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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SAXAGLIPTIN AND DAPAGLIFLOZIN

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ABSTRACT
Saxagliptin and Dapagliflozin are anti diabetic drugs that are used together with proper diet and exercise control high blood sugar levels in people with type 2 diabetes. The present study was to develop a stability indicating RP-HPLC method for simultaneous estimation of Saxagliptin and Dapagliflozin in bulk and tablet dosage forms. The chromatographic separation of these two drugs was achieved on Discovery C_{18} column (250 × 4.6 mm, 5 µ) as the stationary phase with a mobile phase of water (pH 2.2 adjusted with OPA) : Acetonitrile (55:45 v/v) at a flow rate of 1 ml/min and PDA detection at 210 nm. The retention times of Saxagliptin and Dapagliflozin were found to be 3.601 and 2.785 respectively. The proposed method was validated for system suitability, linearity, accuracy, precision, specificity, LOD, LOQ and robustness. The calibration curves were linear in the concentration range of 25-150% of the working concentration (r^2 = 0.999) for both the drugs in binary mixture. The LOD was found to be 0.13 µg/ml and 0.09 µg/ml and LOQ was found to be 0.39 µg/ml and 0.27 µg/ml for Saxagliptin and Dapagliflozin respectively. Accuracy was found to be 99.91 % for Saxagliptin and 100.08% for Dapagliflozin. The percentage recoveries for both drugs were in the range of 99-101%. The method was applied for determination of Saxagliptin and Dapagliflozin in the presence of their degradation products under the variety of stress conditions. The pharmaceutical ingredient was subjected to acid, alkali, water, heat, UV light and oxidative stress conditions and stress samples were analysed by the proposed method. Hence the proposed stability indicating RP-HPLC method can be used in routine analysis of tablets containing Saxagliptin and Dapagliflozin.

KEYWORDS: Saxagliptin, Dapagliflozin, HPLC, stability, method development and validation.

INTRODUCTION
Saxagliptin and Dapagliflozin are anti diabetic drugs that are used together with proper diet and exercise control high blood sugar levels in people with type 2 diabetes. Both the drugs were used in controlling high blood sugar levels helps to prevent kidney damage, blindness, nerve problems, loss of limbs, sexual function problems and proper control of diabetes may also lessen the risk of heart attack or stroke. Saxagliptin is chemically (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxyl-1-adamantyl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile shown in Figure 2. It is dipeptidyl peptidase-4 (DDP-4) inhibitor anti diabetic for the treatment of type-2 diabetes. Dapagliflozin is chemically (2S,3R,4R,5S,6R)-2-[4-Chloro-3-(4-ethoxybenzyl) phenyl]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol shown in Figure 1. It is an oral anti diabetes medicine that inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2) which are responsible for at least 90% of the glucose reabsorption in the kidney. Extensive literature survey revealed that there were few analytical methods for simultaneous estimation of Saxagliptin and Dapagliflozin includes RP-HPLC[1-9] and UV.[10-12]

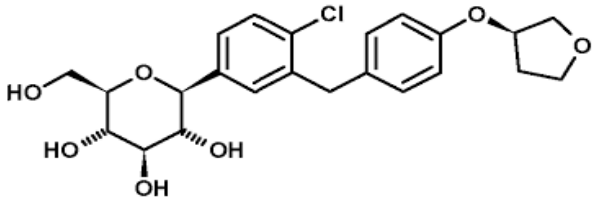

Figure 1: Structure of Dapagliflozin.

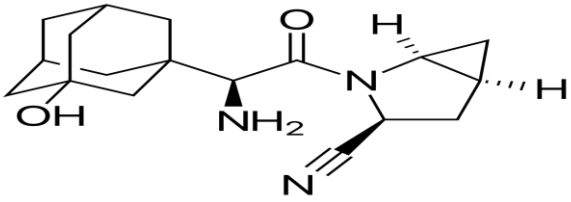

Figure 2: Structure of Saxagliptin.

MATERIALS AND METHODS

Equipment

Waters HPLC system (e 2695 model with Empower 2 software) containing Discovery C_{18} column (250 × 4.6 mm, 5 µ) with PDA detection.

Materials

Pharmaceutical grade pure Saxagliptin and Dapagliflozin were obtained from Spectrum Labs, Hyderabad. Qtern tablets were taken for the study which contains 5mg Saxagliptin and 10mg Dapagliflozin which were procured from local market. Orthophosphoric acid (OPA), HPLC grade water and acetonitrile of analytical grade were procured from Merck.

Diluent: Water: acetonitrile has taken in the ratio of 50:50% v/v.

Methods

Standard stock solution preparation

Accurately weighed 5 mg of Saxagliptin and 10 mg of Dapagliflozin were transferred into a 10 ml clean dry individual volumetric flask separately. 5 ml of diluents was added to both of these volumetric flasks and sonicated for 10 minutes, made up to the final volume with diluent. The volumetric flasks were labelled as standard stock solution 1 and 2. (500 µg/ml of Saxagliptin and 1000 µg/ml of Dapagliflozin).

Preparation of standard working solutions (100 % solution)

1 ml from each stock solution was pipetted out into a 10 ml volumetric flask and made up the final volume with diluent. (50 µg/ml Saxagliptin and 100 µg/ml Dapagliflozin).

Preparation of sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 5 ml of diluent was added and sonicated for 25 min, further the final volume was made up with diluent and filtered through 0.45 µm membrane filters. (500 µg/ml of Saxagliptin and 1000 µg/ml of Dapagliflozin).

Preparation of sample working solutions (100 % solution)

1 ml of the above filtered sample stock solution was transferred to 10 ml volumetric flask and the final volume was made up with diluent. (50 µg/ml of Saxagliptin and 100 µg/ml of Dapagliflozin).

Preparation of buffer

0.1 % OPA Buffer

1 ml of concentrated ortho phosphoric acid was diluted to 1000 ml with water.

Chromatographic Conditions

Chromatographic separation was achieved on Discovery C_{18} column (250 × 4.6 mm, 5 µ) with a mobile phase of water (pH 2.2 adjusted with OPA) : Acetonitrile (55:45 v/v) with PDA detection at 210 nm. The flow rate was set at 1 ml/min for stability indicating RP-HPLC method development and validation for simultaneous estimation of Saxagliptin and Dapagliflozin.

Method Development

Different chromatographic conditions were tried for separation and resolution. Waters Discovery column was found satisfactory. Peak purity of Saxagliptin and Dapagliflozin was checked using photo diode array detector and 210 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. The optimized chromatogram of Standard and sample was shown in Figures 3 and 4. Forced degradation studies were also carried using the developed method and degraded compounds were effectively resolved from Saxagliptin and Dapagliflozin.

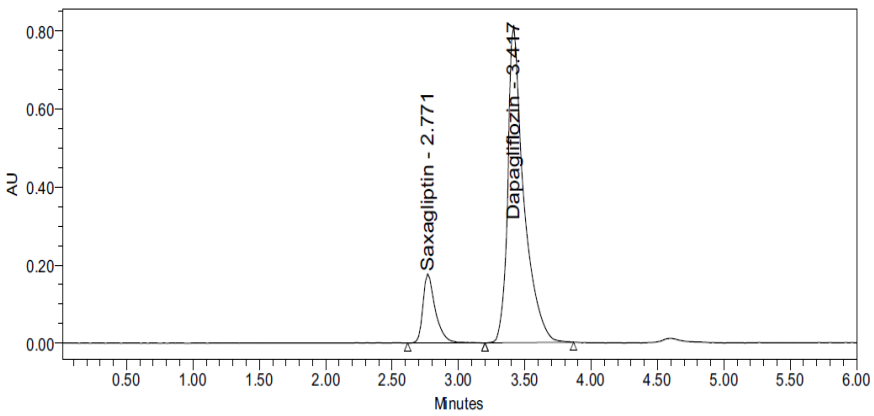

Figure 3: Chromatogram of Saxagliptin and Dapagliflozin in standard preparation.

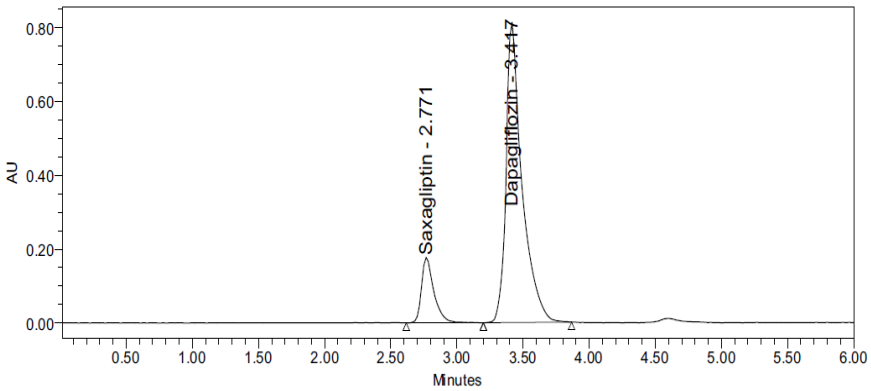

Figure 4: Chromatogram of Saxagliptin and Dapagliflozin in sample preparation.

RESULTS AND DISCUSSION

Method Validation

The developed RP-HPLC method was validated for parameters like system suitability, linearity, accuracy, precision, specificity, LOD, LOQ and robustness according to ICH guidelines.[13]

System Suitability

The system suitability parameters were determined by preparing the standard solutions of Saxagliptin (50ppm) and Dapagliflozin (100ppm) as per the test method and the solutions were injected six times into the system. The system suitability parameters like theoretical plates, resolution, peak tailing, USP plate count and asymmetric factor were evaluated. The results of system suitability parameters for Saxagliptin and Dapagliflozin were tabulated in table 1. All the parameters were found within the limits.

Table 1: Results of System Suitability.

S. No	Saxagliptin				Dapagliflozin			
No. of injections	Rt (min)	USP Plate Count	Peak Tailing	Resolution	Rt (min)	USP Plate Count	Peak Tailing	Resolution
1	2.782	4349	1.31	-	3.428	5084	1.41	3.4
2	2.784	4645	1.33	-	3.433	5131	1.41	3.5
3	2.784	4655	1.33	-	3.437	5042	1.41	3.5
4	2.786	4608	1.30	-	3.459	5241	1.40	3.5
5	2.788	4631	1.34	-	3.554	5211	1.43	4.1
6	2.793	4796	1.28	-	3.359	5030	1.43	4.2

Specificity

The specificity of the analytical method was verified by checking the interference in the optimized method such as degradation products and matrix components. Blank and placebo injections were prepared as per the test method and injected into the system. There were no interference peaks at the retention time of analytes. The chromatograms of blank and placebo were shown in Figures 5 and 6.

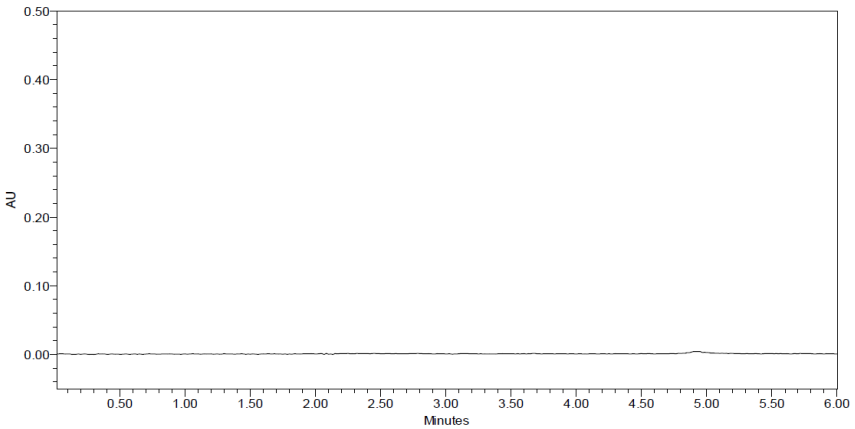

Figure 5: Chromatogram of Blank.

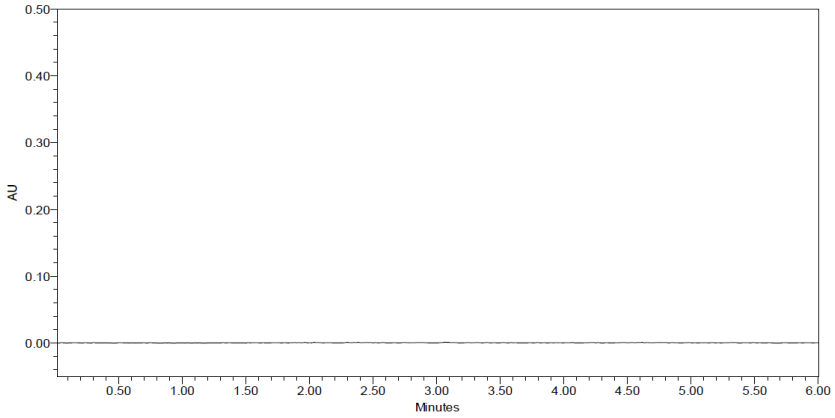

Figure 6: Chromatogram of Placebo.

Accuracy

To ensure the reliability and accuracy of the method, recovery studies were carried out by standard addition method. A known quantity of pure drug was added to concentration levels (50%, 100% and 150%) and contents were reanalyzed by the proposed method and the percentage recovery results were shown in table 2.

Table 2: Results of accuracy of Saxagliptin and Dapagliflozin.

Analytes	%Level	Amount Spiked (µg/ml)	Amount Recovered (µg/ml)	% Recovery	%Mean Recovery
Saxagliptin	50	50	25.17559	100.70	99.91
	100	100	49.61483	99.23	
	150	150	74.84055	99.78	
Dapagliflozin	50	25	50.07194	100.14	100.08
	100	50	101.16393	101.16	
	150	75	148.38476	98.92	

Precision

The reproducibility of the method was verified by calculating the average area, standard deviation and % RSD for both drugs on the same day (interday) and for intraday precision. %RSD was calculated from repeated studies and the results were within the limit. %RSD was reported in the tables 3 and 4.

Table 3: Results of interday precision.

S. No	Area of Saxagliptin	Area of Dapagliflozin
1	478598	791468
2	479111	793690
3	475554	779508
4	478053	787365
5	478313	789431
6	478030	788730
Mean	477943	788365
SD	1237.5	4872.4
RSD (%)	0.3	0.6

Table 4: Results of intraday precision.

S. No	Area of Saxagliptin	Area of Dapagliflozin
1	566082	3466522
2	568211	3459964
3	565666	3472564
4	566160	3489250
5	568956	3453943
6	568930	3466186
Mean	567334	3468072
SD	1528.1	12165.6
RSD (%)	0.3	0.4

Linearity

The linearity of the test solutions for the assay method was prepared from Saxagliptin and Dapagliflozin standard stock solutions at six concentration levels from 25% to 150% of assay concentration. The peak area versus concentration data was treated by least square linear regression analysis. The linearity charts of Saxagliptin and Dapagliflozin was shown in Figures 7 and 8. The results tabulated in table 5 have shown an excellent correlation between peak area and concentration which was within the concentration range of 12.5-75 µg/ml for Saxagliptin and 25-150 µg/ml for Dapagliflozin. The correlation coefficients were found to be 0.999 for both the drugs, which met the method validation acceptance criteria and hence the method was said to be linear for both the drugs.

Table 5: Linearity table for Saxagliptin and Dapagliflozin.

Saxagliptin		Dapagliflozin	
Concentration (µg/ml)	**Peak Area**	**Concentration (µg/ml)**	**Peak Area**
0	0	0	0
12.5	153036	25	1073542
25	288144	50	1888294
37.5	422128	75	2889150
50	565709	100	3852406
62.5	715565	125	4833326
75	854366	150	5907325

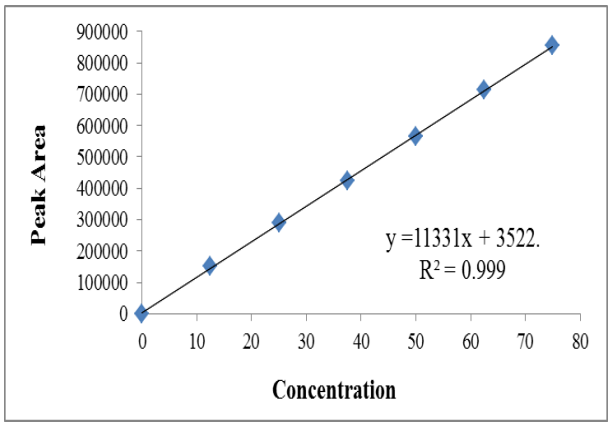

Figure 7: Linearity Chart of Saxagliptin.

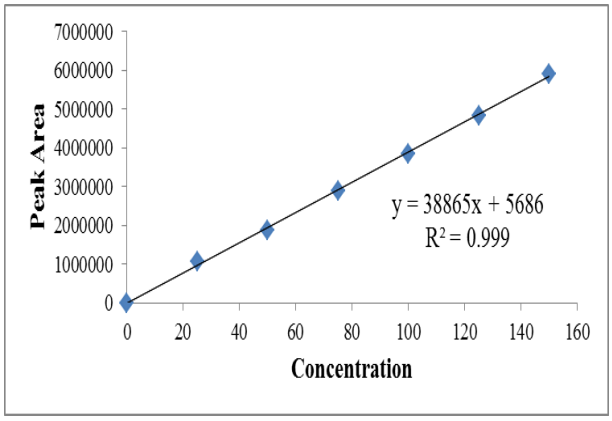

Figure 8: Linearity Chart of Dapagliflozin.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of Quantification (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively. The LOD and LOQ of Saxagliptin and Dapagliflozin were experimentally determined by injecting six injections of each drug. The LOD of Saxagliptin and Dapagliflozin was found to be 0.13 µg/ml and 0.09 µg/ml respectively. The LOQ of Saxagliptin and Dapagliflozin was found to be 0.39 µg/ml and 0.27 µg/ml respectively.

Robustness

Robustness of the method was verified by altering the chromatographic conditions like mobile phase composition, wave length detection, flow rate etc. A deviation of ± 2^0 c in the column temperature, ± 0.2 ml/min in the flow rate and changing the mobile phase ratio's were tried individually. A solution of 100% test concentration with the specified changes in the operational conditions was injected to the instrument in duplicate. %RSD was within the limits which is shown in table 6.

Table 6: Results of Robustness.

Analytes	Flow rate (ml)	%RSD	Column temperature (^{0}C)	%RSD	Mobile phase	%RSD
Saxagliptin	0.9	0.4	25	1.5	50:50	1.2
	1.1	1.4	35	1.6	60:40	1.2
Dapagliflozin	0.9	0.3	25	0.9	50:50	0.7
	1.1	0.6	35	0.3	60:40	0.9

Assay

Qtern, containing the label claim of Saxagliptin 5 mg and Dapagliflozin 10 mg. Assay was performed with the above formulation. The average %assay of Saxagliptin and Dapagliflozin was found to be 99.86% and 98.61% respectively. Results were shown in tables 7 and 8.

Table 7: Assay results of Saxagliptin.

S. No	Standard Area	Sample Area	% Assay
1	562345	566082	99.64
2	563569	568211	100.01
3	570952	565666	99.56
4	563717	566160	99.65
5	570038	568956	100.14
6	574840	568930	100.14
Mean	567577	567334	99.86
SD	5070.2	1528.1	03
RSD (%)	0.9	0.3	0.3

Table 8: Assay results of Dapagliflozin.

S. No	Standard Area	Sample Area	% Assay
1	3480960	3466522	98.56
2	3499039	3459964	98.38
3	3514338	3472564	98.73
4	3536343	3489250	99.21
5	3499800	3453943	98.20
6	3550902	3466186	98.55
Mean	3513564	3468072	98.61
SD	25988.7	12165.6	0.3
RSD (%)	0.7	0.4	0.4

Forced Degradation Studies

The assay method was used to test the drug stability by conducting forced degradation studies for the drug substances under various stress conditions. Stress degradation studies were carried out of acid hydrolysis (2N HCl heated for 30 min at 60^0C), alkali hydrolysis (2N NaOH heated for 30 min at 60^0C), thermal degradation (samples placed in an oven at 105^0C for 1 hr), neutral degradation (drug in water heated for 1 hr at 60^0C), oxidative degradation (20% H_2O_2 heated for 30 min at 60^0C) and for photolytic stress studies, samples were exposed to UV light by keeping them in a UV chamber for 1 day. The chromatograms were shown in Figures 9-14. Results were shown in table 9.

Table 9: Forced Degradation Studies of Saxagliptin And Dapagliflozin.

Analytes	Sample Name	Degradation (%)	Purity Angle	Purity Threshold
Saxagliptin	Acid	4.59	1.264	1.548
	Alkali	3.99	1.234	1.548
	Peroxide	3.04	1.205	1.531
	Thermal	2.45	1.170	1.542
	UV	1.40	1.272	1.534
	Neutral	0.69	1.374	1.690
Dapagliflozin	Acid	5.44	0.224	0.564
	Alkali	3.28	0.154	0.754
	Peroxide	2.36	0.562	0.753
	Thermal	2.06	0.379	0.864
	UV	1.27	0.531	0.852
	Neutral	0.20	0.293	0.672

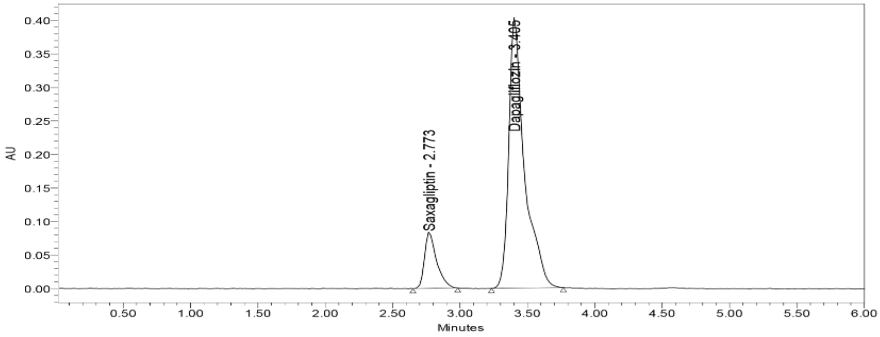

Figure 9: Chromatogram of Acid Degradation.

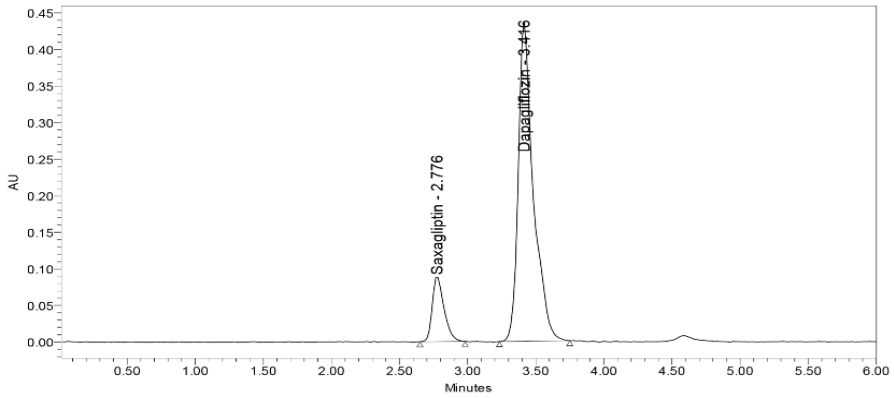

Figure 10: Chromatogram of Base Degradation.

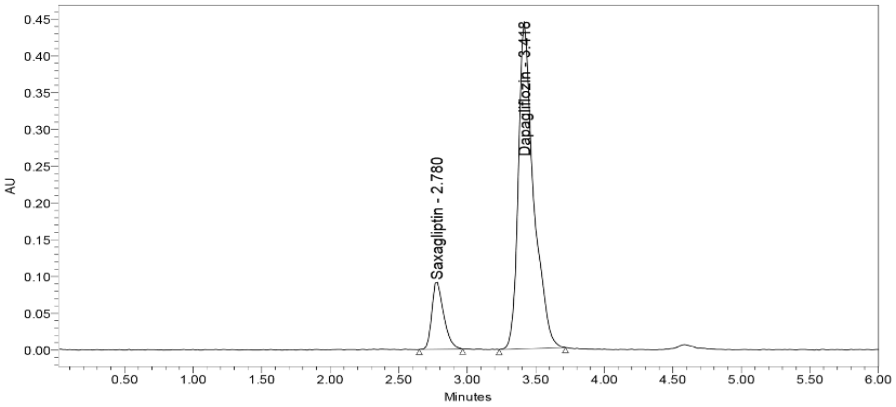

Figure 11: Chromatogram of Peroxide Degradation.

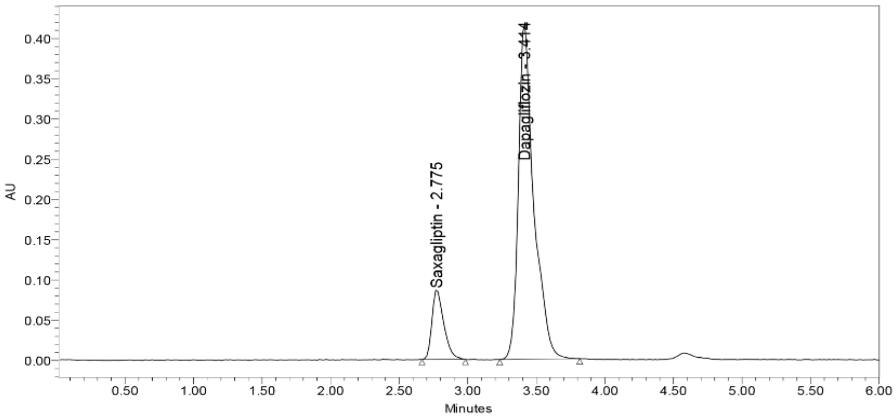

Figure 12: Chromatogram of Thermal Degradation.

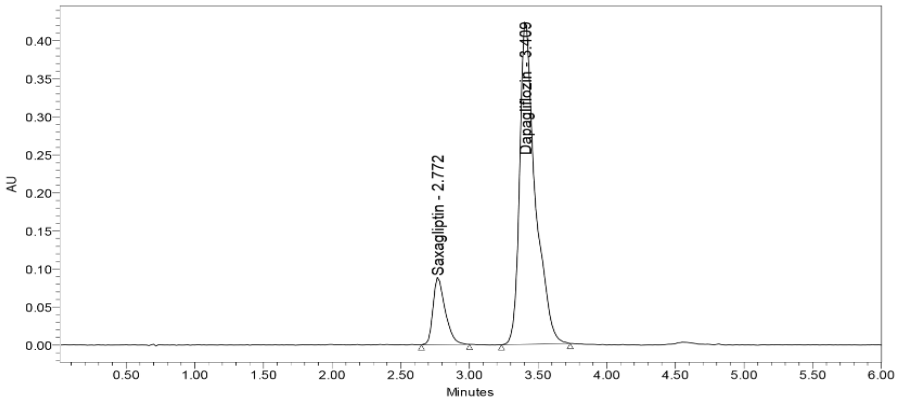

Figure 13: Chromatogram of UV Degradation.

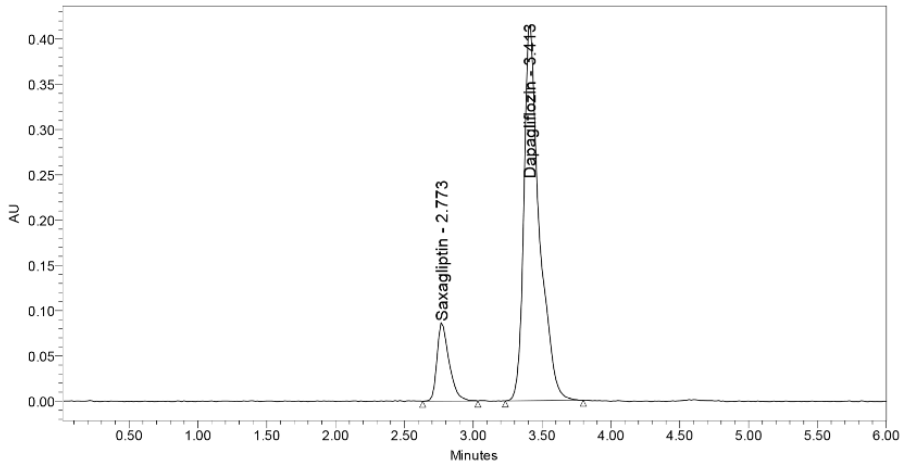

Figure 14: Chromatogram of Water Degradation.

CONCLUSION

The proposed stability indicating RP-HPLC method was found to be simple, accurate, precise, robust, rapid and economical. This method gives good resolution between two compounds with a short analysis time. Hence this method can be used in quality control departments with respect to routine analysis for the assay of the tablets containing Saxagliptin and Dapagliflozin.

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