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DEVELOPMENT OF A NEW STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SAXAGLIPTINE AND DAPAGLIFLOZIN AND ITS VALIDATION AS PER ICH GUIDELINES

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Abstract:

A new stability indicating RP HPLC method has been developed and validated for simultaneous estimation of Saxagliptine and Dapagliflozin in bulk and dosage forms. The method involves separation on XTerra C_{18} column (150mm x 4.6mm x5µm particle size). The optimized mobile phase consists of phoaphate buffer (pH 4) and Acetonitrile (50:50v/v) with a flow rate of 1ml/min and UV detection at 225mn. Retention time was 2.1min (Saxagliptine), 2.8min (Dapagliflozin). Linearity range was 20-60ug/ml (Saxagliptine), 40-120ug/ml (Dapagliflozin). Accuracy was in the range of 99.99-100.50% for both drugs. Precision was 0.78% and 0.44% for Saxagliptine and Dapagliflozin. LOD and LOQ are 1.63ug/ml and 5.39ug/ml for Saxagliptine, 1.94ug/ml and 6.50ug/ml for Dapagliflozin. The method developed is more sensitive, accurate and precise than the methods reported earlier. Retention time and run time were also less and hence the method is economical. When applied for tablet assay, drug content was within 100.24-100.43 % of labeled content. Forced degradation studies indicated the suitability of the method for stability studies.

Key Words: *Saxagliptine, Dapagliflozin, RP-HPLC Method, Simultaneous estimation, Validation as per ICH guidelines, Forced degradation studies.*

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INTRODUCTION:

Type 2 diabetes mellitus (T2DM) is a chronic progressive metabolic disorder characterized by absolute or relative insulin deficiency [1]. Expected rise in prevalence of diabetes is mainly due to increased life span because of better healthcare facilities and increase in diabetic risk factors, especially physical inactivity and obesity due to sedentary life style. Pancreatic β-cell function is gradually deteriorated in patients of T2DM which is reflected into inadequate glycemic control on a long run[2].

Saxagliptin is chemically known as (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1- adamantyl)acetyl]-2 azabicyclo hexane-3-carbonitrile) with molecular formula of C18H25N3O2 and molecular weight of 315.41g/mol[3] . Saxagliptin is a selective and potent dipeptidyl peptidase (DPP)-4 inhibitor, approved as an adjunct to diet and exercise to improve glycemic control in type 2 diabetes mellitus (T2DM). In patients with T2DM, once-daily administration of saxagliptin before breakfast achieves sustained inhibition of plasma DPP-4 activity and reduction of postprandial hyperglycemia, including after dinner, associated with an increase in plasma glucagon-like peptide-1 levels[4,5,6].

Dapagliflozin is chemically known as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol. It has a molecular formula of C24H33ClO8 with molecular weight 408.98 [7]. Dapagliflozin is selective Sodium Glucose Co-Transporter 2 inhibitor (SGLT 2). It acts by reducing the reabsorption of glucose by the kidney, leading to excretion of excess glucose in the urine, there by improving glycemic control in patients with type 2 diabetes mellitus [8].

Though several methods are reported in literature for the estimation of Saxagliptine [9-22] and Dapagliflozin [23-30] individually, no methods are reported for estimation of Saxagliptine and Dapagliflozin in combination. .

The objective of the present study is to develop a novel, simple, accurate, precise, economic method for the simultaneous estimation of Saxagliptine and Dapagliflozin and validate the method with forced degradation studies according to ICH guidelines [31].

EXPERIMENTAL:

Materials and reagents:

HPLC grade Acetonitrile (Lichrosol^R, Merck Lifesciences Pvt. Ltd., Mumbai, India), HPLC water (Lichrosolv^{R}MerckLifesciencesPvt.Ltd., Mumbai, India) Potassium Dihydrogen phosphate (Thermo Fischer Scientific Pvt Ltd., Mumbai, India), and Ortho phosphoric acid (S D Fine –Chem. Ltd., Mumbai, India) were used in the study. The working standards of Saxagliptine and Dapagliflozin were generous gift obtained from HiQ Pharma Labs Pvt Ltd., Hyderabad, India. Qtern tablet containing Saxagliptine 5mg and Dapagliflozin 10mg (Astrazeneca pharmaceutical limited) was procured from UK market.

Instrumentation:

Chromatography was performed on a WATERS 2695 HPLC column (waters corporation, Mildord, USA) with an autosampler and equipped with a 2996 series of PDA detector with a spectral bandpass of 1.2nm. Components were detected using UV and that processing was achieved by Empower 2 software. A hot air oven was used for thermal degradation of the samples and a UV crossinker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300nm was selected for photolytic degradation. Ultrasonic bath (Toshcon by Toshniwal), digital P^H meter(Adwa – AD 1020), UV/VIS spectrophotometer (Labindia UV 3000) were used in the study.

Chromatography conditions:

The chromatographic separation was performed on XTerra C $_{18}$ (4.6 x 150mm, 5µm particle size) at an ambient column temperature. The samples were eluted using Phosphate buffer (pH adjusted to 4 with OPA): Acetonitrile(50:50v/v) as the mobile phase at a flow rate of 1ml/min the mobile phase and samples were degassed by ultrasonication for 20 min and filtered through 0.45µm Nylon (N66) 47mm membrane filter. The measurements were carried out with an injection volume of 10µL, flow rate was set to 1 mL/min, and UV detection was carried out at 225 nm. All determinations were done at ambient column temperature (30°C). The chromatograms of the prepared standard stock solutions of Saxagliptine, Dapagliflozin and Glimiperide were recorded under optimized chromatographic conditions.

Preparation of Buffer and Mobile Phase:

Preparation of 0.025M Phosphate buffer:

3.4g of potassium dihydrogen ortho phosphate was weighed and taken in a 1000ml volumetric flask and P^H was adjusted to 4 with dilute OPA, finally the solution was filtered by using 0.45 micron membrane filter and sonicated for 10 min.

Preparation of mobile phase:

500 ml (50%) of phosphate buffer and 500 ml of Acetonitrile (50%) were mixed and degassed in an

ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent:
Mobile phase was used as diluent

Preparation of Standard Solutions:

Stock solution of Saxagliptine:

Standard stock solution of Saxagliptine was prepared by dissolving 10 mg of Saxagliptine in 10 ml of diluent (Buffer: Acetonitrile, 50:50 v/v) in a 10 ml clean dry volumetric flask and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 1000µg/ml of Saxagliptine. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Saxagliptine.

Stock solution of Dapagliflozin:

Standard stock solution of Dapagliflozin was prepared by dissolving 10 mg of Dapagliflozin in 10 ml of diluent (Buffer: Acetonitrile, 50:50 v/v) in a 10 ml clean dry volumetric flask and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 1000µg/ml of Dapagliflozin. The above standard stock solution suitably diluted with diluents to obtain various concentrations of Dapagliflozin.

Stock solution of Glimiperide:

Standard stock solution of Glimiperide was prepared by dissolving 10 mg of Glimiperide in 10ml of diluent (Buffer: Acetonitrile, 50:50 v/v) in a 10ml clean dry volumetric flask and the standard solutions was filtered through 0.45µm nylon membrane filter and degassed by sonicator to get the concentration of 1000µg/ml of Glimiperide.

Working Standard Solution of Saxagliptine:

Working standard solution of Saxagliptine was prepared by taking 0.4 ml of stock solutions of Saxagliptine in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 40µg/ml of Saxagliptine.

Working Standard Solution of Dapagliflozin:

Working standard solution of Dapagliflozin was prepared by taking 0.8 ml of stock solutions of Dapagliflozin in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 80µg/ml of Dapagliflozin.

Working Standard Solution of Glimiperide:

Working standard solution of Glimiperide was prepared by taking 0.5ml of stock solutions of Glimiperide in to clean dry 10ml volumetric flask and make up volume with diluent to get a concentration of 50µg/ml of Glimiperide.

Preparation of Sample Solutions of Saxagliptine and Dapagliflozin:

Twenty tablets were accurately weighed and powdered and tablet powder equivalent to 262 mg of Saxagliptine and Dapagliflozin was taken into 10 ml clean dry volumetric flask, diluent was added and sonicated to dissolve completely and volume was made up to volume with the diluent. The above sample solution was filtered and suitably diluted to get a concentration of 40 µg/ml of Saxagliptine and 80 µg/ml of Dapagliflozin.

RESULTS AND DISCUSSION:

Optimization of chromatographic conditions:

During the Optimization cycle during the optimization cycle, different columns with different lengths and internal diameters were tried namely, Waters C18 column, hypersil column, lichrosorb, and XTerra column but finally satisfactory separation was obtained on XTerra C 18 (4.6 x 150mm, 5µm) column. Methanol and acetonitrile were examined individually and simultaneously as organic modifiers and acetonitrile was found to be more suitable, individually, as it allowed better separation of the three analytes under investigation. Isocratic mode of elution with different ratios of organic to aqueous phases was tried in order to achieve proper separation of the cited analytes in a reasonable run time. The use of 0.025M Phosphate buffer was necessary in this method in order to influence the ionization of the analytes and to help in their co-elution. Also pH was constant as each of SAXA, DAPA and GMP is obviously affected by the mobile phase composition and pH. The effect of pH on the separation of the analytes was studied. It was found that pH higher than 5.59 was not suitable as due to improper separation of the analyzed compounds. pH was adjusted at 4 for the best separation of the three analytes in a reasonable run time (<10 min) and with good resolution between all peaks. Different flow rates were studied and flow rate of 1 mL min-1 was found to be optimum.
Quantitation was achieved with UV-detection at 225 nm. The column temperature was set at 30ºC. Optimized method was providing good resolution and peak shape for SAXA, DAPA and GMP. Under above described experimental conditions, all the peaks were well defined and free from tailing. The concern of small deliberate changes in the mobile phase composition, flow rates, and column temperature on results were evaluated as a part of testing for methods robustness.

Validation of Method Developed:
The proposed method was validated according to the ICH guidelines for system suitability, specificity, recovery, precision, linearity, and robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

System suitability test:
HPLC system was optimized as per the chromatographic conditions. 10 µl of standard solutions of drugs were injected in triplicate into the chromatographic system. To ascertain the system suitability for the proposed method, the parameters such as retention time, theoretical plates, and tailing factor were calculated.

Specificity:
The specificity of the method was carried out to check whether there is any interference of any impurities with the retention time of analyte peaks. The specificity was performed by the injecting blank, Placebo and standard solutions of drugs.

Precision
Precision is expressed as the closeness of agreement between a series of measurements obtaining from multiple sampling of the same homogeneous sample. Six replicate injections of a known concentration of Saxagliptine (40 µg/mL) and Dapagliflozin (80 µg/mL), have been analyzed by injecting them into a HPLC column on the same day. The intermediate precision was estimated by injecting samples prepared at the same concentrations on three different days by different operators. The peak area ratios of all injections were taken and standard deviation, % relative standard deviation (RSD), was calculated.

Accuracy
Accuracy is tested by the standard addition method at different levels: 50, 100 and 150%. A known amount of the standard drug was added to the blank sample at each level. The mean recovery of Saxagliptine and Dapagliflozin were calculated and accepted with 100±2%.

Linearity
Appropriate volumes of Saxagliptine and Dapagliflozin stock 100(mg/ml) standard solutions were diluted with mobile phase to yield 20,30,40,50,60 µg/mL of Saxagliptine and 40,60,80,100,120 µg/mL Dapagliflozin respectively .Six replicates of each concentration were independently prepared and injected in to HPLC system. The linearity was determined by calculating a regression line from plot of peak area ratio of drug and IS versus concentration of the drug. Regression analysis was computed for Saxagliptine and Dapagliflozin. The method was evaluated by determination of correlation coefficient and intercept values according to ICH guidelines.

Limit of Detection and Limit of Quantification
Limit of detection (LOD) and limit of quantification (LOQ) of Saxagliptine and Dapagliflozin were determined by calibration curve method. Solutions of Saxagliptine and Dapagliflozin were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using the following equations:

LOD= 3 x N/ B

LOQ= 10 x N/ B

Where N is residual variance due to regression;
B is the slope.

Robustness
HPLC conditions were slightly modified to evaluate the analytical method robustness. These changes included the flow rate, column temperature and the Acetonitrile proportion in the mobile phase.

Forced Degradation Study
Alkaline, acidic, oxidative stress, thermal, water and direct exposure to UV were carried out. No internal standard was added in the forced degradation study.

1) **Alkali Hydrolysis:** Ten mL of Saxagliptine and Dapagliflozin stock solution was mixed in a flask with 1N sodium hydroxide (4mL) for 1hr at 50°C. Before analysis, the solution was cooled at room temperature and neutralized with 1N hydrochloric acid. The solution was completed with deionised water to reach the targeted concentration.

2) **Acid Hydrolysis:** Ten mL of Saxagliptine and Dapagliflozin stock solution was mixed in a flask with 1N hydrochloric acid (4mL) for 1hr at 50°C .Before analysis, the solution was cooled at room temperature and neutralized with 1N sodium hydroxide. The solution was completed with deionised water to reach a targeted concentration.

(3) **Oxidative Stress:** Ten mL of the Saxagliptine and Dapagliflozin stock solution was mixed with 1mL of 3% hydrogen peroxide and stored at 50°C for 1hr.The solution was cooled and completed with deionised water until the volumetric flask mark to reach a targeted concentration.

(4) **Sunlight Degradation:** Ten mL of the Saxagliptine and Dapagliflozin stock solution was transferred in to a 200mLvolumetric flask and exposed to direct sunlight for 5days at room temperature. The solution was completed to the

mark with deionised water to reach a targeted concentration.

(5) **Thermal Degradation:** Ten mL of Saxagliptine and Dapagliflozin stock solution was transferred in to volumetric flask (200mL) and kept in air dry oven at 105°C for 5h.Then, the solution was cooled and completed to the flask mark with deionised water to reach a targeted concentration.

(6) **Hydrolytic Degradation:** Ten mL of Saxagliptine and Dapagliflozin solution was transferred in to a volumetric flask and mixed with 10mL of deionised water. The solution was heated on water bath for 1hr.Then, the solution was cooled and completed to the flask mark with deionised water to reach a targeted concentration.

3. Results and Discussion

Validation of Method Developed:

The proposed method was validated according to the ICH guidelines[31] for system suitability, specificity, recovery, precision, linearity, robustness, limit of detection (LOD) and limit of quantification (LOQ). Under the validation study, the following parameters were studied.

System suitability:

The Retention time of Saxagliptine and Dapagliflozin using optimum conditions was 2.10min and 2.81min respectively. For two of them, the peak symmetries were <1.5 and the theoretical plates numbers were >2000 and %RSD of areas of six standard injections of Saxagliptine and Dapagliflozin was less than 2. These values are within the acceptable range of United States pharmacopoeia definition and the chromatographic conditions. The results obtained are shown in **Table 1.**

Table 1: System suitability results of Saxagliptine and Dapagliflozin

Parameter	Saxagliptine	Dapagliflozin	Glimiperide
Peak area	50614 ± 0.1*	97232 ± 0.2*	36692 ± 0.8*
Theoretical plates	3728.32±0.432**	2371.38±0.815**	6446.98±0.629**
Retention time	2.10±0.01**	2.81±0.01**	8.87±0.04**
Tailing factor	1.36±0.04**	1.25±0.03**	1.18±0.03**

*RSD (%)

**Mean of SD of six determinations

Specificity:

The specificity of the method was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. Optimized Chromatogram of Saxagliptine and Dapagliflozin is shown in **Fig. 1** clearly shows the ability of the method to assess the analyte in the presence of other excipients.

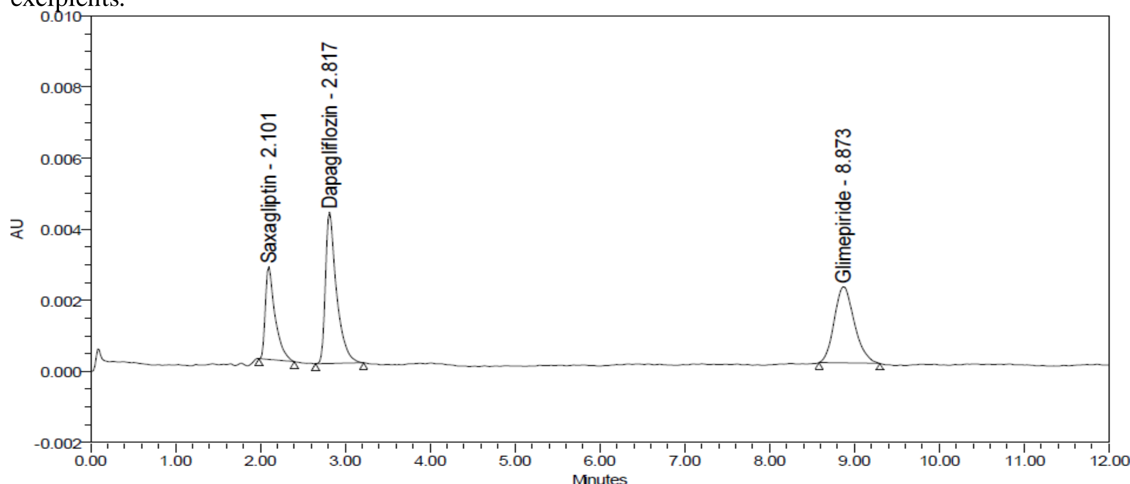

Fig. 1: Optimized Chromatogram of Saxagliptine and Dapagliflozin

Table 2: System Precision data for Saxagliptine and Dapagliflozin

Saxagliptine (40µg/mL)				**Dapagliflozin** (80µg/mL)		
S. No	**Retention time (min)**	**Peak Area**	**P/A Ratio**	**Retention time (min)**	**Peak Area**	**P/A Ratio**
1	2.093	50634	1.38	2.806	97035	2.6446
2	2.094	50543	1.3775	2.806	97154	2.6478
3	2.096	50653	1.3805	2.806	97046	2.6449
4	2.098	50634	1.38	2.806	97534	2.6582
5	2.101	50537	1.3773	2.809	97254	2.6506
6	2.102	50682	1.3813	2.814	97368	2.6537
Avg	2.14	50614	1.4	2.8	97231.83	2.6499
SD	0.004	59.9	0	0	194.768	0.0053
%RSD	0.19	0.1	0.1	0.1	0.2	0.2

Precision:

System Precision:

One dilution of both the drugs in six replicates was injected into HPLC system & was analyzed and the results were found within the acceptance limits (RSD<2) as shown in the **Table 2**.

Method Precision (Repeatability):

Six replicate injections of a known concentration of sample preparation of Saxagliptine (40 µg/mL) and Dapagliflozin (80 µg/mL) have been analyzed by injecting them into a HPLC column on the same day. From the results obtained, %RSD was calculated and was found to be within the limits (<2). The results of precision are given in **Table 3**.

Table 3: Method Precision data for Saxagliptine and Dapagliflozin

Saxagliptine (40µg/mL)				**Dapagliflozin** (80µg/mL)		
S No	Peak Area	P/ A Ratio	% Assay	Peak Area	P/ A Ratio	% Assay
1	50852	1.394	100.27	97290	2.668	99.86
2	50988	1.398	100.54	96467	2.645	99.01
3	50586	1.387	99.74	97515	2.674	100.09
4	50824	1.394	100.21	97230	2.666	99.8
5	50107	1.374	98.8	96629	2.650	99.18
6	50076	1.373	98.74	97372	2.670	99.94
Avg	50572	1.387	99.72	97083	2.662	99.65
SD	394.3	0.011	0.777	428.9	0.011	0.442
%RSD	0.779	0.780	0.780	0.441	0.441	0.443

Ruggedness:
Intermediate precision was accessed injecting sample preparation of Saxagliptine (40 µg/mL) and Dapagliflozin (80 µg/mL) in six replicates in to HPLC column on the same day and on consecutive days and in different laboratories by different analysts . Results were found within the acceptance limits (RSD<2) as shown in the **Tables 4, 5** below.

Accuracy:
A known amount of the standard drug was added to the blank sample at each level. Good recovery of the spiked drugs was obtained at each added concentration, and the mean percentage recovery of Saxagliptine and Dapagliflozin was achieved between 100.21–100.50 ± 0.148% and 99.99 – 100.13±0.74.The results are given in **Tables 6,7**.

Table 4: Ruggedness Data for Saxagliptine

Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
Concentration (µg/ml)	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
40	100.27	99.27	99.83	99.41	99.86	99.83	100.19	99.28
40	100.54	99.86	99.01	99.98	99.01	99.93	99.68	99.45
40	99.74	100.53	100.01	100.24	100.09	99.96	100.44	100.41
40	100.21	100.51	99.8	100.18	99.8	100.28	99.34	100.65
40	98.8	99.54	99.78	100.11	99.18	100.52	100.18	98.95
40	98.74	99.95	99.94	99.87	99.94	99.82	99.13	100.22
Average	99.72	99.94	99.65	99.97	99.65	100.06	99.83	99.83
SD	0.777	0.508	0.442	0.303	0.442	0.282	0.525	0.690
%RSD	0.780	0.508	0.443	0.303	0.443	0.282	0.526	0.691

Table 5: Ruggedness Data for Dapagliflozin

Laboratory-1 (% Assay)-HPLC-1					Laboratory-2 (% Assay)-HPLC-2			
Concentration (µg/ml)	Analyst-1		Analyst-2		Analyst-1		Analyst-2	
	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2	Day-1	Day-2
80	99.86	99.41	100.12	99.43	99.11	100.11	99.13	100.18
80	99.01	99.98	100.54	100.23	100.24	100.13	99.41	99.13
80	100.09	100.24	99.54	100.58	99.15	99.27	99.61	99.45
80	99.8	100.18	99.21	99.1	99.41	100.02	99.38	100.41
80	99.18	100.11	100.64	99.45	100.53	100.5	100.12	100.65
80	99.94	99.87	99.85	99.69	100.8	99.14	100.71	99.41
Average	99.65	99.97	99.98	99.75	99.87	99.86	99.73	99.87
SD	0.442	0.303	0.561	0.555	0.741	0.536	0.585	0.622
%RSD	0.443	0.303	0.561	0.556	0.742	0.537	0.586	0.622

Table 6: Recovery data of Saxagliptine

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	5	5.04	100.72	Mean = 100.50%(n=3) SD =0.2 %RSD =0.2
S2:50%	5	5.02	100.4	
S3:50%	5	5.02	100.4	
S4:100%	10	10.03	100.3	Mean = 100.3%(n=3) SD =0.2 %RSD =0.2
S5:100%	10	10.05	100.5	
S6:100%	10	10.01	100.1	
S7:150%	15	15.05	100.37	Mean = 100.21%(n=3) SD =0.2 %RSD =0.2
S8:150%	15	15.03	100.2	
S9:150%	15	15.01	100.07	

Table 7: Recovery data of Dapagliflozin

Sample name	Amount added (µg/ml)	Amount found (µg/ml)	%Recovery	Statistical Analysis
S1:50%	10	9.97	99.7	Mean = 100.1%(n=3) SD =0.4 %RSD =0.4
S2:50%	10	10.04	100.4	
S3:50%	10	10.02	100.2	
S4:100%	20	20.03	100.15	Mean = 100.13%(n=3) SD =0.1 %RSD =0.1
S5:100%	20	20.01	100.05	
S6:100%	20	20.04	100.2	
S7:150%	30	29.96	99.87	Mean = 99.99%(n=3) SD =0.1 %RSD =0.1
S8:150%	30	30.02	100.07	
S9:150%	30	30.01	100.03	

Linearity and Range:

Linearity was assessed for the two oral anti diabetic drugs at concentration ranges 20-60µg/ml for Saxagliptine and 40-120µg/ml for Dapagliflozin.A linear relationship was established at these ranges between Area under the peak (AUP) and concentration. Good linearity was proved by high values of coefficient of determinations (**Fig.2 and Fig.3**). The results were tabulated in **Table 8.**

Table 8: Linearity data of Saxagliptine and Dapagliflozin

Concentration of Saxagliptine (µg/ml)	Peak area	P/ A Ratio	Concentration of Dapagliflozin (µg/ml)	Peak area	P/ A Ratio
20	25569	0.330	40	49258	0.063
30	37880	0.489	60	72958	0.094
40	50852	0.657	80	97847	0.126
50	62833	0.812	100	120646	0.155
60	75278	0.972	120	146776	0.189

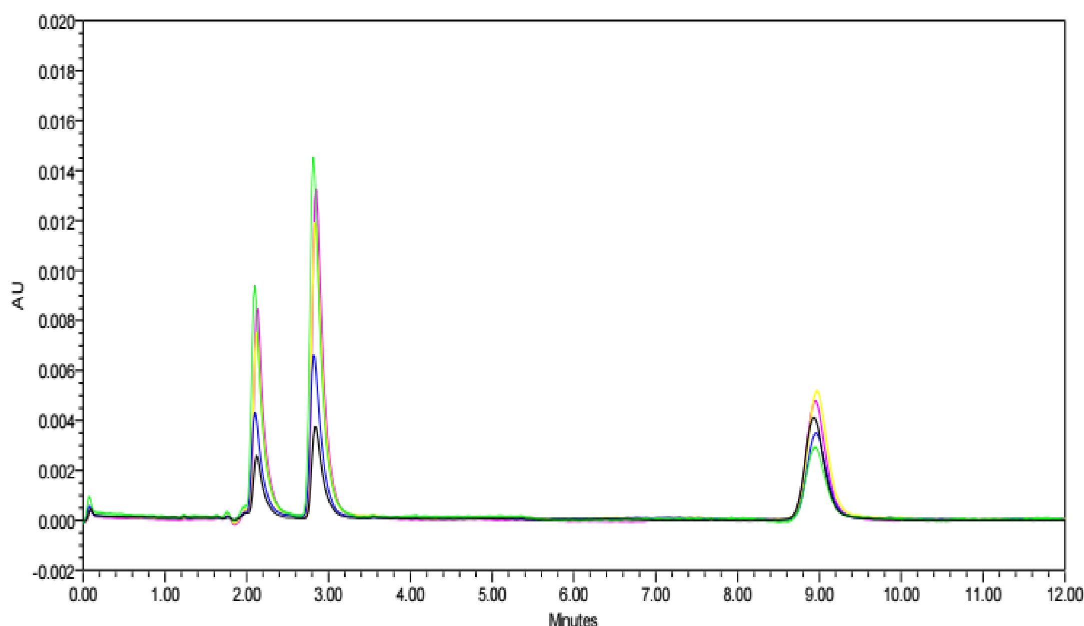

Fig.2.Overlay of Linearity Chromatograms

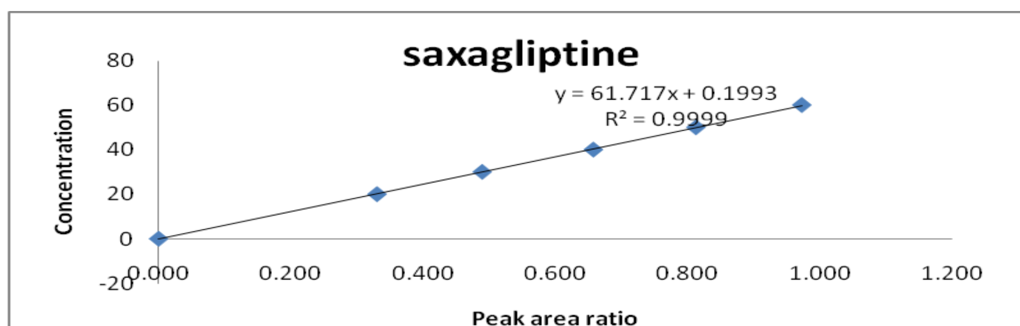

Fig.3. Linearity graph of Saxagliptine

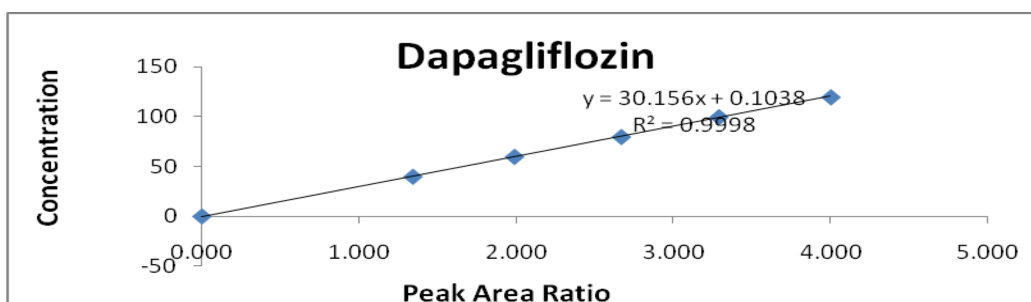

Fig.4. Linearity graph of Dapagliflozin

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The limit of detection and limit of quantification were evaluated by serial dilutions of Saxagliptine and Teneligliptine stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 for LOQ. The LOD value for Saxagliptine and Dapagliflozin was found to be 1.63 µg/mL and 1.94 µg/mL, respectively, and the LOQ value 2.39 µg/mL and 3.50µg/mL, respectively.

Robustness:

The result of robustness study of the developed assay method was established in **Tables9,10,11**. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

Table 9: **Robustness (change in flow rate) for Saxagliptine and Dapagliflozin**

Drug	Change in Flow rate (ml/min)	Change in flow Rate (0.8ml/min to 1.2 ml/min)		
		%Assay	SD	% RSD
Saxagliptine	0.8	100.3	0.412	0.41
	1	100.16	38	0.1
	1.2	99.86	0.2	0.2
Dapagliflozin	0.8	99.32	0.08	0.081
	1	100.35	80	0.1
	1.2	99.32	0.1	0.1

Table 10: Robustness (change in Mobile phase composition) for Saxagliptine and Dapagliflozin

Drug	Change in mobile phase	Change in Mobile phase (10% less organic phase & 10%more organic phase)		
		%Assay	SD	% RSD
Saxagliptine	10% less organic phase	100.1	0.3	0.3
	Actual	100.16	38	0.1
	10% more organic phase	100.1	0.5	0.5
Dapagliflozin	10% less organic phase	99.32	80	0.1
	Actual	100.35	80	0.1
	10% more organic phase	99.7	0.7	0.7

Table 11: Robustness (change in column Temparature) for Saxagliptine and Dapagliflozin

Drug	Change in column temperature	Change in column temperature		
		%ASSAY	SD	%RSD
Saxagliptine	25°C	**99.9**	**0.408**	**0.408**
	30°C	100.16	38	0.1
	35°C	99.59	0.242	0.243
Dapagliflozin	25°C	99.73	0.129	0.129
	30°C	100.35	80	0.1
	35°C	99.03	1.27	1.29

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 for acid hydrolysis (1M HCl heated for 30 min at 60°C), alkali hydrolysis (2 N NaOH heated for 30 min at 60°C), oxidative degradation (20%H2O2 heated at 60°C for 30 min) and thermal degradation (samples placed in an oven at 105°C for 6 h). For photolytic stress studies, samples were exposed to UV light by keeping them in a UV chamber for 7 days. Results are shown in **Tables 12,13**.

Table 12: Forced Degradation studies of Saxagliptine

Sample Name	(%)Assay	Degradation (%)
Unstressed Sample	100	-------
Acid Degradation	95.43	4.57
Alkali Degradation	91.29	8.71
Peroxide Degradation	96.18	3.82
Thermal Stress Sample	94.67	5.33
Photo Stress Sample	97.36	2.64

Table 13: Forced Degradation studies of Dapagliflozin

Sample Name	(%)Assay	Degradation (%)
Unstressed Sample	100	-------
Acid Degradation	93.58	6.42
Alkali Degradation	97.69	2.31
Peroxide Degradation	94.29	5.71
Thermal Stress Sample	95.36	4.64
Photo Stress Sample	94.95	5.05

The retention time of Saxagliptine and Dapagliflozin was found to be 2.10 min and 2.81 min respectively with resolution of 3.26. Linearity was established for Saxagliptine and Dapagliflozin in the range of 20-60µg/ml for Saxagliptine and 40-120µg/ml for Dapagliflozin with correlation coefficients (r^2=0.999) and the percentage recoveries were between 100.21 % to 100.50% and 99.99% to 100.13% for Saxagliptine and Dapagliflozin respectively, which indicate accuracy of the proposed method. The % RSD values of accuracy for Saxagliptine and Teneligliptine were found to be < 2 %. The % RSD values of method precision are 0.780% and 0.443% for Saxagliptine and Dapagliflozin respectively and % RSD values of system precision are 0.1% and 0.2% for Saxagliptine and Dapagliflozin. The % RSD values of reproducibility for Saxagliptine and Dapagliflozin were found to be < 2 %, reveal that the proposed method is precise. LOD values for Saxagliptine and Dapagliflozin were found to be 0.72µg/ml and 0.15µg/ml respectively and LOQ values for Saxagliptine and Dapagliflozin were found to be 2.40µg/ml and 0.51µg/ml respectively. The % RSD values of robustness studies were found to be < 2% reveal that the method is robust enough was shown in (Table 9). These data show that the proposed method is specific and sensitive for the determination of Saxagliptine and Dapagliflozin.

CONCLUSIONS:

1. RP-HPLC method for the simultaneous estimation of Saxagliptine and Dapagliflozin in their combine dosage form was developed and validated as per the ICH guidelines.
2. Linearity was observed in the range of 20-60µg/ml for Saxagliptine and 10-120µg/ml for Dapagliflozin with correlation coefficients (r^2=0.999).
3. The percentage recoveries of Saxagliptine and Dapagliflozin were in the range of 99.99-100.50% which was with in the acceptance criteria.
4. The percentage RSD was NMT 2% which proved the precision of the developed method.
5. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, specific, and robust.

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