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NEW STABILITY INDICATING UPLC METHOD FOR SIMULTANEOUS DETERMINATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN

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Keywords:

Dapagliflozin, Simultaneous Saxagliptin, Acetonitrile, Buffer, UPLC

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ABSTRACT: A new precise, selective, accurate and rapid Ultra Performance Liquid Chromatographic stability indicating method has been developed and validated for simultaneous quantitative determination of Dapagliflozin and Saxagliptin in bulk and pharmaceutical dosage form. The chromatographic separation was achieved with HSS 100 × 2.1 mm, 1.8 m size column. The optimized mobile phase consisting of Phosphate buffer: acetonitrile in the ratio of 50:50 v/v. The flow rate was 0.3 mL/min, and eluents were detected at 260 nm using a PDA detector. The retention time of Dapagliflozin and Saxagliptin were found to be 0.900 and 1.119 min respectively. The percentage recoveries for both the molecules were found to be in the range of 98.61 -100.5%. The calibration curve was constructed between peak areas *vs.* concentration and demonstrated good linearity in the range of (2.5-15 µg/ml Dapagliflozin and 1.25-7.5 µg/ml Saxagliptin). Degradation studies were carried for Dapagliflozin and Saxagliptin under various stress conditions such as acid, base, oxidation, thermal, photochemical and UV degradation peaks were resolved effectively.

INTRODUCTION: Dapagliflozin is a gliflozin class drug which is used to treat type 2 diabetes. It has a chemical name of (2S,3R,4R,5S,6R) -2 - [4-chloro-3 - (4-ethoxy benzyl) phenyl] -6-(hydroxy methyl) tetrahydro -2H -pyran - 3, 4, 5 - triol. The chemical formula of Dapagliflozin is $C_{21}H_{25}ClO_6$, and the molecular weight is 408.873 g/mol [1, 2]. Saxagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor anti-diabetic 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.

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It has a chemical name of (1S,3S,5S)-2-[(2S)-2-amino -2-(3- hydroxy -1- adamantyl) acetyl]- 2 - azabicyclo hexane-3-carbonitrile. The chemical formula is $C_{18}H_{25}N_3O_2$, and the molecular weight is 315.417 g/mol [3, 4].

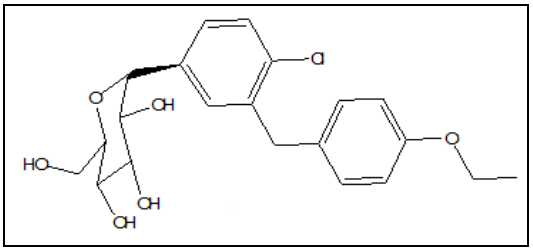

FIG. 1: STRUCTURE OF DAPAGLIFLOZIN

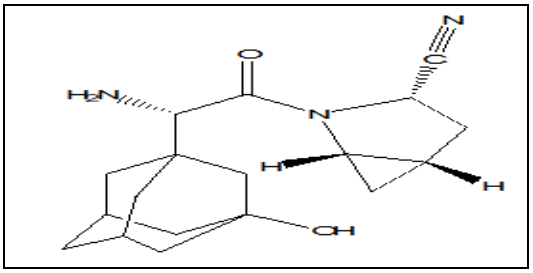

FIG. 2: STRUCTURE OF SAXAGLIPTIN

The combination of Dapagliflozin and Saxagliptin shows a significant effect on symptom relief when compared to individual drugs used in diabetic treatment. Numerous methods are available for the estimation of these drugs individually and in combination with other drugs. Very few methods are available [5, 6, 7, 8] for simultaneous determination of Dapagliflozin and Saxagliptin in pharmaceutical formulations. A successful attempt has been made to estimate the combination of drugs by a stability indicating Ultra Performance Liquid Chromatographic method for simultaneous determination of Dapagliflozin and Saxagliptin in bulk and Pharmaceutical Dosage form.

MATERIALS AND METHODS: [9]

Chemicals and Solvents: Dapagliflozin and Saxagliptin were obtained as gift samples from Eris life sciences Pvt. Ltd., India. The commercial pharmaceutical preparation containing 10 mg of Dapagliflozin and 5 mg of Saxagliptin respectively was procured from a local market. Orthophosphoric acid, acetonitrile, and water used were of HPLC grade.

Instrumentation: The chromatographic separations were performed using UPLC-Waters alliance (Model-2695) consisting of an in-built autosampler, a column oven, and PDA detector. The data was acquired through Empower-2-software. The column used was HSS 100 × 2.1 mm, 1.8 m particle size column. Meltronics sonicator was used for enhancing dissolution of the compounds. Elico pH meter was used for adjusting the pH of the buffer solution. All weighing was done on Sartorius balance (model AE-160).

Chromatographic Conditions: The mobile phase consists of phosphate buffer: acetonitrile in the ratio of 50:50 v/v.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument	Waters 2695, High-Performance Liquid Chromatography
Flow rate	0.3 ml/min
Column	HSS 100 × 2.1 mm, 1.8 m particle size
Detector	wave length of 260 nm
Column temperature	30 °C
Injection volume	3 µl
Run time	3 min
Mobile phase	Buffer: acetonitrile (50:50)
Mode of separation	Isocratic mode

The mobile phase was pumped from the solvent reservoir in the ratio of 50:50 v/v to the column in the flow rate of 0.3 ml/min whereas the run time set was 8 min. The separation was performed on HSS 100 × 2.1 mm, 1.8 m particle size column and the column was maintained at the temperature of 30 °C, and the volume of 3 µl was injected. Before injection, the column was equilibrated for at least 3 min with mobile phase flowing through the system. The eluent was monitored at 260 nm.

Preparation of Buffer Solution (pH 4.0): Orthophosphoric acid 1 ml was taken in a 1000 ml of volumetric flask, 100 ml of milli-Q water was added, and final volume made up to 1000 ml with milli-Q water and degassed using sonicator and finally made up to the volume with water then added 1 ml of triethylamine. The pH adjusted to 4.0 with orthophosphoric acid.

Preparation of Mobile Phase: Buffer and acetonitrile are in the ratio 50:50% v/v, filtered through 0.45 µ filter under vacuum.

Preparation of Standard Solution: Accurately Weighed and transferred 10 mg of Dapagliflozin and 5 mg of Saxagliptin working standards into a 50 ml clean dry volumetric flask, added a 3/4th volume of diluent, sonicated for 15 min and made up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10 ml volumetric flask and made up to 10 ml with diluents [Water and Acetonitrile (50:50)].

Preparation of Sample Solution: Accurately 5 tablets were weighed, powdered and the powder equivalent to 10 mg of Dapagliflozin and 5 mg of Saxagliptin was transferred into a 100 ml volumetric flask, 50 ml of diluent was added and sonicated for 25 min; further the volume was made up to the mark with diluent and filtered. From the filtered solution 1 ml was pipetted out into a 10 ml volumetric flask and made up to 10 ml with diluent.

Validation of Analytical Methods: [10, 11] The developed method was validated according to the ICH (International Conference on Harmonization) guidelines concerning system suitability, precision, specificity, forced degradation studies, linearity, accuracy, limit of detection and limit of quantification.

Linearity: Aliquots of 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 ml were taken from a stock solution of concentration 10 µg/ml of Dapagliflozin and 5 µg/ml of Saxagliptin and then diluted up to mark with diluent to acquire the required concentrations of Dapagliflozin and Saxagliptin respectively. The volume of 3 µl of each sample was injected five times for each concentration level and a calibration curve was constructed by plotting the peak area versus drug concentration. A linear relationship between peak areas *vs.* concentration was observed in the range of study. The observations and calibration curve were shown in **Table 2**, **Fig. 3** and **Fig. 4**.

Accuracy: To determine the accuracy in sample preparation, a method of standard additions was made for measuring the recovery of the drugs. A fixed amount of sample was taken, and the standard drug was added at 50, 100 and 150% levels. The results were analyzed, and the results were found to be within limits. The accuracy was expressed as the percentage of the analytes recovery. The results were shown in **Table 3** and **4**.

System Suitability: The system suitability was determined by making six replicate injections from freshly prepared standard solutions. The observed RSD values were well within usually accepted limits (≤2%). Theoretical plates, tailing factor of Dapagliflozin and Saxagliptin were determined, and all are within limits.

Specificity: The specificity of the method was performed by injecting blank solution (without any sample), and then a drug solution of 10 µl injected into the column, under optimized chromatographic conditions, to demonstrate the separation of the molecules Dapagliflozin and Saxagliptin from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific.

Limit of Detection and Limit of Quantification: LOD and LOQ were calculated using the following formula LOD = 3.3(SD)/S and LOQ = 10 (SD)/S, where SD = standard deviation of response (peak area) and S= slope of the calibration curve. Limit of detection and limit of quantification were found to be 0.03 µg/ml and 0.09 µg/ml Dapagliflozin and 0.02 µg/ml and 0.07 µg/ml Saxagliptin respectively.

Robustness: Robustness was carried by varying three parameters from the optimized chromatographic conditions such as flow rate (± 0.1ml/min), mobile phase composition (± 5%) and column temperature (± 5 ºC). It was observed that the small changes in these operational parameters did not lead to changes in the retention time of the peak of interest and the % RSD was not more than 2. The degree of reproducibility of the results prove that the method is robust. The results were shown in **Table 6**.

Forced Degradation Studies: [12] Forced degradation studies were performed to demonstrate the optimized method is stability indicating. To prove the method which can be able to measure accurately active pharmaceutical ingredient in the presence of degradants which are expected to be formed during different types of degradations applied to the drug sample.

For forced degradation analysis, aliquots of stock (2 mg/ml, 3 mg/ml and 2.5 mg/ml) were separately treated with 1 ml of 2N HCl (Acid stability), 1 ml of 2N NaOH (Alkaline stability), 1 ml of 20% H_2O_2 (Oxidative degradation), exposure of standard drug solution at 105 ºC for 6 h (dry heat degradation), photo stability degradation (exposure of drug at 200 watt/m^2) and neutral degradation (refluxing with water at 60 ºC for 6 h. Stability of these samples was compared with the fresh sample on the day of analysis. The HPLC chromatograms of degraded products show no interference at the analyte peaks, hence the method was specific and stability indicating. The chromatograms were shown in figures and the results were shown in **Table 7**. The detailed degradation of each condition is as follows:

Oxidation: To 1 ml of stock solution of Dapagliflozin and Saxagliptin, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60 °C. For HPLC study, the resultant solution was diluted to obtain 500 µg/ml of Dapagliflozin, 50 µg/ml Dapagliflozin and 100 µg/ml Saxagliptin were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Acid Degradation Studies: To 1 ml of stock solution Dapagliflozin and Saxagliptin, 1 ml of 2N hydrochloric acid was added and refluxed for 30

min at 60 °C. The resultant solution was diluted to obtain 10 µg/ml Dapagliflozin, and 5 µg/ml Saxagliptin solutions were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation Studies: To 1 ml of stock solution Dapagliflozin and Saxagliptin, 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60 °C. The resultant solution was diluted to obtain 10 µg/ml Dapagliflozin and 5 µg/ml Saxagliptin solution, and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Dry Heat Degradation Studies: The standard drug solution was placed in an oven at 105 °C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 10 µg/ml Dapagliflozin, and 5 µg/ml Saxagliptin solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 2 mg/ml, 3 mg/ml and 0.25 mg/ml solution to UV Light by keeping the beaker in UV Chamber for 7 days or 200 Watt-hours/m^2 in photostability chamber.

For HPLC study, the resultant solution was diluted to obtain 10 µg/ml Dapagliflozin and 5 µg/ml Saxagliptin, and 10 µl were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6 h at a temperature of 60 °C. For HPLC study, the resultant solution was diluted to 10 µg/ml Dapagliflozin, and 5 µg/ml Saxagliptin were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION:

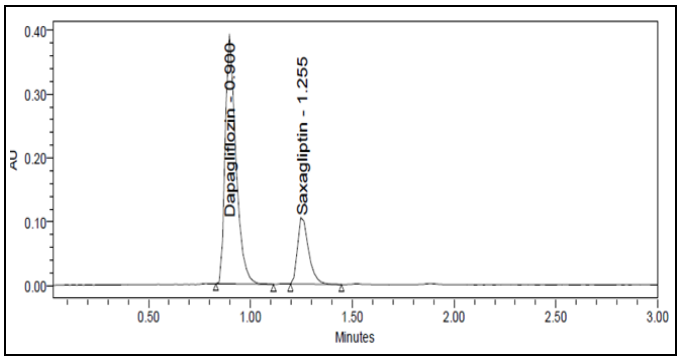

FIG. 3: TYPICAL CHROMATOGRAM OF STANDARD OF DAPAGLIFLOZIN AND SAXAGLIPTIN

TABLE 2: LINEARITY OF DAPAGLIFLOZIN AND SAXAGLIPTIN

S. no.	Dapagliflozin		Saxagliptin	
	Conc. µg/ml	Area	Conc. µg/ml	Area
1	2.5	115605	1.25	26143
2	5	221279	2.5	52316
3	7.5	335058	3.75	78702
4	10	441573	5	103415
5	12.5	564049	6.25	130277
6	15	661010	7.5	155489

TABLE 3: ACCURACY RESULTS OF DAPAGLIFLOZIN

Accuracy %	Conc. in µg/ml	Peak area	% Recovery
50	5	664985	99.43
100	10	885909	99.60
150	15	1105111	99.39
AVG			99.57
SD			0.57
% RSD			0.57

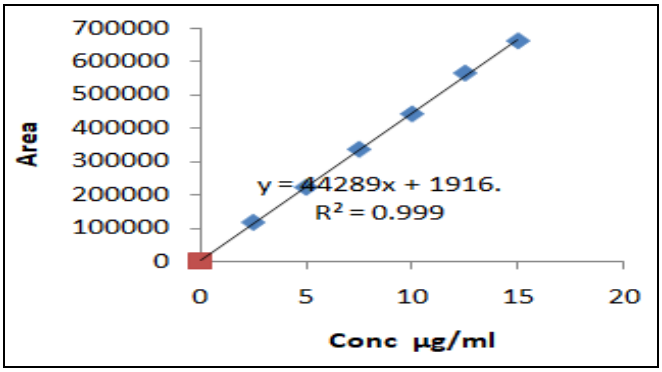

FIG. 4: LINEARITY OF DAPAGLIFLOZIN

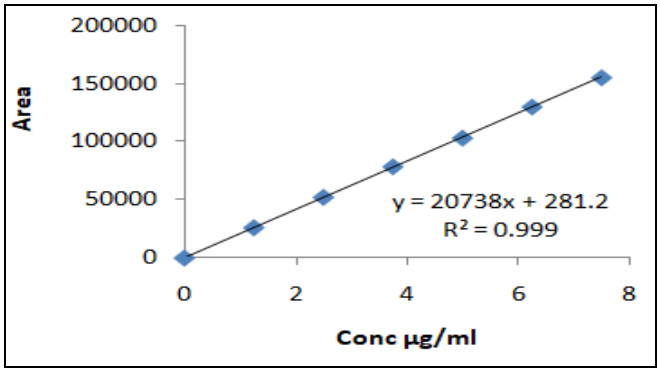

FIG. 5: LINEARITY OF SAXAGLIPTIN

TABLE 4: ACCURACY RESULTS OF SAXAGLIPTIN

Accuracy %	Conc. in µg/ml	Peak area	% Recovery
50	2.5	156758	101.82
100	5	207372	99.72
150	7.5	259058	99.71
AVG			99.71
SD			99.82
% RSD			100.37

TABLE 5: PRECISION RESULTS OF DAPAGLIFLOZIN AND SAXAGLIPTIN

S. no.	Dapagliflozin			Saxagliptin		
	System Precision	Inter day Precision	Intraday Precision	System Precision	Inter-day Precision	Intra-day Precision
1	441603	435561	442183	103965	101387	104726
2	441330	435667	443800	104536	101809	104540
3	441881	434143	442738	104606	103433	105048
4	443753	434482	443807	104829	101843	105048
5	445182	436652	443807	105211	102702	106052
6	441836	435978	442146	105894	103119	104679
AVG	442598	435414	443080	104840	102382	105016
SD	1529.9	940.3	820.9	657.5	819.6	547.6
% RSD	0.3	0.2	0.2	0.6	0.8	0.5

TABLE 6: ROBUSTNESS RESULTS OF DAPAGLIFLOZIN AND SAXAGLIPTIN

Drug Name	Parameter	Optimized Condition	Used Condition	Peak Area	Retention Time	Plate Count	Tailing Factor
Dapagliflozin	Flow rate (±0.1 ml/min)	0.3 ml/min	0.2 ml/min	1320016	0.911	1338	1.40
			0.3 ml/min	1316796	0.912	1312	1.41
			0.4 ml/min	1327793	0.913	1281	1.42
Column temp.	(±5 °C)	30 °C	25 °C	1116426	0.781	1064	1.55
			30 °C	1123203	0.782	1046	1.57
			35 °C	1116314	0.783	1144	1.52
Mobile phase composition	(5% v/v)	50:50	45:55	169206	1.365	1203.4	1.0
			50:50	787449	1.536	3106.2	1.8
			65:35	446312	0.934	1973.2	1.2
Saxagliptin	Flow rate (±0.1 ml/min)	0.3ml/min	0.2 ml/min	377853	1.201	2645	1.49
			0.3 ml/min	376415	1.202	2500	1.45
			0.4 ml/min	377705	1.203	2473	1.43
Column temp.	(±5 °C)	30 °C	25 °C	317219	1.039	2273	1.48
			30 °C	321000	1.039	2031	1.39
			35 °C	317009	1.041	2160	1.35
Mobile phase composition	(5% v/v)	50:50	45:55	18886	2.867	2248.5	1.4
			50:50	19487	2.435	4066.2	0.8
			65:35	105021	1.108	772. 9	1.4

TABLE 7: DEGRADATION STUDIES RESULTS

Drug	Parameters	Acid	Alkali	Peroxide	Thermal	UV	Water
Dapagliflozin	Peak area	422253	428857	431300	430256	431890	441079
	% Assay	95.31	96.80	97.35	97.11	97.48	99.56
	% Degradation	4.69	3.20	2.65	2.89	2.52	0.44
	Purity angle	1.997	1.5	2.661	3.132	2.118	1.293
	Purity threshold	2.008	1.7	2.899	3.437	2.736	2.585
Saxagliptin	Peak area	99902	99509	101200	101588	102308	104167
	% Assay	95.19	94.82	96.43	96.8	97.49	99.26
	% Degradation	4.81	5.18	3.57	3.20	2.51	0.74
	Purity angle	10.995	13.1	12.396	3.220	12.013	25.024
	Purity threshold	25.025	24.9	25.384	4.502	9.276	25.354

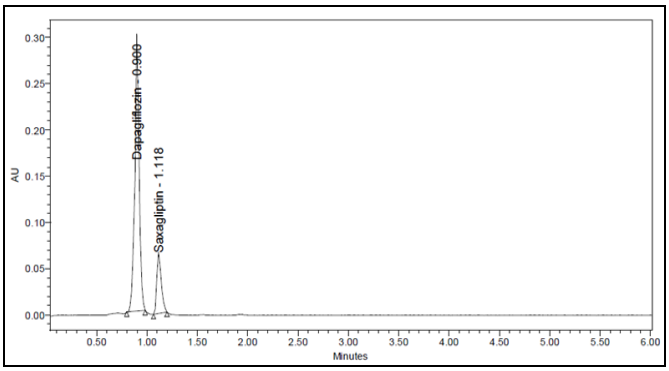

FIG. 6: TYPICAL CHROMATOGRAM SHOWING ACID DEGRADATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN

CONCLUSION: The developed method is accurate, precise, rapid and selective and proved to be stability indicating for the simultaneous estimation of Dapagliflozin and Saxagliptin in the bulk and pharmaceutical dosage form. The sample preparation is simple, the analysis time is short, and the elution is by the isocratic method. The retention time of Dapagliflozin and Saxagliptin were found to be 0.900 and 1.118 min respectively.

The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these drugs. Forced degradation studies of different conditions shows that all the degradants were well resolved from these main drug peaks and able to quantify the Dapagliflozin and Saxagliptin in the presence of degradants and excipients proved that the method is found to be stability indicating. Hence, the proposed method can be conveniently adopted for the routine quality control analysis in bulk and combined formulations. The developed method was validated according to ICH guidelines. As the method could effectively separate the degradation products from active ingredient, it can be used for routine analysis of drug both in bulk and pharmaceutical dosage form.

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CONFLICT OF INTEREST: Nil

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