RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF SAXAGLIPTIN & DAPAGLIFLOZIN IN PHARMACEUTICAL DOSAGE FORM

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Abstract : A new, simple and accurate, precise RP-HPLC method was developed for simultaneous determination of Dapagliflozin and Saxagliptinin bulk and in pharmaceutical dosage form. The separation of Dapagliflozin and Saxagliptin was achieved within 8 minutes on Symmetry C18 150mm X 4.6mm and 5µm Particle Size, Make Waters column using Methanol: Water (75:25v/v) as the mobile phase. Detection was carried out using wavelength at 270nm. Retention time of Dapagliflozin and Saxagliptinwas found to be 2.029 and 3.290min, respectively. The validation of the developed method was performed in terms of accuracy, precision, linearity, limit of detection, limit of quantification as mentioned in International Conference on Harmonization (ICH) guidelines. The method showed adequate sensitivity concerning linearity, accuracy and precision over the range 50-150µg/ml and 25-75µg/ml for Dapagliflozin and Saxagliptin, respectively. The percentage recoveries obtained for Dapagliflozin and Saxagliptin were found to be in range of 98.00 – 102.00 %. The proposed method is hence suitable for use in quality-control laboratories for quantitative analysis of both the drugs bulk and in combination, since it is simple and fast with good accuracy and precision.

Key Words: Dapagliflozin and Saxagliptin, RP-HPLC, Accuracy, Precision.

I. INTRODUCTION

Dapagliflozin is an antihyperglycemic agent which selectively inhibitsodium-glucose co-transporter subtype 2 (SGLT2). It is potently inhibits SGLT2 compared to SGLT1, which is the cotransporter of glucose in the gut. Dapagliflozin is a C-glycosyl comprising beta-D-glucose in which the anomeric hydroxy group is replaced by a 4-chloro-3-(4-ethoxybenzyl) phenyl group. Used (in the form of its propanediol monohydrate) to improve glycemic control, along with diet and exercise, in adults with type 2 diabetes. SGLT2 facilitates 90% of renal glucose resorption and hence its inhibition allows for glucose to be excreted via urine1. This excretion allows for better glycemic control and potentially weight loss in patients with type 2 diabetes mellitus1.

Saxagliptin is a cyanopyrrolidine-based potent, selective and competitive inhibitor of dipeptidyl peptidase 4 (DPP-4), with hypoglycemic activity. DPP-4 inhibitors affect the action of natural hormones in the body called incretins. Incretins decrease blood sugar level by increasing utilization of sugar by the body, through increasing insulin production in the pancreas, and by reducing production of sugar by the liver. DPP-4 has two mode of action, an enzymatic function and another mechanism where DPP-4 binds adenosine deaminase, which conveys intracellular signals via dimerization when activated. The Chemical Structures of Saxagliptin and Dapagliflozin were follows:

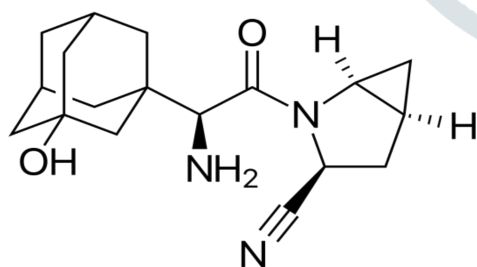

Fig-1: Structure of Saxagliptin

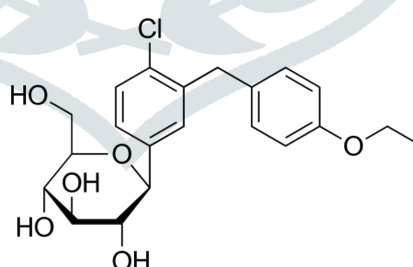

Fig-2: Structure of Dapagliflozin

It was observed that there is no method available for quantitative simultaneous estimation of Dapagliflozin and Saxagliptinin bulk and pharmaceutical dosage forms by RP-HPLC using PDA detector, hence the present work was undertaken to develop and validate a simple, accurate and economical method by RP-HPLC using PDA detector which can be used for routine analysis in quality control and research laboratory for assay of Dapagliflozin and Saxagliptin in bulk and pharmaceutical dosage forms.

II. MATERIAL AND METHODS

Chemicals and Reagents: Both Dapagliflozin and Saxagliptinstandard &API were obtained as a gift sample from Sura Pharma Labs, Hyderabad, India. The marketed formulation in the brand name Qtern (Dapagliflozin-10mg & Saxagliptin-5 mg) procured from the local pharmacy. All the chemicals and reagents used in this work were HPLC grade water, Acetonitrile, phosphate buffer, methanol, potassium dihydrogen orthophosphate bufferand orthophosphoric acid was obtained from Rankem.

Instrumentation: A HPLC system with waters 2695 separation module provided with a photodiode array detector, auto sampler injection with Empower-2 software. Electronic balance, Ultra Sonicator, Hot air oven was used.

Chromatographic Conditions: The chromatographic separations achieved on a Symmetry C18 150mm X 4.6mm and 5µm Particle Size, Make Waters as a stationary phase. The mobile phase was composed of Methanol: Water (75:25% v/v)at a flow rate

of 0.9mL/minute and injection volume is 10µL. The column oven temperature was maintained at Ambient, and the drugs were detected at 270 nm.

Preparation of Mobile Phase: Mobile phase was prepared by mixing 750mL of Methanol and HPLC Grade water in the ratio of 75:25 v/v. The mobile phasewas sonicated for 15 min. and filtered through a 0.45µm membrane filter.

Preparation of Diluent: A mixture of Methanol and HPLC Grade water are taken in the ratio of 75:25 v/v was used as a diluent.

Preparation of Standard Stock Solution: Accurately weighed and transferred 100 mg of Dapagliflozin and 50 mg of Saxagliptin working standard into a 100mL of clean &dry volumetric flasks,added about 70 mL of diluent and sonicated to dissolve and made volume up to the mark with diluent.

Preparation of Standard Solution: Further pipetted 1mL of the Dapagliflozin and Saxagliptinstandard stock solution into a 10mL volumetric flask and diluted up to the mark with diluent (100 ppm of Dapagliflozin and 50 ppm of Saxagliptin).

Preparation of samplesolution:10 tablets were accurately weighed and crushed to a fine powder. A portion of tablet powder equivalent to 100mg of Dapagliflozin and 50 mg Saxagliptin were weighed accurately and transferred into a 100 mL volumetric flask. Added about 70 mL of diluent and sonicated for 30 minutes and made up to volume with diluent. The solution was filtered through 0.45µm PVDF filter (Sample stock solution).

Further pipetted 1mL of the Dapagliflozin and Saxagliptin stock solutions into a 10mL volumetric flask and diluted up to the mark with diluent (100 ppm of Dapagliflozin and 50 ppm of Saxagliptin).

Validation of the RP-HPLC Method: The developed RP-HPLC method was validated as per ICH guidelines.

System Suitability Parameters: The system suitability parameters were determined by preparing standard solutions of Dapagliflozin (100µg/mL) and Saxagliptin (50µg/mL), and the solutions were injected six times and the parameters like retention time, peak tailing, resolution and USP plate count were determined.

Specificity: As per ICH guidelines "Specificity" can be defined as the ability of the method to specifically separate the particular API or analyte in the presence of other components.

Linearity: The stock solution of Dapagliflozin and Saxagliptin was prepared using diluents. From it, various working standard solutions were prepared in the range of 50-150µg/mL, 25-75µg/mL and injected into the HPLC system. The calibration plot (peak area vs. concentration) was generated by replicate analysis (n=3) at all concentration levels. The linearity of the method evaluated using the least square method within Microsoft excel program.

Accuracy: The accuracy method was carried out using one set of different standard addition methods at different concentration levels 50%, 100% and 150% and then comparing the theoretical value and found value.

Precision: The precision of the method was ascertained from the peak area obtained by actual determination of six replicates ofsample of the drug (100µg/mL Dapagliflozin, 50µg/mL Saxagliptin). The precision of the assay also determined in terms of intraday and interday. The peak area of a set of sample solutions was calculated in terms of relative standard deviation (% RSD).

Robustness: The Robustness of the proposed method carried out by small but deliberate changes in method parameters such as flow rate (±0.1), Mobile Phase organic phase ratio (±5%). System suitability parameters were evaluated.

Forced Degradation Tests: The specificity of the method was demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions and the main peak of the drug was studied for peak purity thatindicating the method effectively separated the degradation products from the pure active ingredient.

Degradation by acidic condition:

Further pipetted 1mL of the Dapagliflozin and Saxagliptin sample stock solutions into a 10mL volumetric flask. Added1 mL of 2N HCL solution & refluxed for 30 minutes at 60 ºC. The resultant solution was neutralized with 1 mL of 2N NaOH and diluted up to the mark with diluent.Finally, sample solution was filtered through 0.45-micron PVDF syringe filter. (100 ppm of Dapagliflozin and 50 ppm of Saxagliptin).

Degradation by alkaline condition:

Further pipetted 1mL of the Dapagliflozin and Saxagliptin sample stock solutions into a 10mL volumetric flask. Added1 mL of 2N NaOH solution & refluxed for 30 minutes at 60 ºC. The resultant solution was neutralized with 1 mL of 2N HCL and diluted up to the mark with diluent.Finally, sample solution was filtered through 0.45-micron PVDF syringe filter. (100 ppm of Dapagliflozin and 50 ppm of Saxagliptin).

Oxidative degradation:

Further pipetted 1mL of the Dapagliflozin and Saxagliptin sample stock solutions into a 10mL volumetric flask. Added1 mL of 3% H_2O_2solution & refluxed for 15 minutes at 60 ºCand diluted up to the mark with diluent.Finally, sample solution was filtered through 0.45-micron PVDF syringe filter. (100 ppm of Dapagliflozin and 50 ppm of Saxagliptin).

Thermal degradation:

The Dapagliflozin and Saxagliptin sample was taken in petridish and kept in Hot air oven at 110^0 C for 24 hours.

Photolytic degradation:

The photo stability of the drug was studied by exposing the stock solution to UV light for 200 Watt-hours/m^2 in photo stability chamber.

Further pipetted 1mL of the Dapagliflozin and Saxagliptin stock solutions into a 10mL volumetric flask and diluted up to the mark with diluent (100 ppm of Dapagliflozin and 50 ppm of Saxagliptin).

Water Degradation (Hydrolysis):

Further pipetted 1mL of the Dapagliflozin and Saxagliptin sample stock solutions into a 10mL volumetric flask. Added1 mL of water & refluxed for 30 minutes at 60 ºCand diluted up to the mark with diluent.Finally, sample solution was filtered through 0.45-micron PVDF syringe filter. (100 ppm of Dapagliflozin and 50 ppm of Saxagliptin).

III. RESULT AND DISCUSSION

Method Validation:

Validation of analytical method is a process to establish that the performance characteristics of the developed method meet the requirement of the standard analytical application.

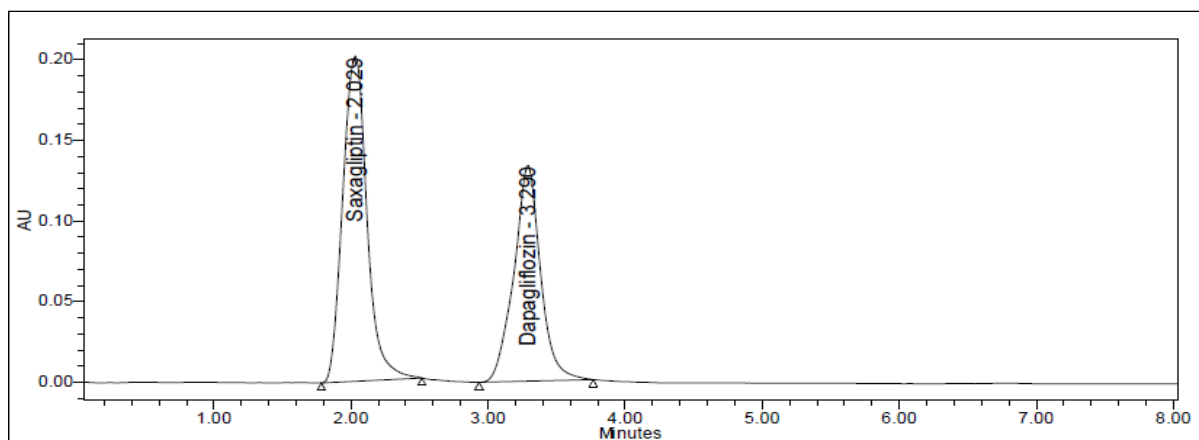

Figure 3: Chromatogram of standard solution

Table 1: Results of system suitability from standard solution injection

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Saxagliptin	2.029	2465189	201933	NA	1.14	3258
2	Dapagliflozin	3.290	1800616	133805	3.66	1.00	4267

Specificity: Method validation was performed according to ICH Q2 guidelines. In the blank chromatogram, there were no peaks observed at the retention times of Dapagliflozin and Saxagliptin.

System Suitability: System suitability was performed to evaluate the parameters like tailing factor, theoretical plates, resolution and % RSD for replicate injections. The results were within limits and were given in Table 2.

Table2: Results of system suitability from standard solution injections for Saxagliptin

S.No.	Name	Rt	Peak Area	Height	USP plate Count	USP Tailing
1	Saxagliptin	2.029	2465189	201933	3286	1.14
2	Saxagliptin	2.032	2458656	202495	3258	1.13
3	Saxagliptin	2.032	2458656	202495	3259	1.13
4	Saxagliptin	2.029	2465189	201933	3325	1.14
5	Saxagliptin	2.029	2465189	201933	3298	1.14
6	Saxagliptin	2.032	2454789	204176	3325	1.13
Mean			**2461278**			
Std. Dev			**4510.97**			
% RSD			**0.18**			

Table3: Results of system suitability from standard solution injections for Dapagliflozin

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Dapagliflozin	3.290	1800616	133805	4275	1.00	3.66
2	Dapagliflozin	3.291	1798469	134987	4236	1.00	3.70
3	Dapagliflozin	3.291	1798469	134987	4287	1.00	3.70
4	Dapagliflozin	3.290	1800616	133805	4312	1.01	3.66
5	Dapagliflozin	3.290	1800616	133805	4299	1.00	3.66
6	Dapagliflozin	3.294	1798535	133830	4315	0.99	3.71
Mean			**1799554**				
Std. Dev			**1164.16**				
% RSD			**0.064**				

Specificity: Retention times of Dapagliflozin and Saxagliptin were 2.029 min and 3.290 min for standard and 2.032 min and 3.294 min for sample respectively.

We did not found any interfering peaks in blank at retention times of these drugs in this method. Hence this method was said to be specific.

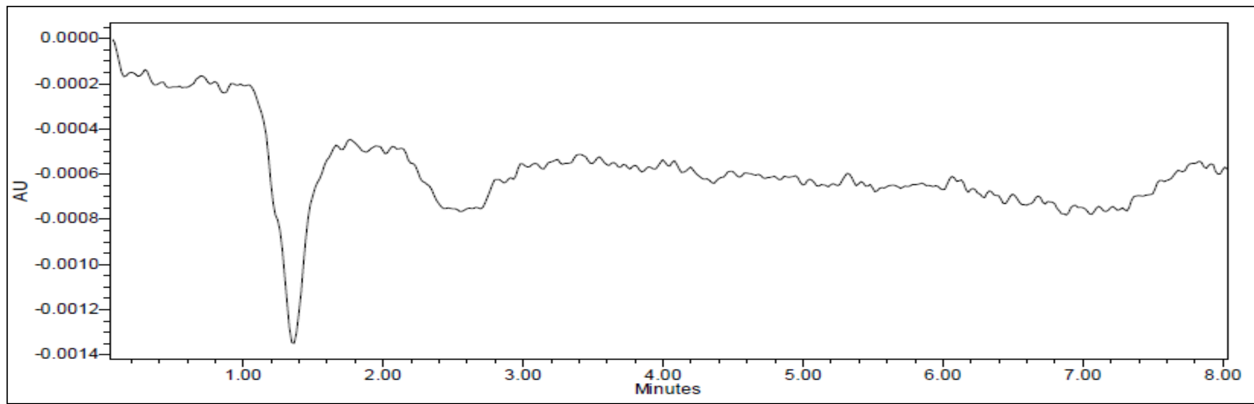

Figure 4: Chromatogram of blank (diluent)

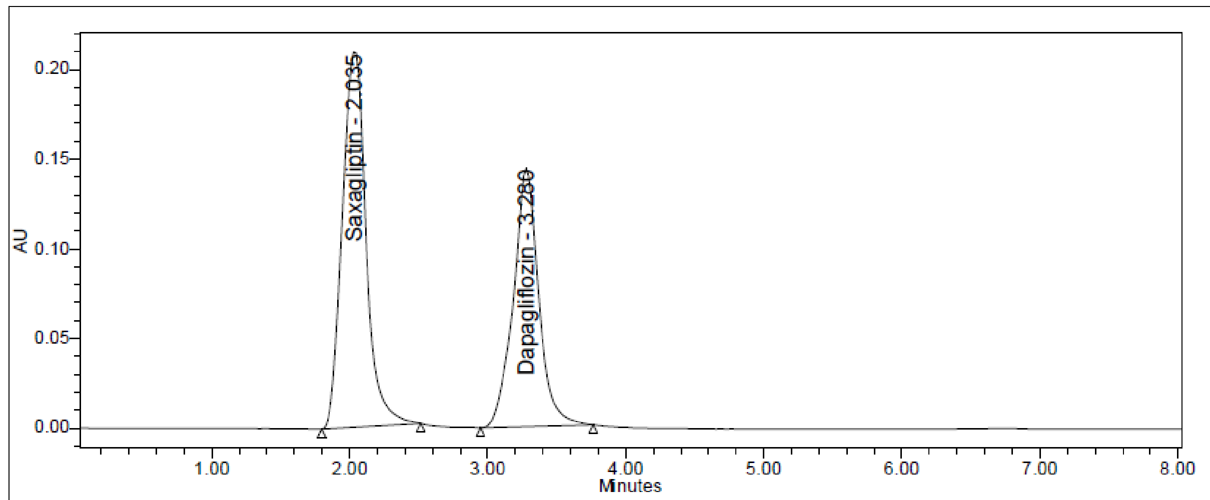

Figure 5: Chromatogram of system suitability standard solution (1st Injection)

Linearity: The linearity of the measurement was evaluated by analyzing different concentrations (50% to 150%) of the standard solutions of Dapagliflozin and Saxagliptin. The calibration curve was constructed by plotting concentrationagainst mean peak area, and the regression equation was computed. The coefficient of correlation (R2) for Dapagliflozin and Saxagliptin were found to be 0.998 and 0.999 respectively. The summary of the parameters is given in Table 4 and 5 and shown in Fig. 6, 7.

Table 4: Linearity results for Saxagliptin:

S.No.	Linearity Level	Standard stock solution taken (mL)	Diluted volume up to mark (mL)	Concentration (ppm)	Area
1	50%	5.0	100	25.0	938169
2	75%	7.5	100	37.5	1416504
3	100%	10.0	100	50.0	1854151
4	125%	12.5	100	62.5	2292866
5	150%	15.0	100	75.0	2723570
Correlation Coefficient					0.999

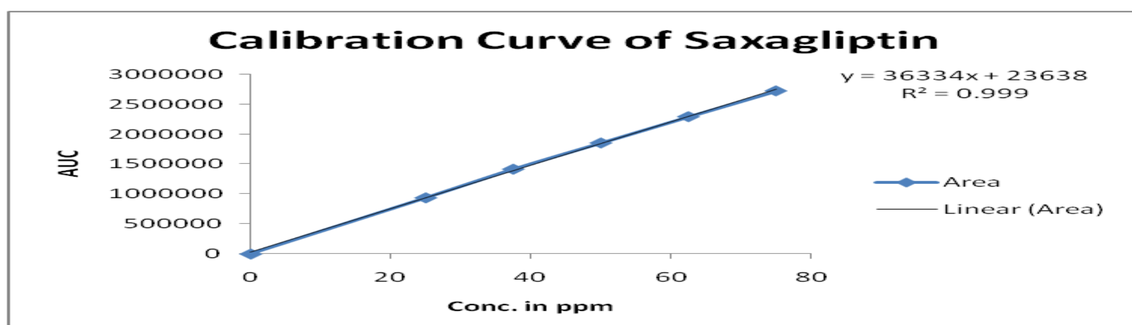

Fig.6: Calibration Graph for Saxagliptin

Table 5: Linearity results for Dapagliflozin:

S.No.	Linearity Level	Standard stock solution taken (mL)	Diluted volume up to mark (mL)	Concentration (ppm)	Area
1	50%	5.0	100	50	1220786
2	75%	7.5	100	75	1797031
3	100%	10.0	100	100	2318334
4	125%	12.5	100	125	2862857
5	150%	15.0	100	150	3398694
Correlation Coefficient					0.998

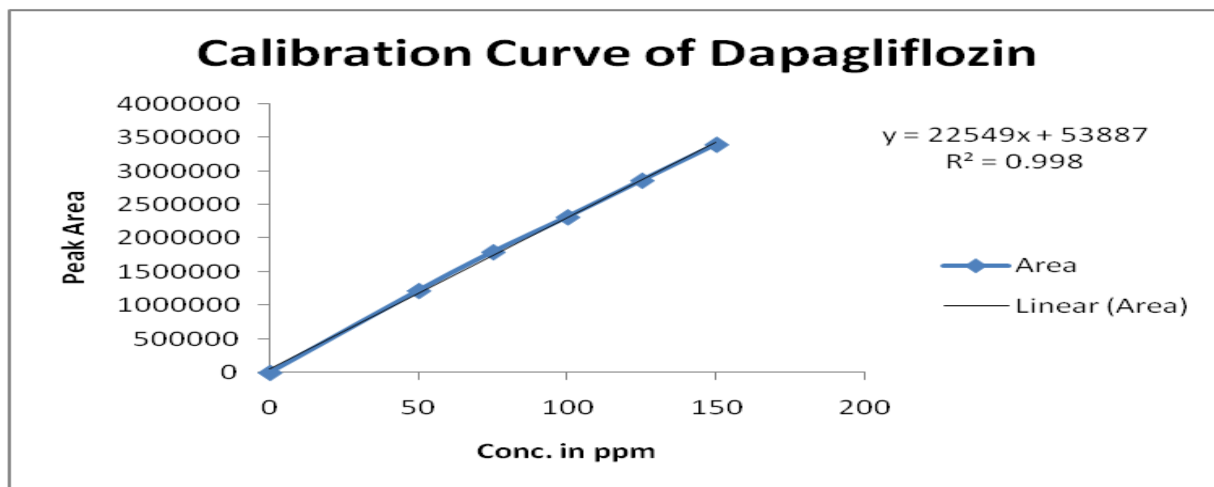

Fig.7: Calibration Graph for Dapagliflozin

Accuracy: To determine the accuracy of the proposed method, recovery studies were conducted at three different levels 50 %, 100 % and 150% andwere calculated. Accuracy was calculated as the percentage of recovery, and the results were shown in Table 6 and 7.

Table6: The Accuracy Results for Saxagliptin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1184152	50	50.124	100.248	100.149%
100%	2314820	100	100.267	100.267	
150%	3434041	150	149.902	99.934	

Table7: The accuracy results for Dapagliflozin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	929664	25	24.924	99.696	99.900%
100%	1840767	50	49.956	99.912	
150%	2754891	75	75.071	100.094	

Precision: Precision was carried out in terms of system precision, repeatability, and intermediate accuracy. These are assessed by using six replicates at a concentration of 100µg/mL of Dapagliflozin and 50µg/mL of Saxagliptin. The data was given in Table 8,9,10&11. The % RSD was found to be <2, indicating the repeatability of the method.

Method Precision:

Table8: Results of Repeatability (Method precision) for Saxagliptin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Saxagliptin	2.032	2492676	208462	3265	1.15
2	Saxagliptin	2.034	2483562	209318	3246	1.16
3	Saxagliptin	2.034	2483562	209318	3325	1.15
4	Saxagliptin	2.032	2460183	209311	3266	1.12
5	Saxagliptin	2.032	2475230	205903	3256	1.15
6	Saxagliptin	2.032	2475230	205903	3327	1.15
Mean			**2478407**			
Std. Dev			**11036.79**			
% RSD			**0.44**			

Table9: Results of Repeatability (Method precision) for Dapagliflozin

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Dapagliflozin	3.283	1811283	139108	4265	1.01	3.79
2	Dapagliflozin	3.286	1798838	138689	4259	1.01	3.82
3	Dapagliflozin	3.286	1798838	138689	4265	1.04	3.82
4	Dapagliflozin	3.285	1797891	138999	4326	1.01	3.84
5	Dapagliflozin	3.289	1791547	136101	4258	1.01	3.74
6	Dapagliflozin	3.289	1796598	136101	4258	1.01	3.74
Avg			**1799166**				
Std. Dev			**6531.55**				
% RSD			**0.36**				

Intermediate Precision:

Table 10: Results of Repeatability (Intermediate Method precision) for Saxagliptin:

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Saxagliptin	2.032	2512327	218674	4856	1.18
2	Saxagliptin	2.032	2525468	218965	4986	1.19
3	Saxagliptin	2.029	2547845	219876	4875	1.18
4	Saxagliptin	2.029	2539853	218654	4986	1.17
5	Saxagliptin	2.029	2543543	219865	4857	1.18
6	Saxagliptin	2.029	2525978	214787	4962	1.19
Mean			**2532502**			
Std. Dev			**13493.84**			
% RSD			**0.53**			

Table 11: Results of Repeatability (Intermediate Method precision) for Dapagliflozin:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Dapagliflozin	3.262	1861553	155797	4965	1.16	4.07
2	Dapagliflozin	3.260	1876592	158659	4875	1.17	4.06
3	Dapagliflozin	3.260	1865985	158748	4869	1.16	4.07
4	Dapagliflozin	3.260	1865748	156985	4758	1.18	4.08
5	Dapagliflozin	3.259	1848547	156254	4875	1.19	4.07
6	Dapagliflozin	3.259	1854786	157487	4698	1.16	4.08
Mean			**1862202**				
Std. Dev			**9755.35**				
% RSD			**0.52**				

Robustness: The robustness of the method was evaluated by the method conditions such as, flow rate (± 0.1) and solvent composition (± 5%) were altered, and the influence of these changes on peak tailing, number of theoretical plates and peak area were evaluated. The results were shown in Table 12& 13.

Table-14: Results for Robustness for Saxagliptin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9 mL/min	2465189	2.029	3258	1.14
Less Flow rate of 0.8 mL/min	3251476	2.510	3568	1.24
More Flow rate of 1.0 mL/min	2189585	1.700	3658	1.17
Less organic phase	2621559	2.031	3856	1.14
More organic phase	2525923	2.035	4168	1.14

Table-15: Results for Robustness for Dapagliflozin:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9 mL/min	1800616	3.290	4267	1.00
Less Flow rate of 0.8 mL/min	2452484	4.023	4859	1.27
More Flow rate of 1.0 mL/min	1662127	2.721	4965	1.02
Less organic phase	1971102	3.374	4896	1.00
More organic phase	1869626	3.155	4759	1.27

Degradation Studies: Since no interference of blank and degradants, the HPLC results showed that the active ingredients Dapagliflozin and Saxagliptin purity angle was less than the purity threshold and hence the proposed method was the specific and revealed its stability-indicating nature. The results were summarized in Table 16.

Fig-16: Results of Forced Degradation Studies

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	2465189	0	100%	100%
2	Acidic	1675342.44	32.04	67.96	100%
3	Basic	1699747.81	31.05	68.95	100%
4	Oxidative	2334533.98	5.30	94.70	100%
5	Thermal	1674109.84	32.09	67.91	100%
6	Photolytic	1735986.09	29.58	70.42	100%
7	Water	1859738.58	24.56	75.44	100%

The drug Dapagliflozin and Saxagliptin were found to be more degraded when exposed to acidic, basic, photolytic, hydrolysis and thermal conditions and least degraded when exposed to oxidative degradation.

IV. CONCLUSION

A simple, specific and reliable reverse phase HPLC method was developed for the estimation of Dapagliflozin and Saxagliptin in their pharmaceutical dosage form. The method was validated over a concentration range 50µg/mL and 150µg/mL for Dapagliflozin and 25µg/mL and 75µg/mL for Saxagliptin. The two compounds were subjected to forced degradation applying several stress conditions. The proposed method successfully separated the two compounds with degradants. The proposed method was specific and stability-indicating. Hence the developed method can be adapted to regular quality control analysis.

V. ACKNOWLEDGMENT

The authors thankful to Sura Pharma Labs., Hyderabad, India for providing gift samples

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