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A NEW STABILTY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF SAXAGLIPTINE AND DAPAGLIFLOZIN IN BULK AND COMBINED TABLET DOSAGE FORMS

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ABSTRACT

A simple, rapid, precise, accurate and robust stability-indicating RP-HPLC method has been developed and validated to estimate Saxagliptin hydrochloride and Dapagliflozin in bulk and in tablet form. The samples were isocratically eluted using a C_{18} (250 cm x 4.6cm*5µ) Primesil ODS column with mobile phase Potassium dihydrogren phosphate Buffer (pH 6.0): Acetonitrile (45:55 v/v) at wavelength 247 nm. A good linear response was obtained in the range from 5-30 µg/mL of Saxagliptin hydrochloride and 10-60 µg/mL of Dapagliflozin. The method was quantitatively evaluated in terms of linearity, precision, accuracy (recovery), selectivity and robustness as per ICH guideline. Forced degradation conditions of hydrolysis (neutral, acidic and alkaline), oxidation, photolysis and thermal stress, as suggested in the ICH guideline Q1A (R2) were estimated. The drug showed instability in hydrolysis (neutral, acidic and alkaline), oxidation, photolysis and thermal stress conditions.

KEYWORDS

Stability indicating RP-HPLC**,** Saxagliptin Hydrochloride, Dapagliflozin, PDA Detection and Tablet Dosage Forms.

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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[2,3].

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 re absorption of glucose by the kidney, leading to excretion of excess glucose in the urine, thereby 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].

Experiment

Chemicals and reagents

All reagents and solvent were of analytical grade; they included water (HPLC grade), Acetonitrile (HPLC grade-Merck), Potassium dihydrogen phosphate (AR Grade), ortho phosphoric acid (AR grade) which were purchased from Merck Ltd., Mumbai, India. The Saxagliptin hydrochloride and Dapagliflozin was procured from Hetero Pharma. PVT LTD (Gift sample from MSN Pharma chem., Telangana, India). The tablets containing Saxagliptin hydrochloride 5 mg and Dapagliflozin 10 mg under brand name QTERN was procured from Astra Zaneca Pharma.Pvt. Ltd. India. The solution filtered through 0.45–l filters.

Instrumentation

A Young lin instruments, CYBER LAB-USA with Chrome-3000 Operation software, containing isocratic system and UV-VIS detector were used for the study. Chromatographic separation was performed by Prime sil C_{18} (250 x 0.46 cm) ODS column.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Mobile phase	:	Acetonitrile: Potassium dihydrogren phosphate (75:25 v/v)
System	:	Isocratic.
Column Stationary Phase	:	Primesil Column: (ODS C_{18} (4.6mm x 250mm, 5 µ).
Flow rate	:	1.0 mL/min
Detection wavelength	:	247 nm
Injection volume	:	10µL
Column oven temperature	:	40°C
Diluent	:	Aceetonitrile: water (90:10V/V)
Run Time	:	6 Minutes
Retention Time	:	2.3 and 3.2 min. for saxagliptin and Dapagliflozin

Preparation of the stock solution

25 mg of each of Saxagliptin and Dapagliflozin were accurately weighed and transferred 25 mL Volumetric flask, added 15 ml diluent (Acetonitrile : water 75:25 v/v) sonicated to dissolve. Make up to the volume with diluent and mixed to obtain 1000 µg/mL of Saxagliptin hydrochloride and Dapagliflozin respectively.

Preparation of the standard working solution

Dilute 2.5 mL of standard stock solution to 25 mL volumetric flask, make up to the volume with

diluent and mixed to obtain 100 µg/mL of Saxagliptin hydrochloride and Dapagliflozin.

Preparation of the working sample solution

The powder from twenty tablets were weighed and take equivalent to 12.5 mg of Saxagliptin hydrochloride and 25 mg of Dapagliflozin was transferred to a 25 ml volumetric flask and make up volume up to the mark with diluent. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 5 ml of this solution was diluted to 25 ml with diluent. The solution was injected 10 µl. The areas of resulting peak were measured at 247 nm.

Method validation

The chromatographic conditions described in the present manuscript were found to be appropriate for the quantitative determination. After the analytical conditions had been optimized, certain parameters such as the linearity, precision, accuracy (recovery), selectivity, and robustness were evaluated to validate the method[5-6].

Linearity and range

The linearity for Saxagliptin hydrochloride and Dapagliflozin were assessed by analysis of combined standard solution in range of 5-30 µg/mL and 10-60 µg/mL respectively by taking 1, 2, 3, 4, 5, 6 ml solutions were pipette out from the Stock solution of Saxagliptin hydrochloride and Dapagliflozin. The solutions were injected into the HPLC system. The calibration curves were constructed by plotting the peak area versus the concentrations of Saxagliptin hydrochloride and Dapagliflozin and the regression equations were determined. Linearity data for Saxagliptin Hydrochloride and Dapagliflozin are shown in Table No.1. Linearity spectra of both drug and calibration curve of Saxagliptin Hydrochloride and Dapagliflozin are shown in Figure No 6, 7, 8.

Accuracy

Accuracy of the method was confirmed by recovery study at three level of standard addition drug solution was taken in three volumetric flasks. Spike 50%, 100%, 150% of standard solution in volumetric flask and diluted up to mark with diluent. The area of each solution peak was measured at 247nm. The amount of Saxagliptin HCl and Dapagliflozin was calculated at each level and % recoveries were calculated. Results of recovery study are shoen in Table No.2.

Intraday and Interday Precision

Precision for 3 intraday and interday was estimated using three concentrations in triplicate. Results for intraday and interday precision are shown in Table No.3 and 4 respectively.

LOD and LOQ (limit of Detection and Quantification)

LOD and LOQ are calculated as per ICH guideline. Results of LOD and LOQ are shown in Table No.5.

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (± 0.2) 5.8 and 6.2.
3. Ratio of Mobile phase was changed (±2) Buffer: Acetonitrile (75:25) and Buffer: Acetonitrile 73:27).

Estimation of marketed formulation

Take synthetic mixture equivalent to 12.5 mg of Saxagliptin HCl and 25 mg of Dapagliflozin was transferred to a 25 mL volumetric flask, and make up volume up to the mark with diluent. The solution was filtered through Whatman filter paper no. 42 and first few drops of filtrate were discarded. 5 ml of this solution was diluted to 25 mL with diluent. The solution was injected 10 µl. The areas of resulting peak were measured at 247 nm. Results are shown in Table No.6.

Force Degradation study

Degradation of Saxagliptin HCl and Dapagliflozin in 5ml 2 N HCl for 30 minutes at 80ºC under reflux condition. The results showed one peak for the degradation products. Major degradation peaks were found at 3.617. Retention time of Saxagliptin HCl and Dapagliflozin was found to be 2.78 and 2.44 min respectively. The % drug degradation was found to be 3.23% for Saxagliptin HCl and 3.12% for Dapagliflozin in standard solution and 3.23% for Saxagliptin HCl and 4.05% for Dapagliflozin in sample solution. HPLC chromatogram of acid

degradation of both the drug are shown in Figure No.9, 10 in standard and sample solution respectively.

Degradation of Saxagliptin HCl and Dapagliflozin 10 ml 0.1 N NaOH for 15 minutes at 80ºC reflux condition

The results showed multiple peaks for the degradation products. Major degradation peaks were found at 2.36, 5.183 and 7.600 mins. Retention time of Saxagliptin HCl and Dapagliflozin was found to be 2.74 and 4.43 min respectively. The % drug degradation was found to be 21.36% for Saxagliptin HCl and 3.94% for Dapagliflozin in standard solution and 20.35% for Saxagliptin HCl and 3.13% for Dapagliflozin in sample solution. HPLC chromatogram of alkali degradation in standard and sample are shown in Figure No.11 and 12 respectively.

Oxidation degradation of Saxagliptin HCl and Dapagliflozin using 5ml 6%H2O2

The results showed one peak for the degradation products. Major degradation peaks were found at 2.275 min. Retention time of Saxagliptin HCl and Dapagliflozin was found to be 2.78 and 4.44 min respectively. The % drug degradation was found to be 11.96% for Saxagliptin HCl and 1.63% for Dapagliflozin in standard solution and 12.18% for Saxagliptin HCl and 1.49% for Dapagliflozin in sample solution. HPLC chromatogram of peroxide degradation of both the drug are shown in Figure No.13, 14 in standard and sample solution respectively.

Thermal degradation of Saxagliptin HCl and Dapagliflozin for 2 hr 800C

The results showed some peaks for the degradation products. Major degradation peaks were found at 2.192 min. Retention time of Saxagliptin HCl and Dapagliflozin was found to be 2.72 and 3.32 min respectively. The % drug degradation was found to be 8.30% for Saxagliptin HCl and 2.63% for Dapagliflozin in standard solution and 7.03% for Saxagliptin HCl and 1.84% for Dapagliflozin in sample solution. HPLC chromatogram of peroxide degradation of both the drug are shown in Figure No.15, 16 in standard and sample solution respectively.

Table No.1: Linearity data of Saxagliptin and Dapagliflozin

S.No	Concentration (µg/ml) Saxagliptin Hydrochloride	Avg area ±SD (n=3) Saxagliptin Hydrochloride	Concentration (µg/ml) Dapagliflozin	Avg area ±SD (n=3) Dapagliflozin
1	5	40495	10	78378
2	10	41622	20	80147
3	15	41596	30	80982
4	20	40637	40	78964
5	25	40989	50	80021
6	30	41256	60	

Table No.2: Accuracy data of Saxagliptin and Dapagliflozin

S.No	Name of the drug	Conc. Level (%) (n=3)	Sample added (µg/ml) (n=3)	Amount Recovered (µg/ml) (n=3)	% Recovery (n=3)	% Mean Recovery ± S.D (n=3)
1	Saxagliptin HCl	50	2.5	2.48	99.93	99.89±0.34
		100	5.0	4.96	98.99	98.90±0.76
		150	7.5	7.48	99.90	99.56±0.78
2	Dapagliflozin	50	5	4.97	99.97	98.79±90
		100	10	9.95	99.96	99.78±0.78
		150	15	14.93	99.89	99.79±0.23

Table No.3: Robustness data- flow rate 0.9 mL/min

S.No	Drug	Retention time (min)	Tailing factor	Number of theoretical plates	Resolution
1	Saxagliptin	2.3	1.2	2159	------
2	Dapagliflozin	3.2	1.0	2110	7.9

Table No.4: Robustness data- flow rate 1.1 mL/min

S.No	Drug	Retention time (min)	Tailing factor	Number of theoretical plates	Resolution
1	Saxagliptin	2.27	1.1	2231	------
2	Dapagliflozin	3.28	1.45	3470	7.35

Table No.5: Data of LOD and LOD

S.No	Drugs	LOD	LOQ
1	Saxagliptin.HCL	1.3	3.96
2	Dapagliflozin	0.76	1.57

Table No.5: Robustness data- less organic composition

S.No	Drug	Retention time (min)	Tailing factor	Number of theoretical plates	Resolution
1	Saxagliptin	2.27	1.1	2643	-----
2	Dapagliflozin	3.28	1.5	2110	7.45

Table No.6: Robustness data- More organic composition

S.No	Drug	Retention time (min)	Tailing factor	Number of theoretical plates	Resolution
1	Saxagliptin	2.27	1.1	2770	------
2	Dapagliflozin	3.28	1.3	2790	7.6

Table No.6: Estimation of Marketed Formulations

S.No	Brand Name	Label claim		Assay (%of Label Claim) Mean± S.D .(n=6)	
1	QTERN	Saxagliptin	Dapagliflozin	Saxagliptin	Dapagliflozin
		5% w/w	10%w/w	98.99±0.237	99.26±0.29

Table No.7: Percentage of degradation of Saxagliptin and Dapagliflozin

Drugs	Sample treatment	% Assay	% degradation	Purity angle	Purity threshold
Saxagliptin	Acid	98.65	2.44	1.04	0.37
	Base	99.74	0.36	0.90	0.43
	Peroxide	97.37	1.63	1.25	0.39
	Thermal	99.41	0.59	0.94	0.37
	Photolytic(UV)	99.52	0.48	0.77	0.38
	Water	98.66	2.34	1.01	0.37
Dapagliflozin	Acid	97.82	2.18	0.82	0.44
	Base	98.56	1.44	1.01	0.53
	Peroxide	95.91	4.09	1.26	0.53
	Thermal	99.75	0.25	1.01	0.51
	Photolytic(UV)	98.16	1.84	0.87	0.51
	Water	98.11	1.89	3.1	0.60

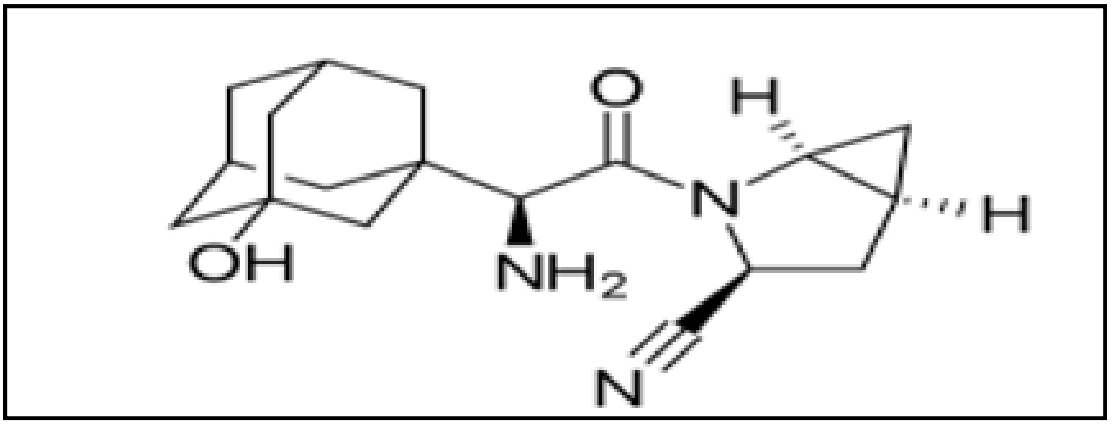


Figure No.1: Chemical structure of Saxagliptin Hydrochloride

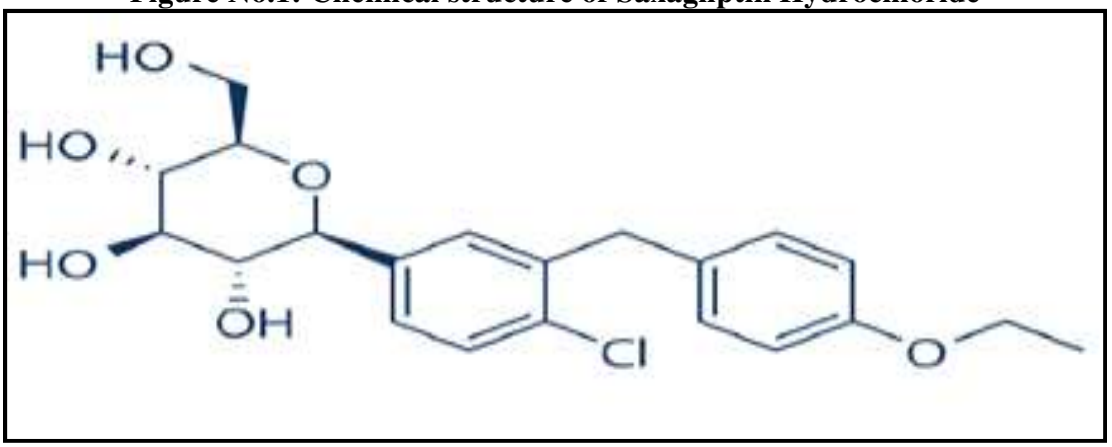
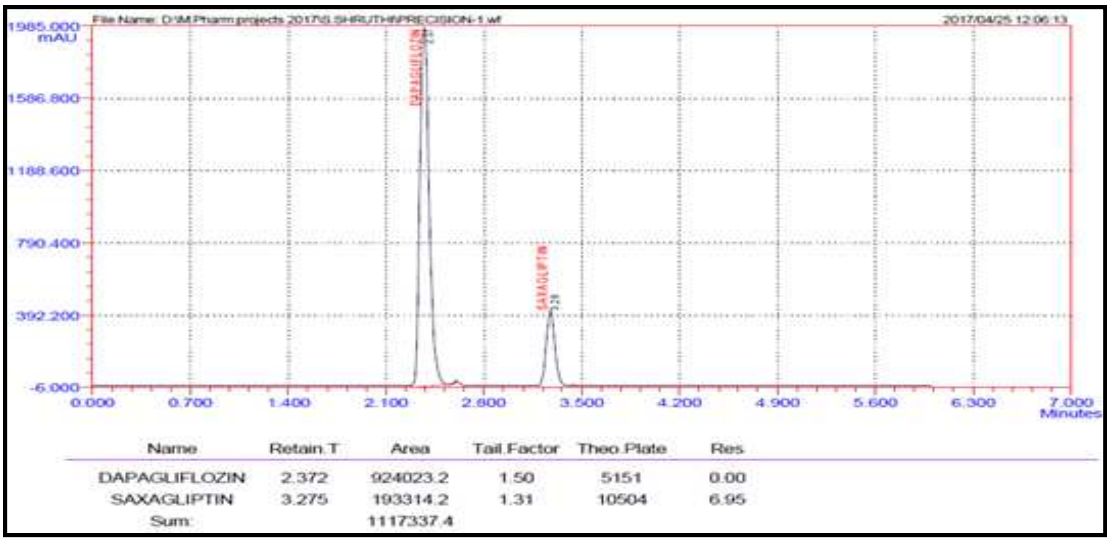


Figure No.2: Chemical structure of Dapagliflozine



Name	Retain.T	Area	Tail.Factor	Theo.Plate	Res.
DAPAGLIFLOZIN	2.372	924023.2	1.50	5151	0.00
SAXAGLIPTIN	3.275	193314.2	1.31	10504	6.95
Sum:		1117337.4			

Figure No.3: Typical chromatogram of Saxagliptin and Dapagliflozin

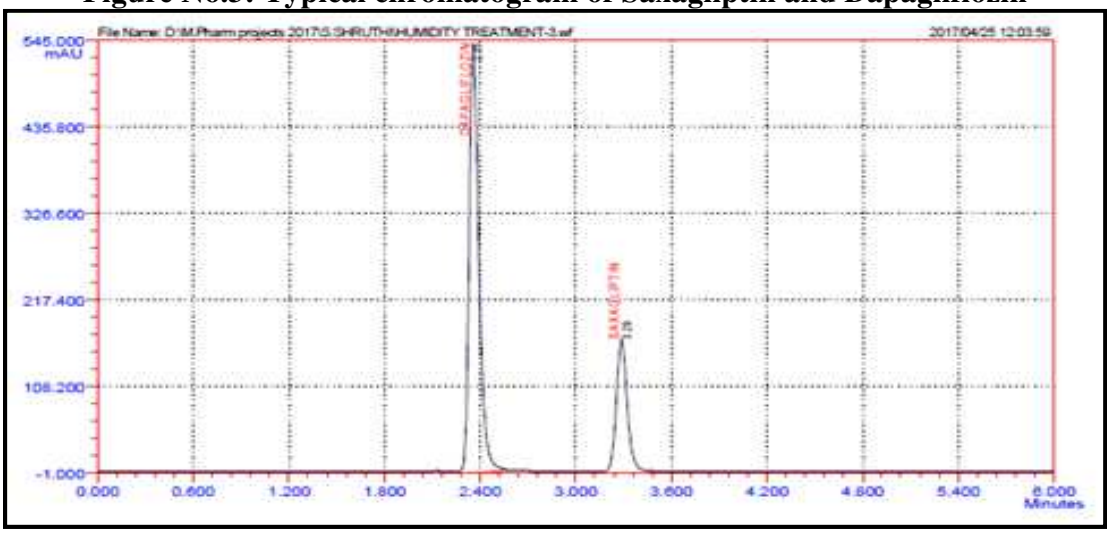


Figure No.11: Chromatogram showing effect of the Acid degradation

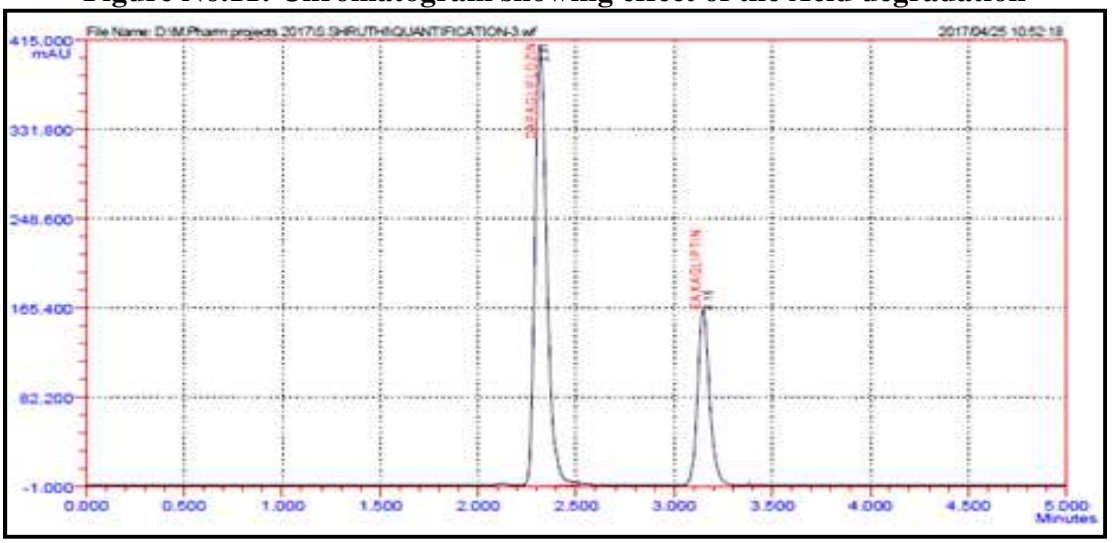


Figure No.12: Chromatogram showing effect of the Alkali degradation

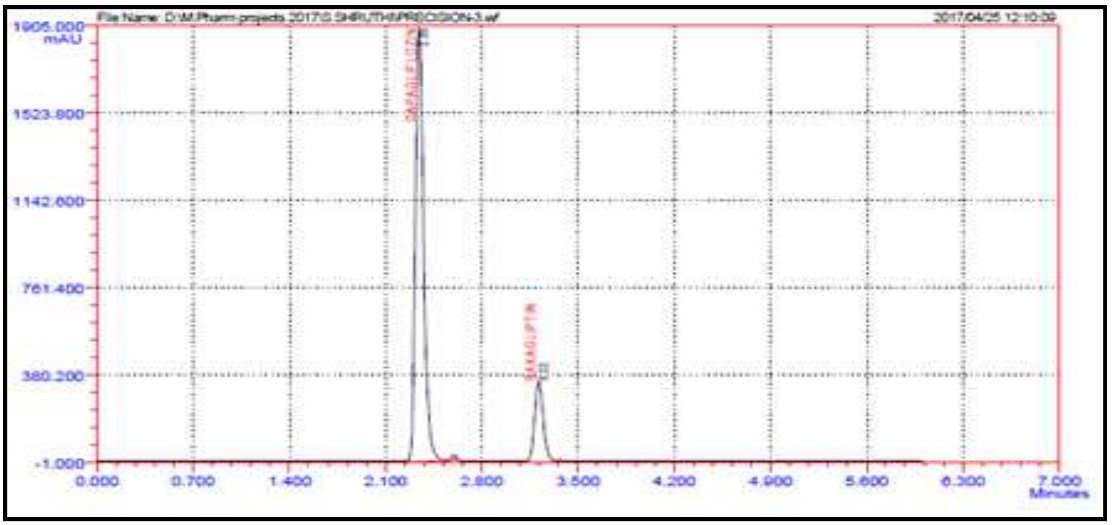


Figure No.14: Chromatogram showing the effect of Peroxide degradation

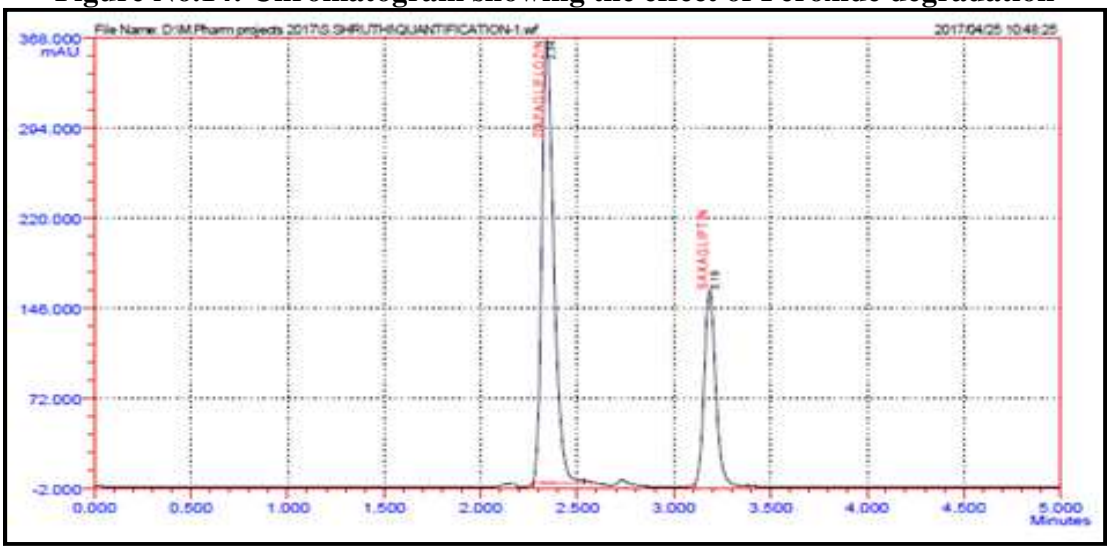


Figure No.15: Chromatogram showing the effect of thermal degradation

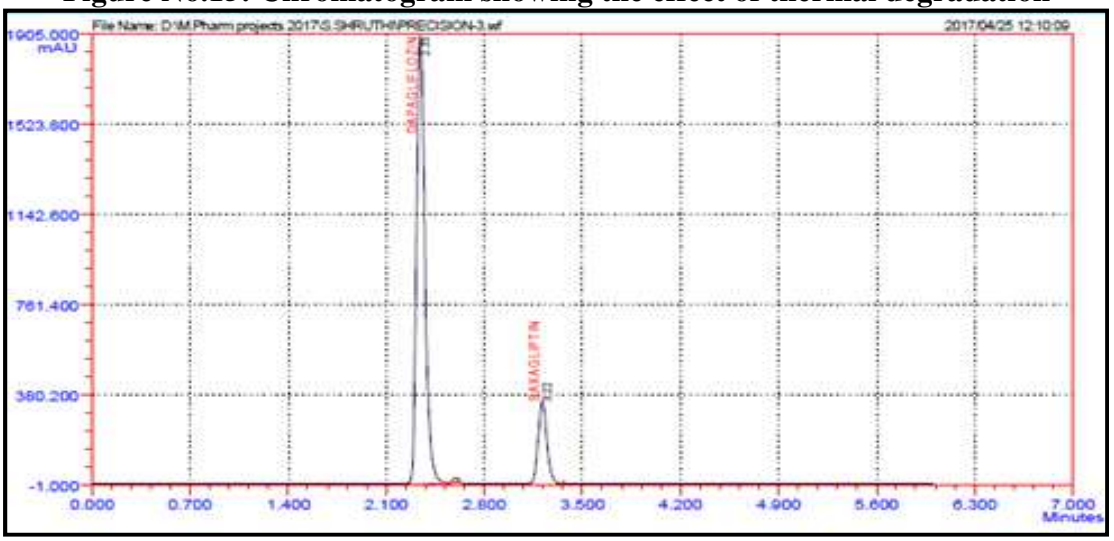


Figure No.16: Chromatogram showing the effect of Hydrolysis degradation

CONCLUSION

The developed RP-HPLC method is accurate, precise, robust, sensitive and selective. And the method is cost effective and less time consuming. It can successfully applied for estimation of Saxagliptin and Dapagliflozin in its pharmaceutical dosage form. The Force degradation studies method was found to be simple, sensitive, selective, and suitable for determination of Saxagliptin and Dapagliflozin in presence of its degraded product.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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