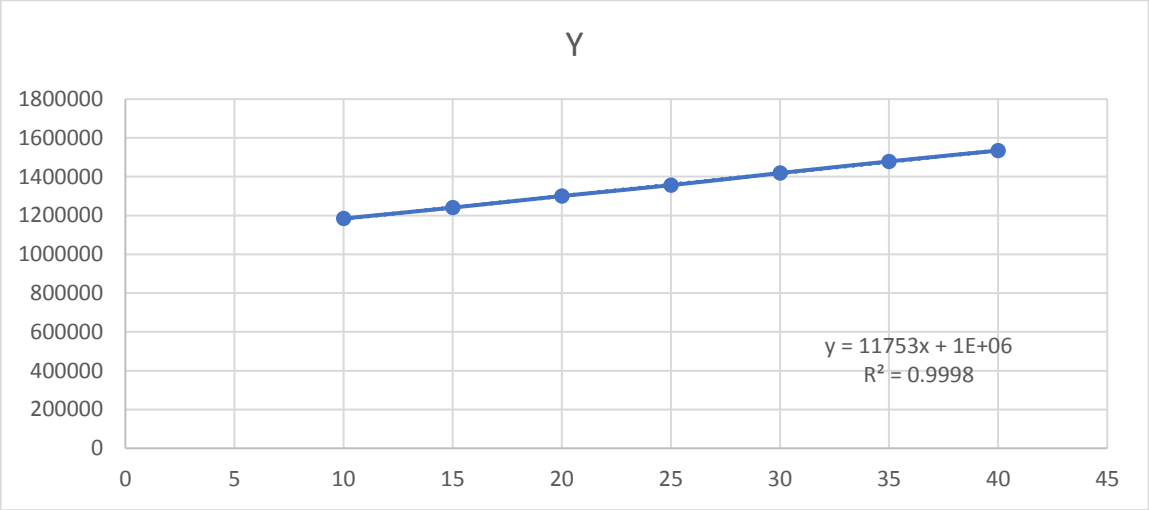


Figure 5: Calibration curve of Saxagliptin

Table 2
Accuracy of Saxagliptin

Drug %	Initial amount (µg/mL)	Amount added	% Recovery	%RSD
80%	10µg/mL	8µg/mL	98.43	0.7
100%	10µg/mL	10µg/mL	100.13	0.05
120%	10µg/mL	12µg/mL	101.96	0.2

Accuracy: Standard addition method was used for the evaluation of accuracy. To fix pre-analyzed drug solution, a known amount of standard drug was added. This method was performed at 80%, 100% and 120% level. The result of accuracy is shown in table 2.

Precision: The precision of the developed method was calculated by obtained peak area of various replications of concentration of the drug (10 µg/mL). It is also determined in terms of inter-day and intra-day precision.

Variations of inter-day and intra-day in the peak areas of same drug concentration on continuous three days were calculated in terms of relative standard deviation. The results are shown in table 3.

Robustness: Robustness of method was determined by making changes in flow rate, temperature and wavelength. Results are obtained by the standard deviation for Empagliflozin. The results are shown in table 4.

Repeatability: It was determined by analyzing sample solution 10 µg/mL of Empagliflozin into system and the peak area was measured. It was repeated six times. Table 5 showed the result of repeatability.

Ruggedness: It was calculated by using the same method by two analysts in different instrument. The result was represented by %RSD. The results were shown in table 6.

Detection limit and quantitation limit: Formulas for limit of detection and the limit of quantitation are:

LOD = 3.3 SD/Slope
LOQ = 10 SD/Slope
SD = Standard deviation of y-intercept of regression line.

According to developed method, the LOD was 2.8µg/mL and the LOQ was 9µg/mL for drug Saxagliptin.

Force Degradation Studies: In these studies according to ICH guidelines[5], Q1B (R2), we can interpret the acid, base, oxidation, thermal, photolytic degradation in the sample. The results of degradation study were shown in table 7.

Acid degradation: The drug interacts with acid producing primary degradation in the desirable range. For acid analysis, HCl or H_2SO_4 (0.1-1M) are widely used. The chromatogram was shown in figure 6 with reaction of H_2SO_4.

Base degradation: When the drug interacts with base, it produces primary degradant in the desirable range. For base analysis, NaOH or KOH (0.1-1M) are widely used. The chromatogram after degradation with NaOH is shown in figure 7.

Oxidative degradation: Hydrogen peroxide is widely used for oxidation degradation. Drug structure will allow selecting concentration and condition of oxidizing agent. The chromatogram after degradation is shown in figure 8.

Photolysis degradation: The drug Saxagliptin was directly keep in sunlight in closed Petri-dish for exposure. The results are shown in figure 9.

Table 3
Inter-day and Intra-day Precision data for Saxagliptin

Concentration 10 µg/ml	Intra-day study			Inter-day study		
	Morning	Afternoon	Evening	Day 1	Day 2	Day 3
Mean (peak area)	1186937	1185504	1186262	1185632	1185760	1184480
SD	494.63	1036.72	923.47	6217.76	653.06	569.03
%RSD	0.04	0.08	0.07	0.5	0.05	0.04

Table 4
Robustness data of Saxagliptin at various temperature, flow rate and wavelength

Concentration	Flow rate		Temperature		Wavelength	
10µg/mL	1.2	0.8	45	25	214	216
Av. Peak area	1179432	1185235	1186080	1185737	1185912	1187935
SD	12927.91	992.870	1554.94	1321.95	1647.14	2199.61
%RSD	1.09	0.08	0.13	0.11	0.13	0.18

Table 5
Repeatability of developed method

S.N.	Peak area
1	1184484
2	1186942
3	1186313
4	1189463
5	1181044
6	1189421
Average	1186278
SD	3196.18
%RSD	0.26

Table 6
Ruggedness of developed method for Saxagliptin

	Peak area	SD	%RSD
Analyst 1	1179481 1181344 1185346	2996.80	0.25
Analyst 2	1184631 1187906 1185436	1706.58	0.14

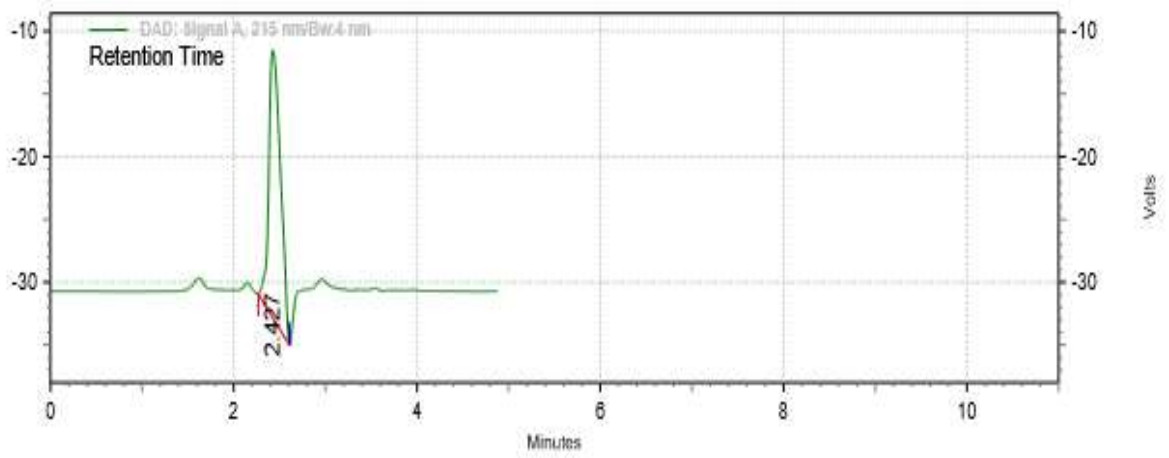


Figure 6: Acid degradation analysis

Table 7
Degradation Analysis

Sample preparation	Area of Saxagliptin in sample	Drug remained%	Drug degraded%	Comments
Control sample	1184481	100	0	No Degradation
Base Stress-4hrs	404114	34.11	65.89	Degradation
Base Stress-8hrs	369491	31.19	68.89	Degradation and other peaks also present
Base Stress-24hrs	84206	7.1	92.90	Completely degraded and other peaks also present
Acid Stress-4hrs	1023024	86.36	13.64	No degradation
Acid stress-8hrs	672726	56.79	43.21	Degradation
Acid stress-24hrs	400565	33.81	66.19	Degradation
6% Peroxide treated-4hrs	386720	32.64	67.36	Degradation
6% Peroxide treated-8hrs	212569	17.94	82.06	Degradation
6% Peroxide treated-24hrs	177139	14.95	85.05	Degradation
Heat-24hrs	1160746	97.99	2.01	No degradation
Heat-48hrs	1122709	94.78	5.22	No degradation
Light-5days	1177869	99.44	0.56	No degradation
Light-10days	1048765	88.54	11.46	No degradation

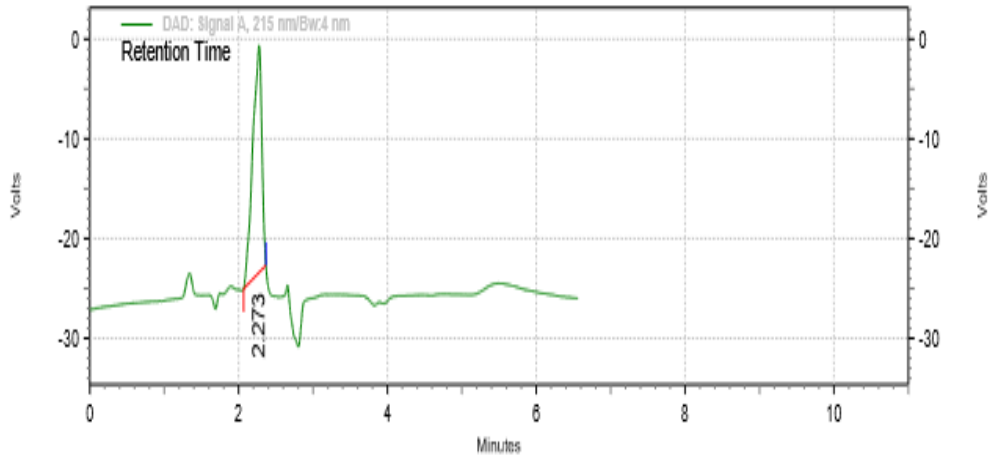


Figure 7: Base Degradation Analysis

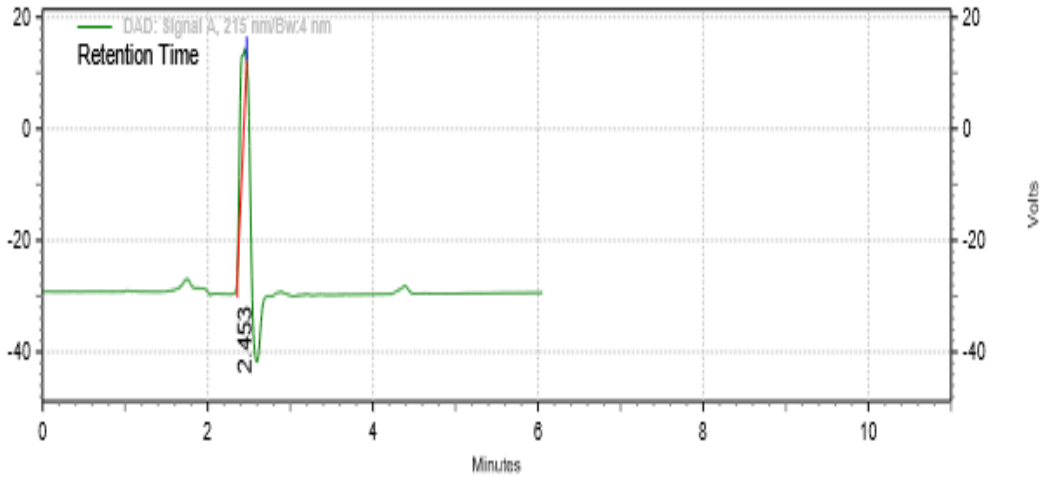


Figure 8: Oxidative degradation analysis

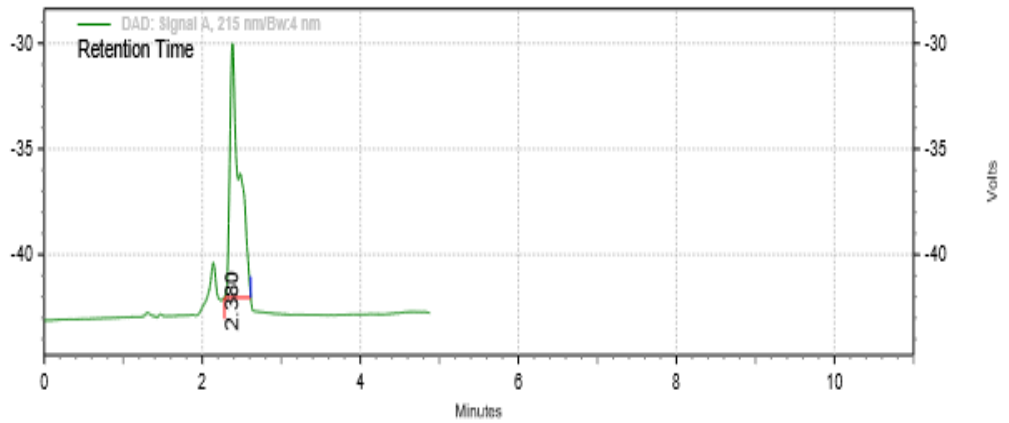


Figure 9: Photolytic degradation analysis

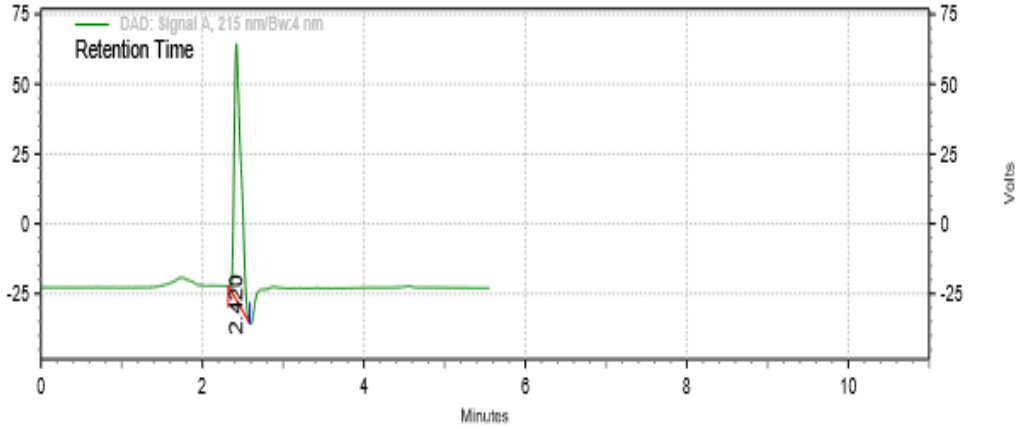


Figure 10: Thermal degradation analysis

Thermal degradation: According to ICH Q1A accelerated testing condition, thermal degradation should be carried out in dry heat or wet heat. Study may be conducted at high temperature for short period. The results are shown in figure 10.

Analysis of dosage form: Drug content was determined 99.70% with a standard deviation of 0.20. Standard deviation error must be less than the two. It is advantageous in accordance with the guidelines.

Application of Developed Method: It is very challenging to use chemicals, drug and solvent of separation method in pharmaceutical product in green chemistry. Mostly volatile organic compounds are used in the laboratory, research and industry for method development. Use of toxic reagents is strictly limited. Nowadays developed methods are very simple and less harmful to environment, human and animal. Green chemistry mainly focused on new analytical method and techniques reducing the unsafe chemicals. Green chemistry should be applied for the drug Empagliflozin in method development and validation.

Conclusion

RP-HPLC-DAD method for estimating Saxagliptin with degradation analysis has been developed. Developed method has been validated and it has been found to be simple, sensitive, reliable and durable and can be used for routine Saxagliptin analysis. The forced degradation studies were conducted in compliance with ICH guidelines and the findings showed the method's suitability to study drug stability under various conditions of degradation such as acid, base, oxidative, thermal, UV and photolytic degradation. The conclusion was that the approach is simple, sensitive and is capable of degradation of drug for separation.

References

1. Abdel-Ghany M.F., Abdel-Aziz O., Ayad M.F. and Tadros M.T., Stability-Indicating Liquid Chromatographic Method for Determination of Saxagliptin and Structure Elucidation of the Major Degradation Products Using LC–MS, *J Chromatogr Sci.*, **53**, 554-56 **(2014)**

2. Cholke P., Shirsath M., Temak Y., Kagde A. and Lagad R., Development and validation of UV-Visible spectroscopy method for simultaneous estimation of Saxagliptin hydrochloride and metformin hydrochloride in tablet dosage form, *Int J Res Pharm Pharm Sci*., **3(4),** 31-34 **(2018)**

3. Deepan T. and Dhanaraju M.D., Stability indicating HPLC method for the simultaneous determination of dapagliflozin and

saxagliptin in bulk and tablet dosage form, *Curr Issues Pharm Med Sci.*, **31(1)**, 39-43 **(2018)**

4. ICH, Q1B (R2) stability testing of new drug substances and products, Proceedings of the International Conference on Harmonization, Geneva **(1996)**

5. ICH Q2A, Harmonised tripartite guideline: text on validation of analytical procedures, Proceedings of the international conference on harmonization, Geneva **(1994)**

6. ICH Q2B, Harmonised tripartite guideline: validation of analytical procedures: methodology, Proceedings of the international conference on harmonization, Geneva **(1996)**

7. Mechanism of action of saxagliptin, available at https://www.drugbank.ca/drugs/DB06335 Accessed 15 April 2018 **(2018)**

8. Quadri S.S., Sonwane L.V., Poul B. and Kamshette S., Review on Stability Indicating Assay Methods (SIAMs), *Pharma Tutor*, **2(8),** 16-31 **(2014)**

9. Scheeren L.E., Marcolino A.I.P., Adams A.I.H. and Rolim C.M.B., Stability indicating RP-LC-PDA method for the quantitative analysis of saxagliptin in pharmaceutical dosage form, *BJPS*, **51(2),** 461-466 **(2015)**

10. Tekkeli S.E.K., Kızıltaş M.V. and Dinçel D., An HPLC Method for the Determination of Saxagliptin in Human Plasma with Fluorescence Detection, *J Adv Chem*., **5(3),** 810-818 **(2013)**.

(Received 14th May 2020, accepted 25th July 2020)