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Method Development and Validation for Simultaneous Estimation of Dapagliflozin and Saxagliptin in Combined Formulation by RP-UPLC Method

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ABSTRACT:

A novel approach was devised to concurrently determine the quantities of Saxagliptin and Dapagliflozin in tablet form using the Rp-Uplc chromatographic technique. The method was implemented using the ACQUITY UPLC HSS C18 Column (2.1 mm X 100 mm X 1.8 µm). The mobile phase, consisting of a mixture of Acetonitrile and KH2Po4 in a ratio of 45:55, was pumped through the column at a flow rate of 0.4 ml/min. Buffer employed in this procedure The pH of the phosphate buffer is adjusted to 5.4 by adding 0.1% formic acid. The temperature was consistently maintained at 40°C. The wavelength selected for optimization was 254 nm. The retention time for Saxagliptin and Dapagliflozin was determined to be 0.578 min and 1.173 min, respectively. The relative standard deviation (RSD) of Saxagliptin and Dapagliflozin was determined to be 1.2 and 1.5, respectively. PercentageThe recovery rates for Saxagliptin and Dapagliflozin were 100.38% and 99.93% respectively. The limits of detection (LOD) and limits of quantification (LOQ) values derived from the regression equations for Saxagliptin and Dapagliflozin were determined to be 0.19 and 0.57, and 0.37 and 1.11, respectively. The regression equation for Saxagliptin is y = 7496x + 449.71. The equation for Dapagliflozin concentration is y = 7519.1x + 3994.9. The retention times and run time were reduced, making the developed method simple and cost-effective for regular adoption in quality control tests in industries.

Keywords: Dapagliflozin, Saxagliptin, RP-HPLC, Method Validation

Introduction:

The combination of Saxagliptin and dapagliflozin has the potential to confer significant benefits in glycemic control without the risk of weight gain and hypoglycemia, which may be associated with other medications used to treat type 2 diabetes. The fixed dose combination containing 10 mg of dapagliflozin and 5 mg of Saxagliptin was recently approved by the US-Food and Drug Administration (FDA) for adults with type-2 diabetes. The combination was available under the brand name Qtern.[2] Dapagliflozin belongs to the sodium glucose co-transporter-2 inhibitors with the chemical name (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl) tetrahydro-2*H*-pyran-3,4,5-triol.[1]

Saxagliptin + Dapagliflozin is a combination of two medicines Saxagliptin & Dapagliflozin. Saxagliptin is a dipeptidyl peptidase-4 inhibitor which works by increasing the release of insulin from pancreas and decreasing the hormones that raise blood sugar levels. This reduces both fasting and post meal sugar levels. Dapagliflozin works by removing excess sugar (glucose) from your body through urine.[2]

Saxagliptin and the fixed dose combination of Daxagliptin/Dapagliflozin are orally active, selective, long-acting, and reversible dipeptidyl-peptidase 4 (DPP4) inhibitors, used for the treatment of type 2 diabetes mellitus. DPP4 inhibitors enhance levels of active glucagon-like peptide 1 (GLP-1) and other incretins, and facilitate glucose-dependent insulin secretion. In addition, GLP-1 inhibits glucagon release, slows gastric emptying, reduces appetite, and regulates the growth and differentiation of the insulin producing β cells in pancreatic islets. In this application note, we describe a simple method for the simultaneous quantification of Saxagliptin and its major active metabolites, 5-Hydroxy Saxagliptin and Dapagliflozin in human plasma. This method uses a fast, selective sample preparation in the 96-well format and high-throughput Ultraperformance Liquid Chromatography tandem mass spectrometry (UPLC-MS/MS) analysis to achieve lower limits of quantification in the sub ng/mL range.[3]

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 C21H25ClO6, 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.[4]

Diabetes mellitus is one of the most common medical conditions globally. The number of people with diabetes is increasing due to population growth, aging, urbanization, increasing prevalence of obesity and physical inactivity. Some conventional therapies for type 2 diabetes mellitus (T2DM) fail to address the progressive nature of the disease, Saxagliptin was approved by the US Food and Drug Administration in July 2009 and by the European

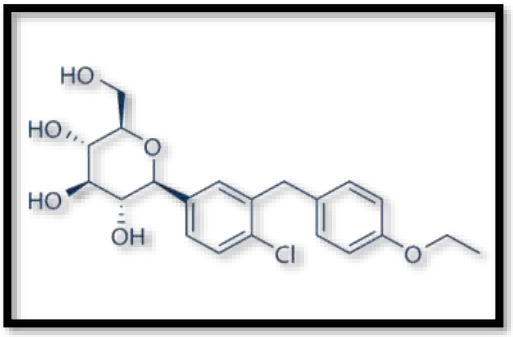
Medicines Evaluation Agency in October 2009 for use as monotherapy or in combination regimens for the treatment of type 2 diabetes mellitus.[5]An extensive literature survey has revealed that there is no reverse phase high performance liquid chromatographic (RP HPLC) method available for individual or simultaneous estimation of SAXA and DAPA in bulk, or pharmaceutical dosage forms use an experimental design approach. A few analytical methods were reported in the literature for the determination of SAXA alone. Stability indicating RP HPLC and RP-LC-PDA methods for determination of SAXA in pharmaceutical dosages were developed. [6,7] SAXA was estimated, with other antidiabetic drugs like vildagliptin, using spectrophotometric and spectrofluorimetric methods from bulk and pharmaceutical dosage forms[8] and also with metformin hydrochloride, using the RP column liquid chromatographic method in binary mixtures.[9] The HPLC method is used in active drug and pharmaceutical dosage forms[10] and stability indicating RP HPLC method for the determination of Saxagliptin and metformin in bulk and pharmaceutical product[11]

Background:

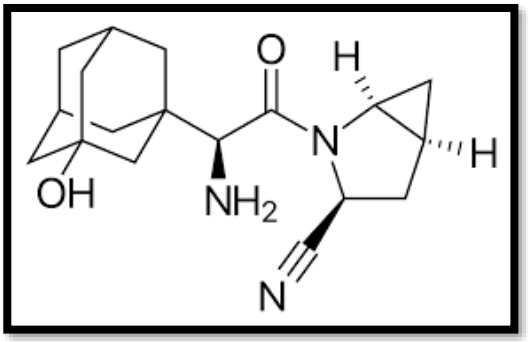
Dapagliflozin-Chemically known as $C_{21}H_{25}ClO_6$, Chemical Nomenclature of the drug given by IUPAC- (2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4ethoxyphenyl) methyl] phenyl}-6-(hydroxymethyl) oxane-3,4,5-. Dapagliflozin is an inhibitor of the sodium-glucose cotransporter 2 (SGLT2), and it was the initial SGLT2 inhibitor to receive approval. Prescribed for the treatment of type 2 diabetes mellitus.[12] When used alongside diet and exercise in adults, dapagliflozin enhances glycemic control by blocking the reabsorption of glucose in the proximal tubule of the nephron, resulting in the excretion of glucose in the urine, it has been studied both as a standalone treatment and as a supplementary therapy alongside insulin or other oral hypoglycemic agents.[13,14]

Saxagliptin- Saxagliptin

Saxagliptin (RINN) is an orally active hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. This medication is utilized for the management of type II diabetes mellitus, specifically in cases where there is a deficiency in the secretion of GLP-1 and impaired insulinotropic effects. Dual therapy with this medication is recommended in adults who have inadequate glycemic control, sulphonyl urea, or a thiazolidinedione. Dapagliflozin, Saxagliptin shown in (figure-1).



Dapagliflozin



Saxagliptin

Figure-1: Structures of Dapagliflozin, Saxagliptin.

A comprehensive literature review revealed that numerous analytical methods have been documented, with the identification of more cost-effective approaches. However, no method has been reported for estimating stability studies. Therefore, a straightforward and economical method for determining the stability of Dapagliflozin, Saxagliptin, and in a pharmaceutical dosage form using RP-UPLC is proposed.[19-25] must be developing and validated as per the guidelines of ICH (Q2 specification) [26].

Materials and Reagents

Dapagliflozin, Saxagliptin, the respective pure drugs were acquired from Spectrum Pharma research solutions. The Dapagliflozin, Saxagliptin combination tablet (QTREN) was purchased from India Mart in Hyderabad. The chemicals and buffers utilized in this estimation were obtained from Rankem, an Indian supplier.

Instrumentation

The development and method validation were conducted using a WATERS HPLC ACQUITY Premier System_Uplc, equipped with a TUv_detector. The system also included an automated sample injector and the Empower 2 software.

Objective:

The primary objective of this study is to create a highly reliable, exact, sensitive, specific, consistent, and expedient analytical method for concurrently determining the quantities of Dapagliflozin and Saxagliptin in both their pure form and tablet formulation.

Chromatographic Conditions:

Flow rate: 0.4ml/min

Column: ACQUITY UPLC HSS C18 Column (2.1 mm X 100 mm X 1.8 µm)

Buffer: Di Potassium phosphate buffer

Detector: 254.0 nm

Temperature: Ambient

Injection volume: 1.0µL

Run time: 3.0 mins

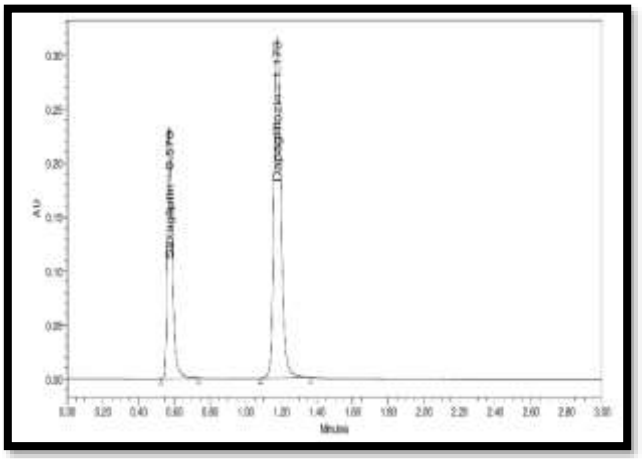


Fig 2 Optimized Chromatogram

Preparation of Buffer

Preparation of 0.01N di-sodium hydrogen phosphate Buffer: Weighed precisely 1.36 grams of potassium dihydrogen Ortho phosphate and added it to a 1000 milliliter Volumetric flask. Approximately 900 milliliters of milli-Q water were then added and degassed through sonication. The flask was filled with water to reach the desired volume, and the pH was adjusted to 5.4 using diluted Formic acid.

Preparation of Standard solution: Precisely measured 5mg of Saxagliptin and 10mg of Dapagliflozin, and then transferred them individually into separate 25ml volumetric flasks. Three-fourths of the diluent was added to both flasks and subjected to sonication for a duration of 10 minutes. The flasks were prepared by combining a diluent and labeled as the Standard stock solution. The concentration of Saxagliptin is 200µg/ml and the concentration of Dapagliflozin is 400µg/ml.

Preparation of Standard working solution: 1ml from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20 µg/ml of Saxagliptin and 40µg/ml of Dapagliflozin)

Preparation of Sample solution: 10 tablets were assessed for weight, and the mean weight of each tablet was determined. Subsequently, the weight corresponding to a single tablet containing Saxagliptin (5mg) and Dapagliflozin (10mg) was transferred into a 50 ml volumetric flask. To this, 50ml of diluent was added, and the mixture was subjected to sonication for 25 minutes. The final volume was adjusted by adding more diluent, and the solution was filtered using UPLC filters. The concentration of Saxagliptin is 100µg/ml and the concentration of Dapagliflozin is 200µg/ml.

Preparation of Sample working solution: 2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Saxagliptin and 40µg/ml of Dapagliflozin)

Method Validation

The HPLC method was validated to simultaneously estimate the drug substances Dapagliflozin and Saxagliptin, following the ICH guidelines. This was done to show that the method is suitable for routine analysis.

System suitability:

The system suitability parameters were determined by preparing standard solutions of Saxagliptin(20ppm) and Dapagliflozin(40ppm). These solutions were then injected six times to determine parameters such as peak tailing, resolution, and USP plate count. The relative standard deviation (RSD) for the area of six standard injections should not exceed 2%. System suitability chromatogram was shown in figure 3 and values are mentioned in the table 1.

Specificity (Selectivity):

Checking of the interference in the optimized method. We have not found interfering peaks in blank and placebo at retention times of these drugs in this Approach. This method was described as specific. Figure 4 displays a representative chromatogram, while Table 2 presents the experimental data.

Table 1: System suitability data

S no	**Saxagliptin**			**Dapagliflozin**			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	0.573	2024	1.53	1.179	4726	1.24	9.9
2	0.574	2046	1.53	1.181	4626	1.25	9.9
3	0.574	2076	1.51	1.181	4602	1.25	9.9
4	0.574	2068	1.52	1.183	4586	1.25	9.9
5	0.575	2078	1.52	1.187	4566	1.25	9.9
6	0.575	2060	1.53	1.19	4520	1.25	9.9

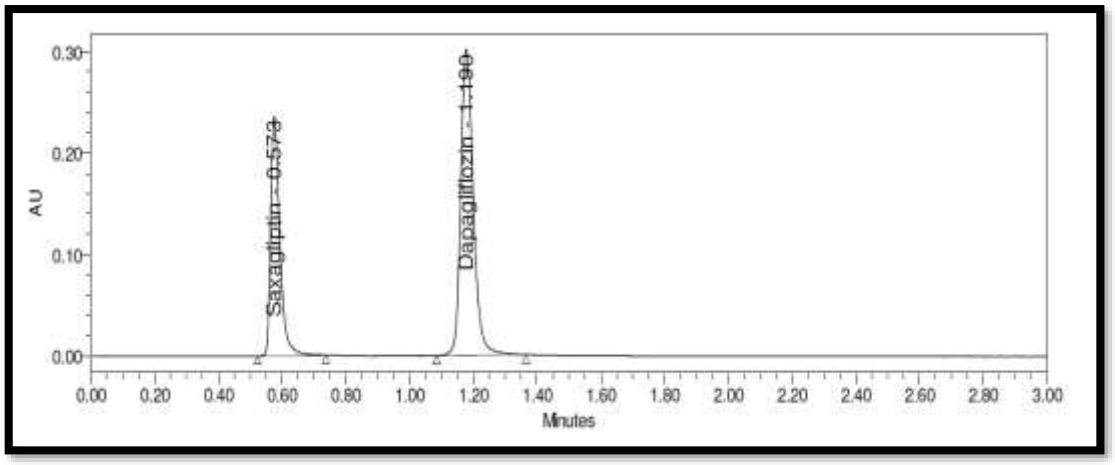
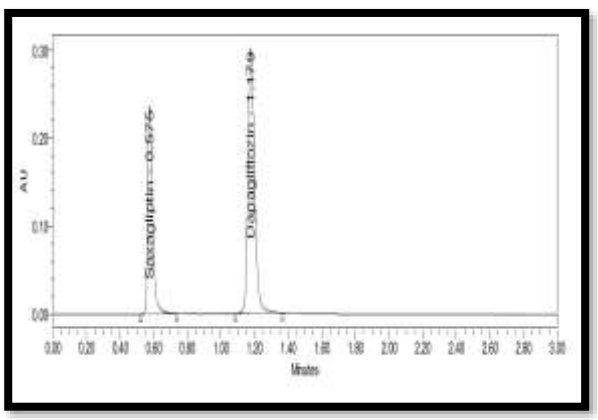


Figure 3: System suitability Chromatogram of Dapagliflozin and Saxagliptin.

Table 2: Specificity data

Sample name	**Retention time(mins)**	**Area**
Dapagliflozin	1.173	315860
Saxagliptin	0.578	145499



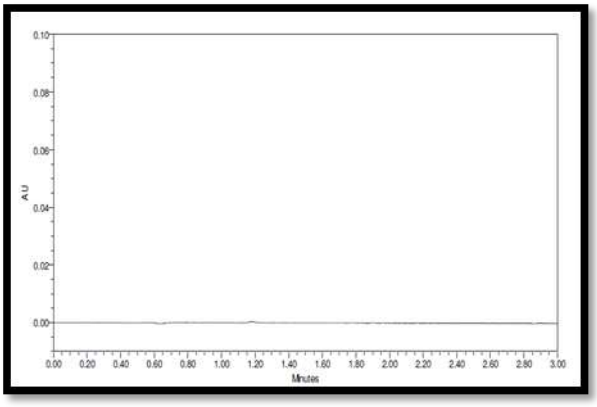


Figure 4: Specificity Chromatogram of Dapagliflozin, and Saxagliptin.

Table 3: Dapagliflozin Linearity

% Level	**CONC**	**Area**
0	0	0
25%	10	79036
50%	20	156314
75%	30	233055
100%	40	307117
125%	50	381734
150%	60	449713
R^2 value		0.999

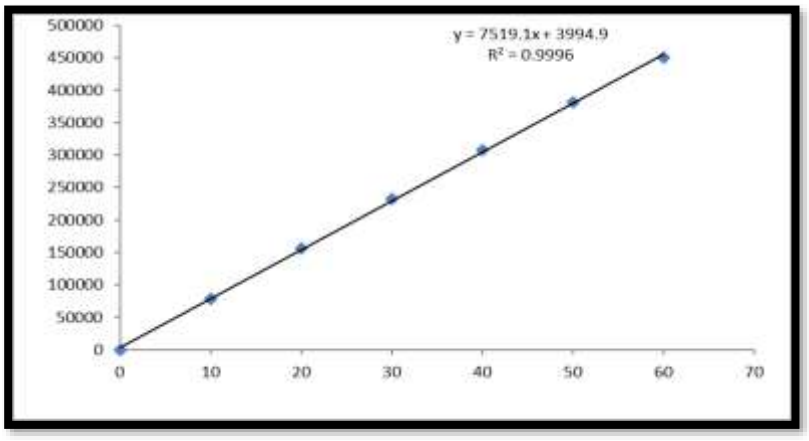


Figure 5: Dapagliflozin Calibration curve

Table 4: Saxagliptin Linearity

% Level	**CONC**	**Area**
0	0	0
25%	5	37734
50%	10	74667
75%	15	115307
100%	20	149649

125%	25	188691
150%	30	224181
R^2 value		0.999

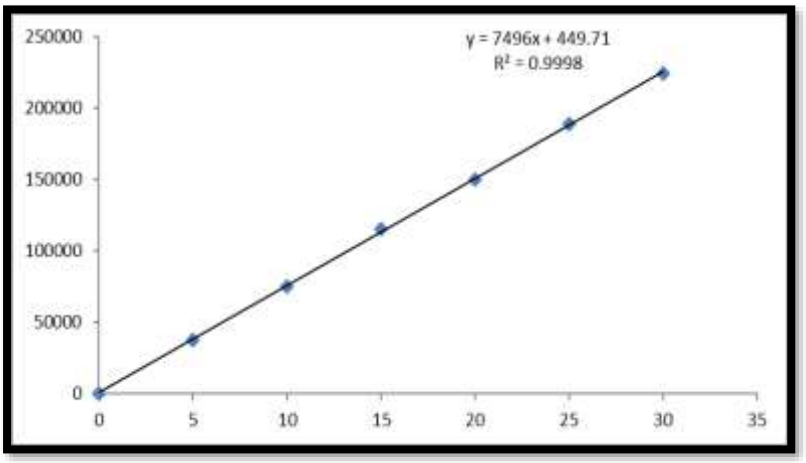


Figure 7: Saxagliptin Calibration curve

Table 6: Accuracy (%Recovery data)

%Level	**Recovery Data**					
	Dapagliflozin			**Saxagliptin**		
	Amt added	Amt found	%Rec	Amt added	Amt found	%Rec
50% Level	20	19.90	99.51	10	10.03	100.28
	20	20.04	100.18	10	10.07	100.68
	20	20.13	100.67	10	9.96	99.64
100%Level	40	40.20	100.51	20	20.17	100.83
	40	39.96	99.91	20	19.98	99.88
	40	39.65	99.11	20	20.15	100.77
150%Level	60	59.80	99.67	30	30.27	100.90
	60	60.02	100.04	30	30.12	100.39
	60	59.87	99.78	30	30.01	100.03
Mean%			**99.93**			**100.38**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Dapagliflozin and Saxagliptin. Results of peak area are summarized in Table 6

Table 7: System precision data

Injection	**Dapagliflozin**	**Saxagliptin**
1	313479	149589
2	323688	146575
3	316006	148582
4	312070	145377

5	314335	149618
6	310270	149501
Avg	314975	148207
Std dev	4694.7	1810.4
%RSD	1.5	1.2

The % RSD for the peak areas of Dapagliflozin and Saxagliptin obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Dapagliflozin and Saxagliptin. (Six individual sample preparations). Data obtained is summarized in Table 8.

Table 8: Method precision data

Injection	**Dapagliflozin**	**Saxagliptin**
1	315037	148682
2	314059	149572
3	318068	147901
4	312337	148931
5	315778	149230
6	317680	149041
Avg	315493	148893
Std dev	2178.0	570.4
%RSD	0.7	0.4

From the above results, the % RSD of method precision study was within the limit for Dapagliflozin and Saxagliptin.

Table 9: Robustness results: - The robustness conditions were upheld, which involved a decrease in flow rate of 0.3ml/min, an increase in flow rate of 0.5ml/min, a decrease in mobile phase composition to 50% B and 50% A, an increase in mobile phase composition to 60% B and 40% A, a decrease in temperature to 38°C, and an increase in temperature to 42°C. The specimens were duplicated upon injection. The system suitability parameters were only slightly affected, and all parameters satisfied the necessary criteria. The %RSD value met the specified limit.

S.no	Condition	%RSD of Saxagliptin	%RSD of Dapagliflozin
1	Flow rate (-) 0.3ml/min	0.5	0.5
2	Flow rate (+) 0.5ml/min	0.9	1
3	Mobile phase (-) 50B:50A	0.9	0.9
4	Mobile phase (+) 60B:40A	1.4	0.6
5	Temperature (-) 38°C	1.2	1
6	Temperature (+) 42°C	1.3	0.7

Table 10: Forced degradation conditions for Dapagliflozin and Saxagliptin.

Stress condition	**Solvent**	**Temp(^{0}C)**	**Exposed time**
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxdation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

From the results, no degradation was observed when the samples were exposed to acid, base, hydrolysis, thermal, light and water. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 11: Degradation profile results

Type of degradation	**Saxagliptin**			**Dapagliflozin**		
	Area	% Recovered	% Degraded	Area	% Recovered	% Degraded
Acid	140852	94.94	5.06	294581	93.43	6.57
Base	146150	98.51	1.49	308146	97.73	2.27
Peroxide	139274	93.88	6.12	299068	94.85	5.15
Thermal	138734	93.51	6.49	294662	93.46	6.54
Uv	147759	99.60	0.40	310571	98.50	1.50
Water	148528	99.60	0.40	313631	99.47	0.53

Table 12: Assay results for Dapagliflozin and Saxagliptin

(QTERN), bearing the label claim Dapagliflozin and Saxagliptin 5MG, 10MG. Assay were performed with the above formulation.

Drug Name	**Label claim dose**	**%Assay**
Dapagliflozin	5mg	100.06
Saxagliptin	10mg	100.36

Assay was performed by: -

The weight of 10 tablets was measured and the average weight of each tablet was calculated. Subsequently, the weight attributed to a single tablet was transferred into a volumetric flask with a capacity of 100 ml. A total of 50 milliliters of diluent was added, and the mixture underwent sonication for a duration of 25 minutes. Afterwards, the volume was modified with a diluent and then passed through HPLC filters for filtration. The Dapagliflozin concentration is 200µg/ml and the Saxagliptin concentration is 100µg/ml. Preparation of the working solution sample: 2 milliliter of the filtered sample stock solution was transferred into a 10-milliliter volumetric flask and then filled with diluent to the top. The concentration of Dapagliflozin is 10 µg/ml and the concentration of Saxagliptin is 100 µg/ml. After injecting six samples of the formulation, the relative standard deviation (RSD) for the area of the six standard injections should not exceed 2%.

Assay was calculated by: -

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{25} \times \frac{1}{10} \times \frac{50}{1} \times \frac{10}{2} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of test solution
AS	Mean peak area of standard solution
WS	Weight of working standard taken in mg
P	Assay of working standard in % on dried basis
L.C	Label Claim
Avwt	Average weight of a drug

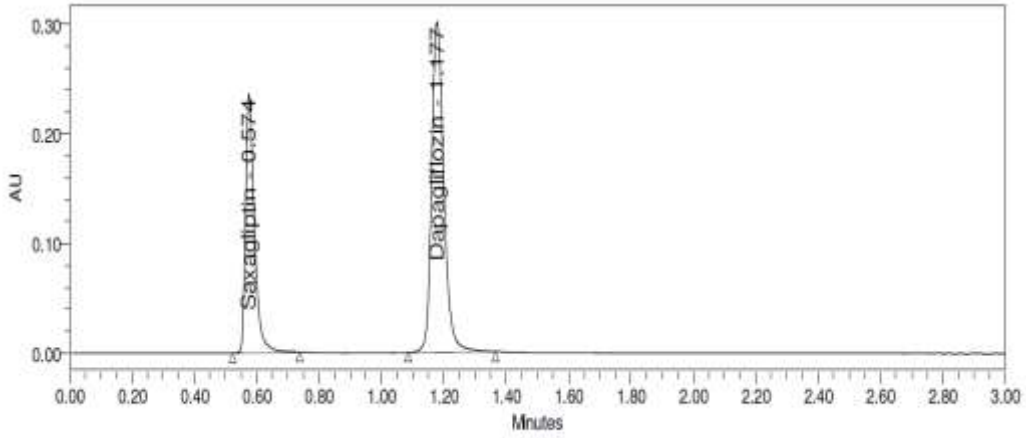


Figure 8: Assay Chromatogram

CONCLUSION

A novel and validated stability indicating analytical approach was developed using RP-UPLC methodology. The study's results will significantly assist in the quality assessment of Dapagliflozin and Saxagliptin in pharmaceutical formulations. This is attributed to the study's uncomplicated sample preparation technique, which employs a minimal quantity of mobile phase and necessitates only a short analysis duration. After examining two medications in a combined dosage form, the results showed a nearly perfect effectiveness of 100% using the newly developed methodology. The recovery studies yielded positive results, suggesting that the excipient has no discernible impact.

REFERENCES:

1. Sharmila D et al., Simultaneous Estimation of Saxagliptin and Dapagliflozin in Human Plasma by Validated High Performance Liquid Chromatography - Ultraviolet Method, Turk J Pharm Sci. 16(2): 227–233 2019.
2. Dr. Anuj Saini, Information About Saxagliptin + Dapagliflozin, 1mg.com 2023
3. Rajesh PMN et al., Simultaneous Quantification of Saxagliptin, 5-Hydroxy Saxagliptin, and Dapagliflozin in Human Plasma Using SPE and UPLC-MS/MS Analysis, waters 2018
4. Y. Surendranath Reddy et al., New Stability Indicating Uplc Method For Simultaneous Determination OF DAPAGLIFLOZIN AND SAXAGLIPTIN, IJPSR Volume 10, Issue 7, (2019).
5. S. Madhavi et al., DEVELOPMENT AND VALIDATION OF A METHOD FOR SIMULTANEOUS DETERMINATION OF DAPAGLIFLOZIN AND SAXAGLIPTIN IN A FORMULATION BY RP-UPLC, WJPR, Vol 6, Issue 12, 2017.
6. Scheeren LE, Marcolino AIP, Adams AIH, Rolim CMB. Stability indicating RP-LC-PDA method for the quantitative analysis of saxagliptin in pharmaceutical dosage form. Braz J Pharm Sci. 2015;51(2):461-6.

7. Konari SN, Jacob JT. Stability indicating validated RP-HPLC technique for the analysis of multicomponent anti-diabetic drug combos in pharmaceutical dosage forms. Karbala Int J Mod Sci. 2015;1(1):39-48.
8. Marwa SM. Spectrophotometric and spectrofluorimetric methods for the determination of saxagliptin and vildagliptin in bulk and pharmaceutical preparations. Bull Fac Pharm, Cairo Univ. 2013;51(2):139-50.
9. Mohammad AA, Ehab FE, Marwa AF. Development and validation of a reversed-phase column liquid chromatographic method for simultaneous determination of two novel gliptins in their binary mixtures with Metformin. Chem Eur J. 2012;3(2):152-5.
10. Hanan AM, Nesrin KR, Sherine SD, Azza AM. Chromatographic methods for the simultaneous determination of binary mixture of saxagliptin HCl and metformin HCl. Bull Fac Pharm, Cairo Univ. 2017;55(2):311-7.
11. Ramesh J, Senthil Kumar N. A validated high performance liquid chromatography method for the determination of saxagliptin and metformin in bulk and pharmaceutical dosage form, a stability indicating study. IOSR J Pharma Biol Sci. 2016;11(6):92-100.
12. . Y. S. Reddy Et Al, New Stability Indicating Uplc Method For Simultaneous Determination Of Dapagliflozin And Saxagliptin, IJPSR, 2019
13. Rajesh PMN Et Al, Simultaneous Quantification Of Saxagliptin, 5-Hydroxy Saxagliptin, And Dapagliflozin In Human Plasma Using SPE And UPLC-MS/MS Analysis, Waters, 2018
14. Sherif A Abdel-Gawad Et Al, Spectrodensitometric And Ultra-Performance Liquid Chromatographic Quantification Of Dapagliflozin And Saxagliptin In Their Dosage Form And Human Plasma, Tropical Journal Of Pharmaceutical Research June 2021; 20 (6): 1223-1231.
15. Karthikeyan. V, A Stability Indicating Rp-Uplc Method For The Simultaneous Estimation Of Metformin, Dapagliflozin And Saxagliptin In Bulk And Tablet Dosage Form, Repository, The Tamil Nadu Dr. M.G.R. Medical University,2020
16. Upender Rao Eslawath Et Al, Stability Indicating Rp-Uplc Method Development And Validation For The Simultaneous Estimation Of Metformin Hydrochloride, Saxagliptin And Dapagliflozin In Their Bulk And Pharmaceutical Dosage Forms, Eur. Chem. Bull. 2023,12(Special Issue 12), 186 – 198
17. Devi Velmurugan et al, A stability indicating RP-UPLC method for the simultaneous estimation of Metformin, Dapagliflozin and Saxagliptin in bulk and tablet dosage form, Vensel Org, 2022
18. Sharmila Donepudi et al, Simultaneous Estimation of Saxagliptin and Dapagliflozin in Human Plasma by Validated High Performance Liquid Chromatography - Ultraviolet Method, Turk J Pharm Sci. 2019 Jun; 16(2): 227–233.
19. Aruna Gundala et al, Application of quality by design approach in RP-HPLC method development for simultaneous estimation of saxagliptin and dapagliflozin in tablet dosage form, Braz. J. Pharm. Sci. 55 • 2019
20. N. singh et al, Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of dapagliflozin and saxagliptin in fixed-dose combination, New J. Chem., 2018.
21. Sayali S. More et al, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Saxagliptin and Dapagliflozin in Tablets, AJPT, 2018
22. B. Reddy Padmaja et al, A Highly Validated Rp-Hplc Method Development for The Simultaneous estimation Of Dapagliflozin and Saxagliptin In Tablet Dosage Forms
23. R. Aswini et al, A Novel RP-HPLC Method For Simultaneous Estimation Of Dapagliflozin And Saxagliptin In Bulk And Pharmaceutical Dosage Form, IJPSR, 2018
24. B. Rama Rao et al, RP- HPLC Method for Simultaneous Estimation of Dapagliflozin and Saxagliptin in Bulk Samples, J. Pharm. Sci. & Res. Vol. 11(1), 2019, 254-257
25. Rama Kumar Kandula et al, Stability Indicating and Simultaneous Determination of Saxagliptin, Dapagliflozin and Metformin in Tablet dosage form using RP-HPLC, RJPT, 2021
26. ICH, Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, IFPMA , Geneva , (1996).