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RP-HPLC Method Development and Validation for the Simultaneous Estimation of Dapagliflozin and Saxagliptin in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the Dapagliflozin and Saxagliptin in Tablet dosage form. Chromatogram was run through Std BDS 150 x 4.6 mm, 5µ. Mobile phase containing Buffer Perchloric acid: Acetonitrile taken in the ratio 50:50 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA. Temperature was maintained at 30°C. Optimized wavelength selected was 220 nm. Retention time of Dapagliflozin and Saxagliptin were found to be 2.266min and 2.805min. %RSD of the Dapagliflozin and Saxagliptin were and found to be 0.9 and 0.6 respectively. %Recovery was obtained as 99.72% and 99.60% for Dapagliflozin and Saxagliptin respectively. LOD, LOQ values obtained from regression equations of Dapagliflozin and Saxagliptin were 0.12, 0.36 and 0.02, 0.06 respectively. Regression equation of Dapagliflozin is y = 20173x + 18271, and y = 4124x + 2572 of Saxagliptin. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Dapagliflozin, Saxagliptin, RP-HPLC.

1. INTRODUCTION

Chemically Dapagliflozin is (2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol as shown in figure 1. Dapagliflozin inhibits subtype 2 of the sodium-glucose transport proteins (SGLT2), which is responsible for at least 90% of the glucose reabsorption in the kidney. Use of Dapagliflozin leads to blood glucose to be eliminated through the urine, which can lead to weight loss and tiredness [1-2]. Dapagliflozin was approved by FDA on 8th January 2014.

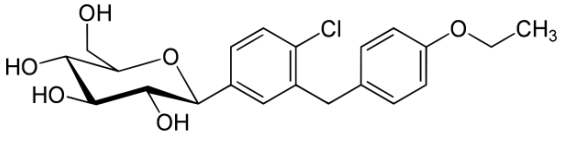

Figure-1: Chemical Structure of Dapagliflozin

While Saxagliptin is (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile as shown in figure 2. Saxagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor antidiabetic for the treatment of type 2 diabetes. DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones DPP-4 inhibitors are a class of compounds that work by affecting the action of natural hormones [3-4]. Saxagliptin HCl was approved by FDA on 31st July 2009.

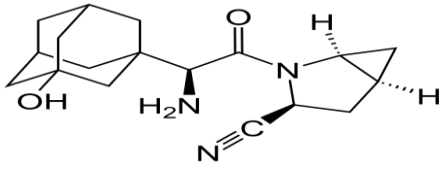

Figure-2: Chemical Structure of Saxagliptin

Both the drugs either alone or in combination therapy are used to treat type 2 Diabetes mellitus. Literature survey revealed estimation of Dapagliflozin and Saxagliptin HCl by HPLC method either in alone or in combination with other drugs [5-10]. Literature survey revealed that there were no any official or reported methods available for the estimation of both the drugs in combination.

The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for simultaneous estimation of Dapagliflozin, Saxagliptin in bulk ant tablet dosage form.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Dapagliflozin and Saxagliptin pure drugs (API), Combination Dapagliflozin and Saxagliptin tablets (QTERN), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem- Mumbai.

2.2 Instruments and Chromatographic Conditions

Electronics Balance-Denver, P^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC Aquity system equipped with quaternary pumps, UV detector and Auto sampler integrated with Empower 2 Software was used for LC peak integration and Data processing. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV-win 6 Software was used for measuring absorbance of Dapagliflozin and Saxagliptin solutions. The mobile phase used was 50% Ortho phosphoric acid:50% Acetonitrile at a flow rate of 1ml/min. samples were analyzed at 220 nm detector wave length and at an injection volume of 10 µL using BDS C18 4.6 x 150mm, 5µm.with run time of 6 min.

2.3 Preparation of Solvents and Solutions

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of buffer:

0.1% OPA Buffer: 1ml of Conc Ortho Phosphoric acid was diluted to 1000ml with water.

Preparation of Standard stock solutions: Accurately weighed 10 mg of Dapagliflozin, 5 mg of Saxagliptin and transferred to individual 10 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (1000µg/ml of Dapagliflozin and 500µg/ml of Saxagliptin)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml Dapagliflozin of and 50µg/ml of Saxagliptin)

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, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000µg/ml of Dapagliflozin and 500µg/ml of Saxagliptin)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of Dapagliflozin and 50µg/ml of Saxagliptin)

2.4 Method Validation

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

2.4.1 Specificity: It is the ability of analytical method to measure the response of the analyte and have no interference from other extraneous components and well resolved peaks are obtained.

2.4.2 Linearity:

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Dapagliflozin and 12.5 µg/ml of Saxagliptin)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (50µg/ml of Dapagliflozin and 25µg/ml of Saxagliptin)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Dapagliflozin and 37.5µg/ml of Saxagliptin)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (100µg/ml of Dapagliflozin and 50µg/ml of Saxagliptin)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (125µg/ml of Dapagliflozin and 62.5µg/ml of Saxagliptin)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (150µg/ml of Dapagliflozin and 75µg/ml of Saxagliptin)

2.4.3 Accuracy:

Preparation of Standard stock solutions: Accurately weighed 10 mg of Dapagliflozin, 5 mg of Saxagliptin and transferred to individual 10 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (1000µg/ml of Dapagliflozin and 500µg/ml of Saxagliptin)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask,

to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

2.4.4 Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

2.4.5 LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Dapagliflozin, Saxagliptin, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

2.4.6 LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Dapagliflozin, Saxagliptin, and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

2.4.7 System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Dapagliflozin (100ppm) and Saxagliptin (50ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined to check whether the results complies with Recommended limits.

2.5 Assay of Dapagliflozin and Saxagliptin

An Accurately measured weight equivalent to (Qtern) 10 mg and 5mg of Dapagliflozin and Saxagliptin respectively was used to perform assay by utilizing the method developed and under the optimized chromatographic conditions. Sample solutions were injected in to the HPLC system and scanned at 220 nm from which the % of drug was estimated.

3. RESULTS AND DISCUSSION

3.1 Optimization of Chromatographic Conditions:

To develop and establish a suitable RP-HPLC method for simultaneous estimation of Dapagliflozin and Saxagliptin in bulk and Tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 50% Ortho phosphoric acid:50% Acetonitrile at a flow rate of 1ml/min. samples were analyzed at 220 nm detector wave length and at an injection volume of 10 µL using BDS C18 4.6 x 150mm, 5µm.with run time of 6 min. The proposed method was optimized to give sharp peak with good resolution and minimum tailing effect for Dapagliflozin and Saxagliptin, the optimized chromatogram was obtained as shown in (Figure-3).

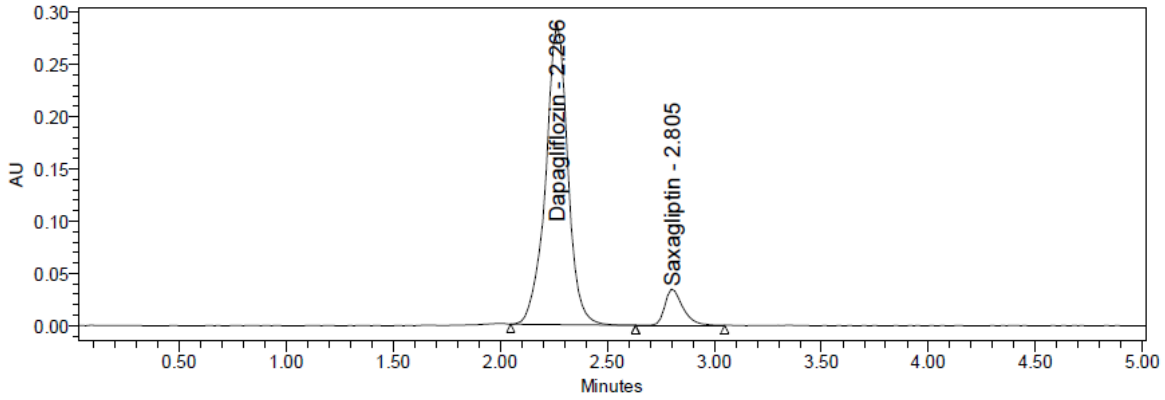

Figure-3: Optimized Chromatogram of Dapagliflozin and Saxagliptin

Table-1: Optimized Chromatographic Conditions

Parameter	Condition
RP-HPLC	WATERS HPLC SYSTEM equipped with quaternary pumps with PDA detector
Mobile phase	50% OPA (0.1%) : 50% Acetonitrile
Flow rate	1ml/min
Column	BDS C18 (4.6 x 150mm, 5µm)
Detector wave length	220nm
Column temperature	30°C
Injection volume	10µL
Run time	6 min
Diluent	Water and Acetonitrile in the ratio 50:50
Retention Time	Dapagliflozin 2.266 min and Saxagliptin 2.805min
Theoretical Plates	Dapagliflozin 5717 and Saxagliptin 2612

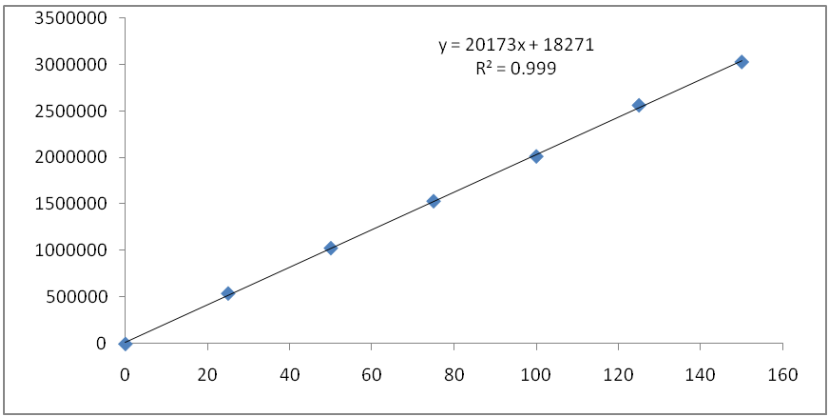

Figure-4: Linearity Curve of Dapagliflozin

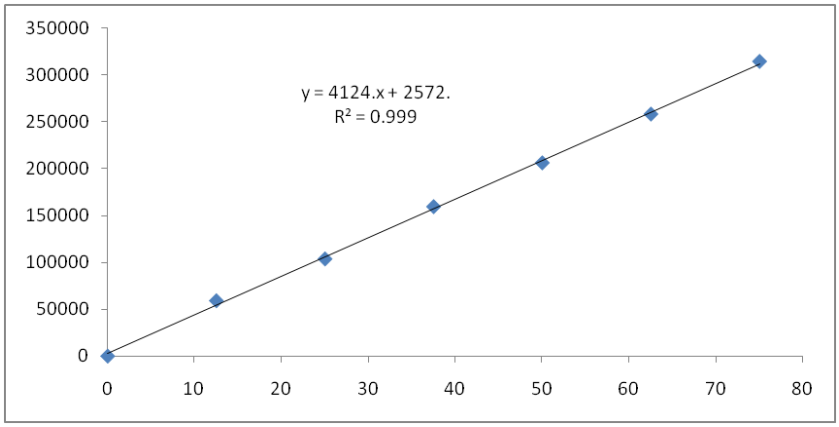

Figure-5: Calibration Curve of Saxagliptin

3.2 Validation

Linearity was established for Dapagliflozin (25-150µg/ml) and Saxagliptin (12.5-75 µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as y = 20173x + 18271for Dapagliflozin and y = 4124x + 2572 for Saxagliptin, Correlation coefficient (R^2) was determined as 0.999 for the two drugs. The Linearity calibration curves were plotted as shown in (Figure-4&5) for Dapagliflozin and Saxagliptin, respectively. Retention times of Dapagliflozin and Saxagliptin were 2.266min and 2.805min min respectively. Where no interfering peaks in blank and placebo at retention times of these drugs were not found in this method. So this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared and Triplicates of injections were given for each level of accuracy and mean %Recovery was obtained as 99.72% and 99.60% for Dapagliflozin and Saxagliptin respectively were shown in (Table-2).% RSD was calculated from the corresponding peaks obtained by injecting six times a known concentration of Dapagliflozin and Saxagliptin the repeatability was obtained as 0.9% and 0.6% respectively for Dapagliflozin and Saxagliptin and Low % RSD values indicates that the method developed was precise as shown in (Table-3). The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Dapagliflozin and Saxagliptin. The detection limit values were obtained as 0.12 and 0.02 and Quantitation limit were fund to be 0.36 and 0.06 for Dapagliflozin and Saxagliptin Respectively as given in (Table-4).

Table-2: Accuracy results of Dapagliflozin and Saxagliptin

Conc.	Dapagliflozin			Saxagliptin		
	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery
50%	50	49.75	99.51	50%	25	25.06
	50	50.25	100.50		25	24.89
	50	49.94	99.88		25	24.75
100%	100	99.95	99.95	100%	50	49.08
	100	99.25	99.25		50	49.40
	100	99.47	99.47		50	49.08
150%	150	150.69	100.46	150%	75	75.54
	150	149.87	99.91		75	75.80
	150	147.87	98.58		75	75.50
Mean % Recovery			**99.72%**	**Mean % Recovery**		**99.60%**

Table-3: Precision Results of Dapagliflozin and Saxagliptin

S.No	Repeatability		Intermediate precision	
	Area of Dapagliflozin	Area of Saxagliptin	Area of Dapagliflozin	Area of Saxagliptin
1.	2034898	201096	2010157	206036
2.	2018179	201429	2005117	206070
3.	2023139	202992	2033139	201432
4.	2000187	201587	2050187	201587
5.	2008785	200262	2008785	202105
6.	2048445	203566	2048445	201801
Mean	2022272	201822	2025972	203172
S.D	17502.0	1231.3	20606.8	2243.2
%RSD	0.9	0.6	1.0	1.1

Table-4: LOD and LOQ values of Dapagliflozin and Saxagliptin

Molecule	LOD	LOQ
Dapagliflozin	0.12	0.36
Saxagliptin	0.02	0.06

Table-5 Robustness Data of Dapagliflozin and Saxagliptin

S.No.	Condition	%RSD of Dapagliflozin	%RSD of Saxagliptin
1	Flow rate (-) 0.9ml/min	1.2	1.4
2	Flow rate (+) 1.1 ml/min	1.3	1.1
3	Mobile phase (-) 60B:40A	1.0	1.0
4	Mobile phase (+) 50B:50A	1.1	0.4
5	Temperature (-) 25°C	0.5	0.7
6	Temperature (+) 35°C	0.8	0.8

Table-6: System Suitability Parameters Results of Dapagliflozin and Saxagliptin

S No	Dapagliflozin			Saxagliptin			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.802	5623	1.33	2.257	2612	0.98	3.2
2	2.803	5703	1.33	2.261	2701	0.96	3.1
3	2.803	5261	1.37	2.266	2708	0.96	3.0
4	2.804	4997	1.39	2.270	2570	0.95	3.0
5	2.804	5232	1.32	2.272	2506	1.03	3.0
6	2.805	5717	1.37	2.275	2477	1.00	3.1

Table-7: Assay results of Dapagliflozin and Saxagliptin

S.No	Dapagliflozin			Saxagliptin		
	Standard Area	Sample area	% of Drug	Standard Area	Sample area	%of Drug
1.	2004545	2034898	100.02	202059	201096	99.00
2.	2026029	2018179	99.20	203810	201429	99.17
3.	2045009	2023139	99.45	201711	202992	99.94
4.	2037805	2000187	98.32	204921	201587	99.24
5.	2058268	2008785	98.74	201655	200262	98.59
6.	2022506	2048445	100.69	203366	203566	100.22
Mean	2032360	2022272	99.40	202920	201822	99.36
S.D	18824.1	17502.0	0.86	1326.5	1231.3	0.61
%RSD	0.9	0.9	0.87	0.7	0.6	0.61

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (65:35) mobile phase plus (55:45) temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner Table -5). System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table -6). Dapagliflozin and Saxagliptin pure drugs (API) were obtained from spectrum Pharma research solutions and AstraZeneca pharmaceuticals (Qtern), bearing the label claim Dapagliflozin 10mg, Saxagliptin 5mg. Assay was performed with the above formulation. Average % Assay for Dapagliflozin and Saxagliptin obtained was 99.40% and 99.36% respectively the results were shown in (Table-7) and the chromatograms for Dapagliflozin and Saxagliptin standard drugs and pharmaceutical dosage forms were shown in (Figure-6, 7) respectively.

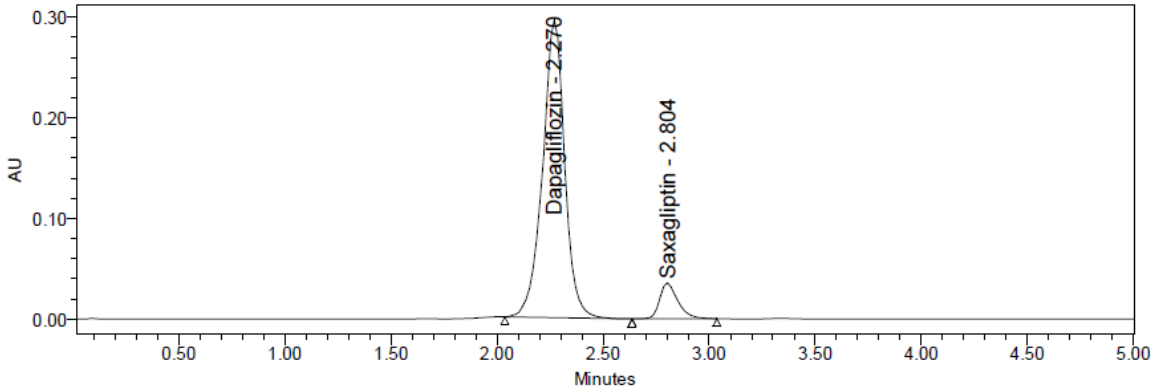

Figure-6: Standard Chromatogram of Dapagliflozin and Saxagliptin

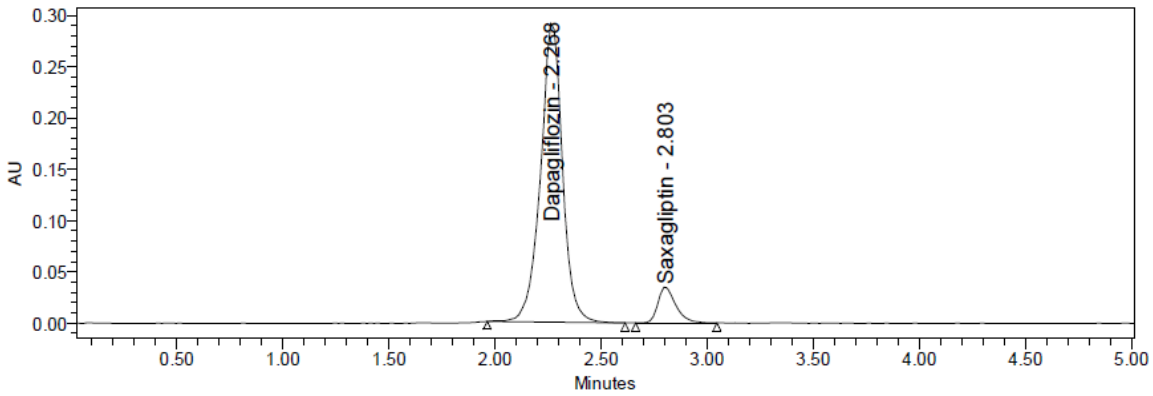

Figure-7: A Sample Chromatogram of Dapagliflozin and Saxagliptin in Pharmaceutical Dosage Form

3.3 Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation (Table 8).

Table 8: Degradation Data of Dapagliflozin & Saxagliptin

S.No	Degradation Condition	%Drug Degraded	
		Dapagliflozin	Saxagliptin
1	Acid	4.54	4.89
2	Alkali	2.52	2.80
3	Oxidation	1.61	1.82
4	Thermal	0.95	0.78
5	UV	0.83	0.76
6	Water	0.97	0.56

4. CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Dapagliflozin and Saxagliptin in Tablet dosage form. Retention time of Dapagliflozin and Saxagliptin were found to be 2.266min and 2.805min. %RSD of the Dapagliflozin and Saxagliptin were and found to be 0.9 and 0.6 respectively. %Recovery was obtained as 99.72% and 99.60% for Dapagliflozin and Saxagliptin respectively. LOD, LOQ values obtained from regression equations of Dapagliflozin and Saxagliptin were 0.12, 0.36 and 0.02, 0.06 respectively. Regression equation of Dapagliflozin is y = 20173x + 18271, and y = 4124x + 2572 of Saxagliptin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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