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A VALIDATED RP HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SAXAGLIPTIN AND DAPAGLIFLOZIN IN ITS BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT:

A simple, selective, precise, accurate and sensitive for the estimation of Saxagliptin and Dapagliflozin was done by RP-HPLC. The assay of Saxagliptin and Dapagliflozin was performed with tablets and the % assay was found to be 99.58 and 100.21 which shows that the method is useful for routine analysis. The linearity of Saxagliptin and Dapagliflozin was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.4 and 0.5 for Saxagliptin and Dapagliflozin which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.3 and 0.3 for Saxagliptin and Dapagliflozin which shows that the method is repeatable when performed in different days also.

KEYWORDS: Saxagliptin and Dapagliflozin, Validation, stability indicating method, degradation products.

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INTRODUCTION

Dapagliflozin, (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl}-6-(hydroxymethyl)oxane-3,4,5-triol, Dapagliflozin is indicated for the management of diabetes mellitus type 2, and functions to improve glycemic control in adults when combined with diet and exercise. Dapagliflozin is a sodium-glucose co transporter 2 inhibitor, which prevents glucose reabsorption in the kidney. Using dapagliflozin leads to heavy glycosuria (glucose excretion in the urine), which can lead to weight loss and tiredness. Dapagliflozin was approved by the FDA on Jan 08, 2014. Dapagliflozin is not recommended for patients with type 1 diabetes mellitus or for the treatment of diabetic ketoacidosis.

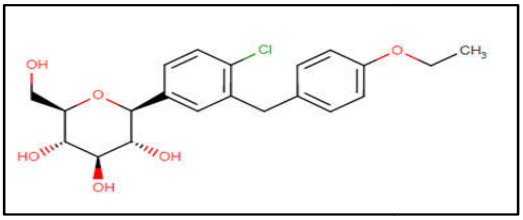

Figure no: 1 Dapagliflozin

Saxagliptin, (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxyadamantan-1-yl) acetyl]-2-azabicyclo [3.1.0]hexane-3-carbonitrile is an orally active hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. FDA approved on July 31, 2009. Post-administration of saxagliptin, GLP-1 and GIP levels rise up to 2- to 3- fold. Because it is very selective of DPP-4 inhibition, there are fewer systemic side effects. Saxagliptin inhibits DPP-4 enzyme activity for a 24-hour period. It also decreased glucagon concentrations and increased glucose-dependent insulin secretion from pancreatic beta cells. The half maximal inhibitory concentration (IC50) is 0.5 nmol/L. Saxagliptin did not prolong the QTc interval to a clinically significant degree.

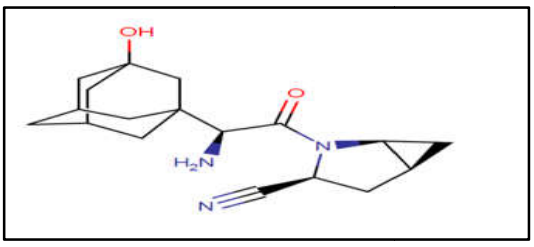

Figure no: 2 Saxagliptin

EXPERIMENTAL

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient
Column	:	Inertsil ODS(4.6 x 100mm, 5µm)
Buffer	:	Phosphate buffer
pH	:	3.5
Mobile phase	:	30% buffer 70% Acetonitrile+Methanol
Flow rate	:	1 ml per min
Wavelength	:	220 nm
Injection volume	:	20 µl
Run time	:	10 min.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of phosphate buffer pH 3.5:

Weigh accurately about 3.5gmsof potassium di hydrogen ortho phosphate dissolved in 1000 ml of HPLC water Ph was adjusted up to 3.5 with ortho phosphoric acid. Final solution was filtered through 0.44 μm Membrane filter and sonicate it for 10 mins.

Preparation of mobile phase:

Accurately measured 300 ml (30%) of above buffer and 700 ml (70%) of Acetonitrile+Methanol HPLC were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration. The same was used as diluents.

PREPARATION OF THE SAXAGLIPTIN & DAPAGLIFLOZIN STANDARD & SAMPLE SOLUTION:

Standard Solution Preparation:

Accurately weigh and transfer 10 mg of Saxagliptin and 20 mg of Dapagliflozin working standard into a 100 ml clean dry volumetric flask add about 7 mL

of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Saxagliptin and 20 mg Dapagliflozin sample into a 100 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate it up to 15 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is Filtered through 0.45 micron Injection filter. (Stock solution)

Further pipette 3ml of Saxagliptin and Dapagliflozin from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for Saxagliptin and Dapagliflozin peaks and calculate the %Assay by using the formulae.

RESULTS AND DISCUSSIONS

Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

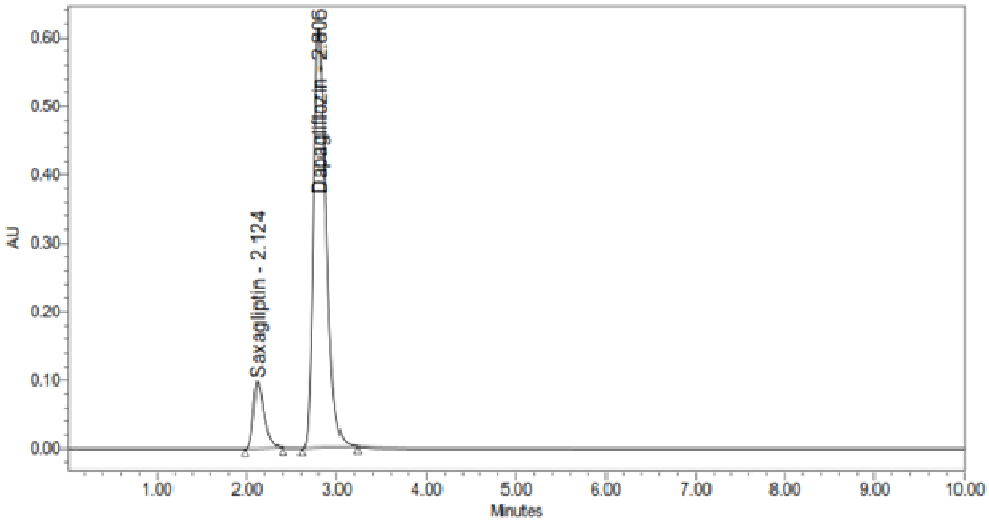

Figure no: 3 Standard Chromatogram of Saxagliptin and Dapagliflozin

TABLE NO: 1 RESULTS OF SYSTEM SUITABILITY PARAMETERS

S.No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Saxagliptin	2.105	784954	96962		1.45	3568.55
2	Dapagliflozin	2.785	5528694	612232	3.04	1.35	5239.73

TABLE NO: 2 RESULTS OF LINEARITY OF SAXAGLIPTIN AND DAPAGLIFLOZIN

Sl. No.	Saxagliptin		Dapagliflozin	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	10	268654	20	1832427
2	20	520739	40	3726834
3	30	783140	60	5582709
4	40	1061084	80	7407799
5	50	1342518	100	9322648

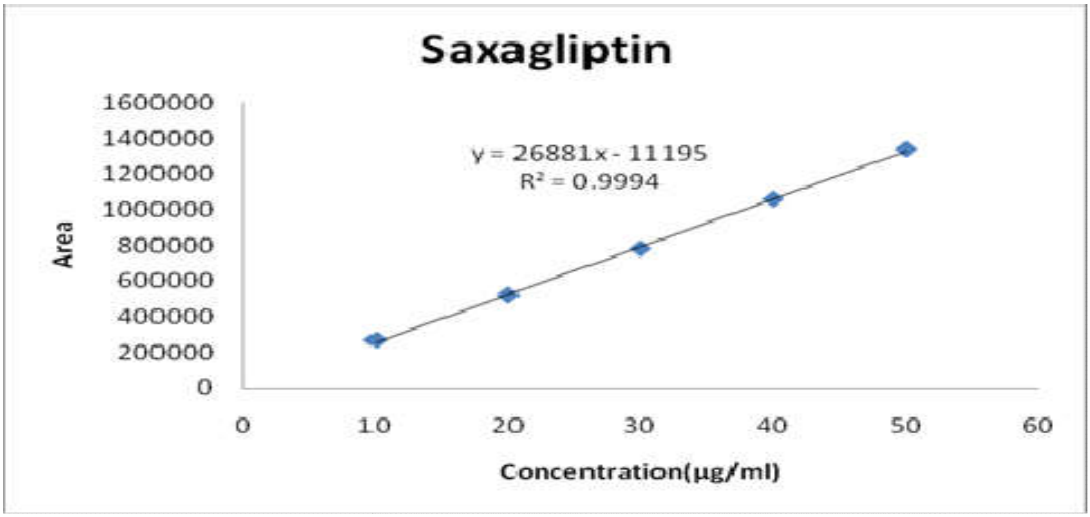

Figure no: 4 Calibration graph for Saxagliptin

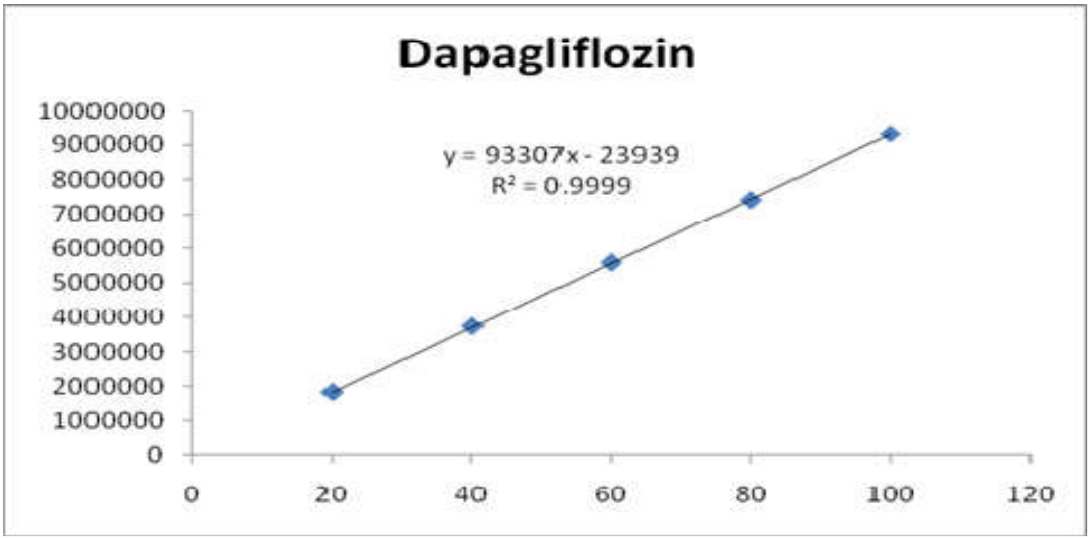

Figure no: 5 Calibration graph for Dapagliflozin

TABLE NO: 3 RESULTS OF PRECISION FOR SAXAGLIPTIN

Injection	Area
Injection-1	789316
Injection-2	785334
Injection-3	780020
Injection-4	786180
Injection-5	781227
Injection-6	782839
Average	784152.7
Standard Deviation	3450.5
%RSD	0.4

TABLE NO: 4 RESULTS OF PRECISION FOR DAPAGLIFLOZIN

Injection	Area
Injection-1	5523508
Injection-2	5528488
Injection-3	5591669
Injection-4	5523942
Injection-5	5539053
Injection-6	5567550
Average	5545701.7
Standard Deviation	27917.4
%RSD	0.5

TABLE NO: 5 ACCURACY (RECOVERY) DATA FOR SAXAGLIPTIN

% Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	396812	5	5.04	100.85	100.09
100%	787039	10	10.00	100.01	
150%	1173386.0	15	14.91	99.40	

*Average of three determinations

TABLE NO: 6 ACCURACY (RECOVERY) DATA FOR DAPAGLIFLOZIN

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	2754176	10	9.97	99.73	99.86
100%	5551291.7	20	20.10	100.51	
150%	8229366.3	30	29.80	99.33	

TABLE NO: 7 RESULTS OF LOD

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Saxagliptin	51	148	2.90
Dapagliflozin	51	158	3.10

TABLE NO: 8 RESULTS OF LOQ

Drug name	Baseline noise (µV)	Signal obtained (µV)	S/N ratio
Saxagliptin	51	505	9.90
Dapagliflozin	51	515	10.10

DEGRADATION STUDIES:

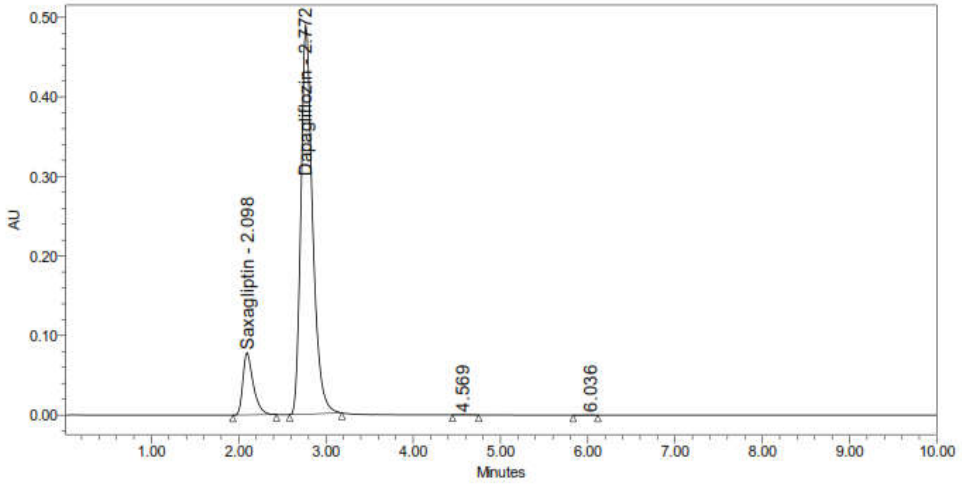

Figure no: 6 Chromatogram showing Acid degradation

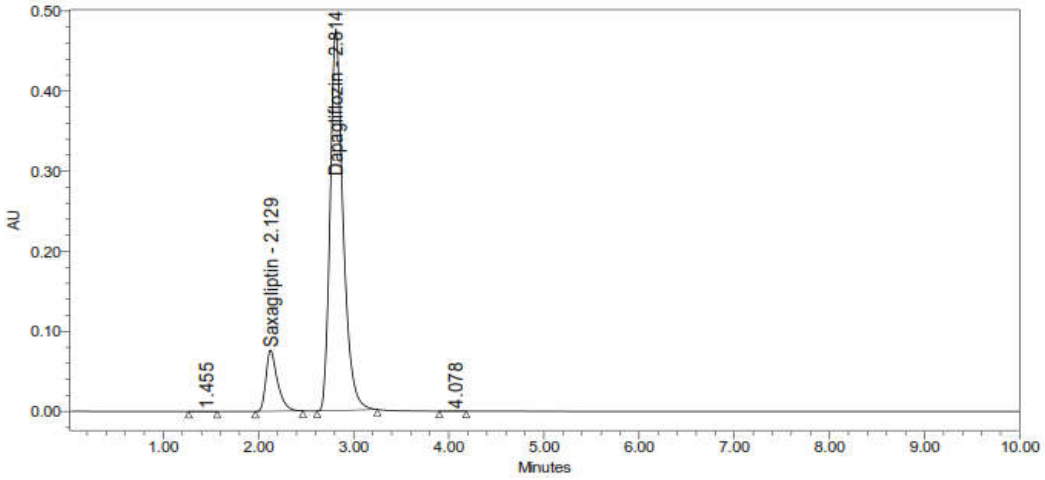

Figure no: 7 Chromatogram showing Base degradation

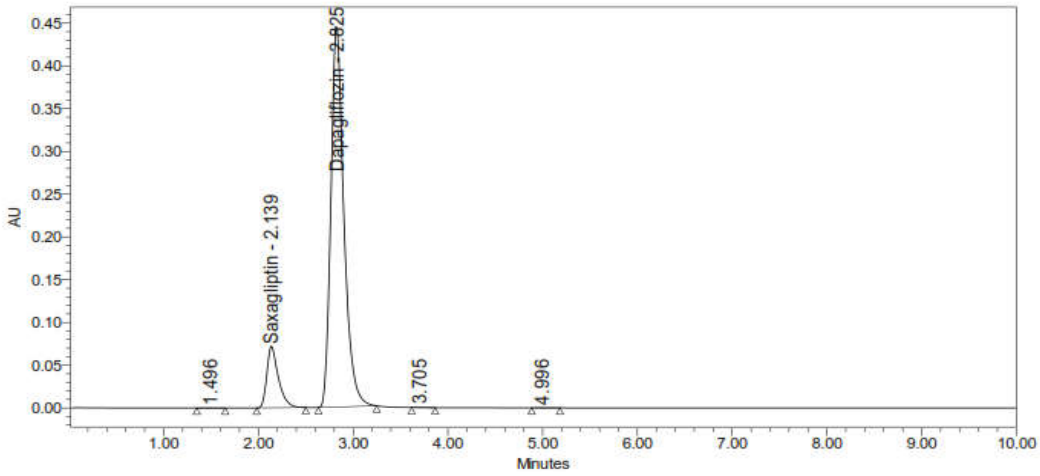

Figure no: 8 Chromatogram showing Peroxide degradation

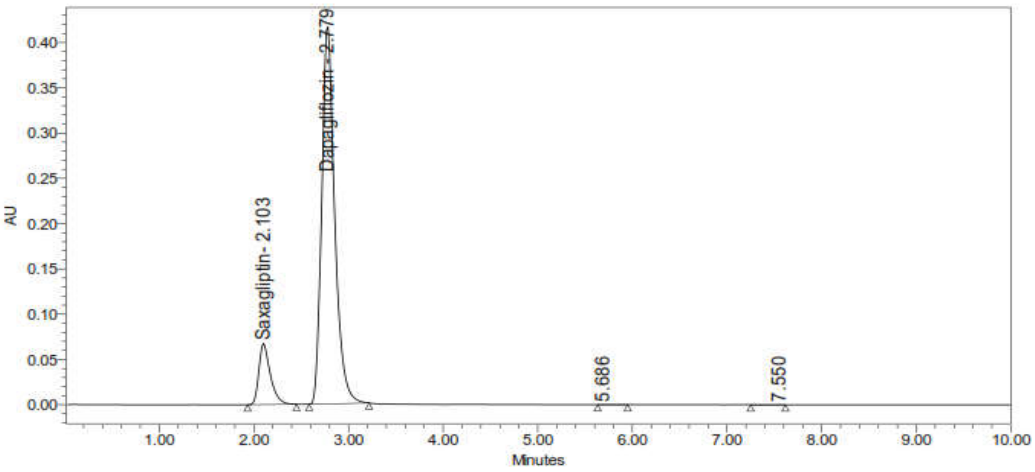

Figure no: 9 Chromatogram showing Thermal degradation

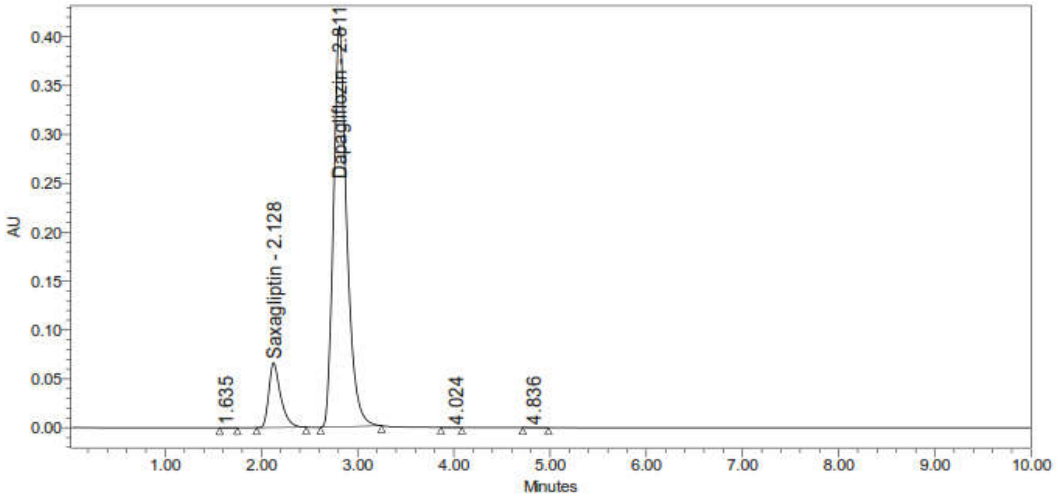

Figure no: 10 Chromatogram showing Photo degradation

Table 9: Results for Stability of Saxagliptin and Dapagliflozin

Sample Name	Saxagliptin		Dapagliflozin	
	Area	% Degraded	Area	% Degraded
Standard	785386		5512235	
Acid	763563	2.78	5312622	3.62
Base	757893	3.50	5286737	4.09
Peroxide	759376	3.31	5297856	3.89
Thermal	735422	6.36	5215762	5.38
Photo	745353	5.10	5257689	4.62

Table 10: Results of Assay for Saxagliptin and Dapagliflozin

	Label Claim (mg)	% Assay
Saxagliptin	5	99.58
Dapagliflozin	10	100.21

CONCLUSION

The estimation of Saxagliptin and Dapagliflozin was done by RP-HPLC. The assay of Saxagliptin and Dapagliflozin was performed with tablets and the % assay was found to be 99.58 and 100.21 which shows that the method is useful for routine analysis. The linearity of Saxagliptin and Dapagliflozin was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.4 and 0.5 for Saxagliptin and Dapagliflozin which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more

than 2.0% and the method show precision 0.3 and 0.3 for Saxagliptin and Dapagliflozin which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.09% and 99.86% for Saxagliptin and Dapagliflozin. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ are 3 and 10. The LOD and LOQ for Saxagliptin was found to be 2.90 and 9.90 and LOD and LOQ for Dapagliflozin was found to be 3.10 and 10.10. The robustness limit for mobile phase variation and flow rate variation are well within the limit, the % degradation results are in limits. Which shows that, the method has a good system suitability and precision under given set of conditions.

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CONFLICT OF INTEREST REPORTED: NIL ; SOURCE OF FUNDING: NONE REPORTED