Development of Stability Assessment UHPLC Method for Simultaneous Quantification of Saxagliptin and Dapagliflozin in Pharmaceutical Formulations

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***Abstract*—A simple, accurate, precise method was developed for the simultaneous estimation of Saxagliptin and Dapagliflozin in Tablet dosage form. Chromatogram was run through Agilent Poroshell 120 column 2.7µ Particle) the Mobile phase containing acetonitrile: methanol (80:20% v/v). The solution was pumped through the column at a flow rate of 1 ml/min. The column temperature was maintained at 30°C. Optimized wavelength selected was 210 nm. Retention time of Saxagliptin and Dapagliflozin were found to be 6.61min and 11.50 min. % RSD of the Saxagliptin and Dapagliflozin were found to be less than 2%. respectively. % Recovery was obtained as 100.42 % - 102.25% and 100.45% - 102.57% for Saxagliptin and Dapagliflozin respectively. the LOD and LOQ 3.37 µg/ml and 11.24 µg/ml for Saxagliptin and 1.30 µg/ml 4.34 µg/ml were observed for dapagliflozin respectively. So, the developed method was simple and economical that can be applied successfully for simultaneous estimation of both Saxagliptin and Dapagliflozin in Pharmaceutical formulation. The force degradation studies using UHPLC technique revealed the possible degradation of selected drug Saxagliptin and Dapagliflozin under the given stress condition including effect of acid hydrolysis (0.1N HCl), alkali hydrolysis (0.1N NaOH), oxidative degradation (6%H2O2) and thermal degradation. Hence, the method was successfully implied for stability-indicating studies and for routine examination of Saxagliptin and Dapagliflozin in pharmaceutical formulation.**

***Index Terms*—UHPLC, ICH guidelines, Validation, Saxagliptin, Dapagliflozin, Method Development, Stability studies.**

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic progressive metabolic disorder characterized by absolute or relative insulin deficiency [1,2.] Several new drug molecules were introduced in recent years for effective control of diabetes mellitus by using them alone or in combination. One such combination is Saxagliptin and Dapagliflozin combination introduced in to market in 2017. Saxagliptin is chemically known as (1S,3S,5S)-2-[(2S)-2 amino-2-(3-hydroxy-1- adamantyl) acetyl]-2 hexane-3-carbonitrile) with molecular azabicyclo formula of C18H25N3O2 and molecular weight of 315.41g/mol [3] (fig.1). 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].

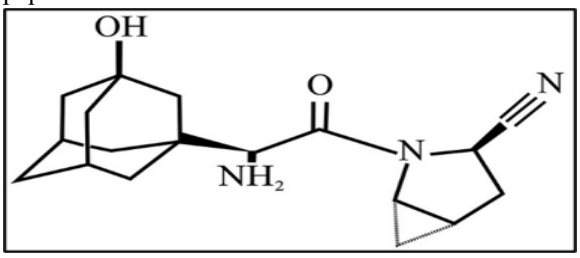

Fig. 1. Structure of Saxagliptin

Dapagliflozin (fig.2) is chemically known as (1s)-1, 5- unhydro 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, thereby improving glycaemic control in patients with type 2 diabetes mellitus [8]. Though several methods are reported in literature for the estimation of Saxagliptin and Dapagliflozin 23-30 individually and in combination with other drugs. No UHPLC methods are reported for estimation of Saxagliptin and Dapagliflozin in combination. The objective of the present study was to develop and validate a new UHPLC method for the simultaneous estimation of Saxagliptine and Dapagliflozin in dosage forms and to evaluate its application in pharmacokinetic studies for estimation of Saxagliptine and Dapagliflozin in plasma samples. [11-24]

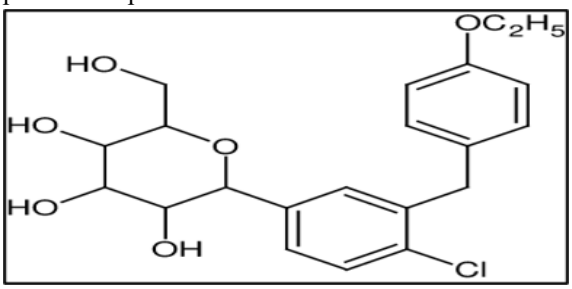

Fig. 2. Structure of Dapagliflozin

II. MATERIAL AND METHOD

A. Reagent and Chemical:

Saxagliptin and dapagliflozin is obtained as gift sample from Umedica R & D Centre Mumbai, Mumbai, methanol (HPLC grade), double distilled water, Acetonitril, marketed formulation. All the chemicals and reagents are used in this experiment were analytical grade.

III. INSTRUMENTATION

The UHPLC chromatographic separation was performed on standard Agilent-Poroshell (3.0 x 50 mm; 2.7µ particle size) column with UV Detector the flow 1.0ml/min and injection volume 10Ul at an ambient temperature.

IV. EXPERIMENTAL WORK

A. Standard stock solution:

Exactly, 5 mg of Saxagliptin and 10 mg Dapagliflozin standards were weighed separately and dissolved into two different 100ml of calibrated volumetric flask containing 50ml of methanol, sonicated for 10minutes. Finally, volume make up with acetonitrile: methanol (80:20% v/v) to calibrated mark obtaining 50 µg/ml and 100 µg/ml of Saxagliptin and Dapagliflozin as a standard solution.

B. Standard Working Solution:

A working standard solution of Saxagliptin and Dapagliflozin were prepared by diluting accurate volume of 1.0 mL into 10 mL of two different calibrated volumetric flask from standard stock solutions and volume was diluted up to the mark to get the 5 µg/mL and 10 µg/mL concentrations of Saxagliptin and Dapagliflozin.

C. Optimization of chromatographic conditions:

Various combination was performed with respective to all parameters. The Solvent A; 10mM ammonium format (AF) and solvent B; Acetonitrile: Methanol (80:20% v/v) get the good chromatogram. So, acetonitrile: methanol (80:20% v/v) is a choice of solvent system with pH 5.5. (Fig.3)

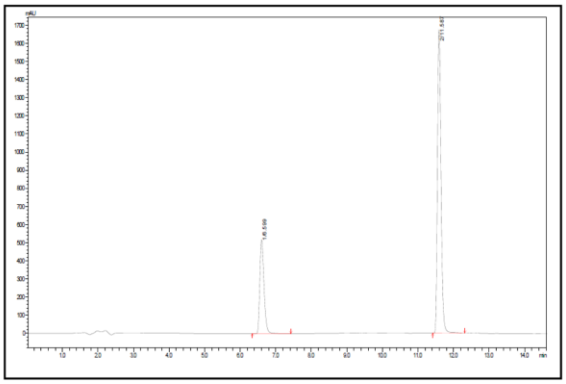

Fig. 3 Optimized Spectra of Saxagliptin and Dapagliflozin

V. RESULT AND DISCUSSION

VALIDATION OF DEVELOPED METHOD:

SYSTEM SUITABILITY:

The homogenous mixture of freshly prepared stock solution of equal concentration of Saxagliptin (5 µg/ml) and Dapagliflozin (10 µg/ml) were injected 6

times to determine the closeness of results achieved for relative standard deviation (RSD) in percentage; The calculated values should always less than 2%. Moreover, other system suitability parameters including, retention or capacity factor (k'), resolution (Rs) and theoretical plates (N), tailing factor/peak asymmetry (As) and separation factor were tested and evaluated. The results are given in table no 1.

Table no: 01 Result for system suitability test

Parameter	Saxagliptin	Dapagliflozin
Retention Time	6.61	11.50
Theoretical Plates	15030	60202
Tailing Factor	1.35	1.29
Resolution		

LINEARITY:

Linearity: The linearity was evaluated by determining five standard working solution drugs, over range 1.65 - 25ug/ml and 3.75 - 50ug/ml and found to be linear with linear regression value 0.9991 and 0.999 respectively. The results are given in table no 2, Fig.4 and Fig.5.

Table no. 2. Result for linearity

Sr No	Saxagliptin		Dapagliflozin	
01	25	2580147	50	8204483
02	12.5	1358658	25	4319041
03	6.25	712622	12.5	2306777
04	3.12	367345	6.25	1231974
05	1.65	184141	3.75	647887

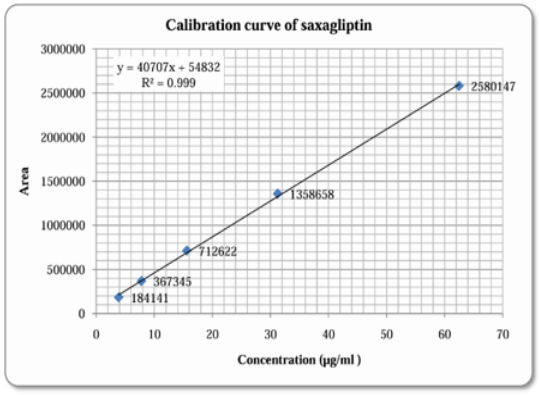

Fig.4. Linearity Graph of Saxagliptin

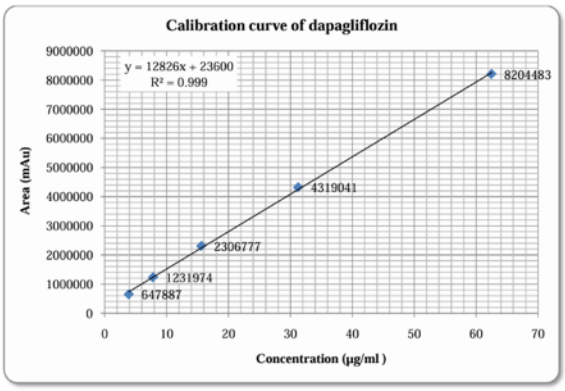

Fig.5. Linearity Graph of Dapagliflozin

PRECISIN:

The homologous mixture of saxagliptin and dapagliflozin of three replicates of selected similar concentrations; were tested and evaluated in three successive days (interday/ intermediate precision). The %RSD was calculated and it is found less than 2%. The results are given in table no 3 and Fig 5 and 6.

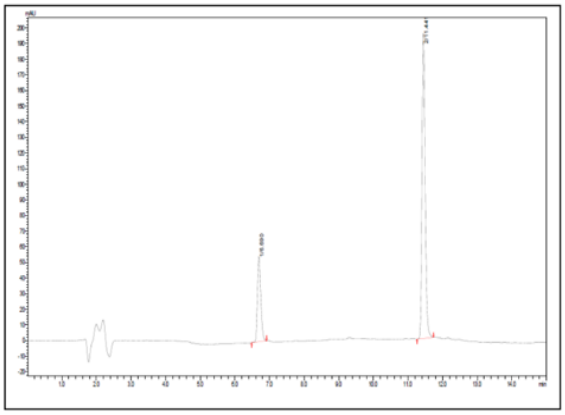

Fig. 5 Interday Precision spectra of Saxagliptin and Dapagliflozin

Fig. 5 Interday Precisiospectra of Saxagliptin and Dap

Table No: 3 Intermediate Precision Data of Saxagliptin And Dapagliflozin

Saxagliptin				
Sr No	Concentration (ug/ml)	Area	Standard Deviation (SD)	% RSD
DAY 1	50	4227471	77,528.89	1.80%

	50	4362341		
	50	4359004		
DAY 2	50	4274199	17,041.75	0.3969 %
	50	4306662		
	50	4299424		
DAY 3	50	4390750	51969.81	1.17%

Table No: 4 Intermediate Precision Data of Dapagliflozin

Sr No	Concentration (ug/ml)	Area	Standard Deviation (SD)	% RSD
DAY 1	100	119209	1450.10	1.20 %
	100	120792		
	100	122105		
DAY 2	100	119809	1149.48	0.95 %
	100	120856		
	100	122105		
DAY 3	100	117,900	2100	1.75 %
	100	120,000		
	100	122,100		

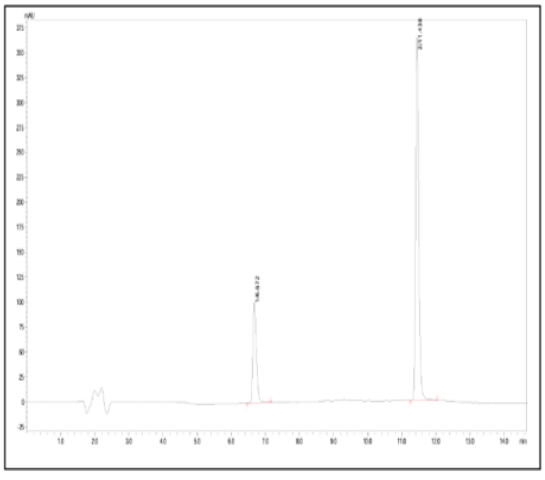

Fig. 6 Interday Precision spectra of Saxagliptin and Dapagliflozin

ACCURACY

Percentage drug accuracy of three different concentrations; 80%, 100% and 120%. to estimate the Saxagliptin and dapagliflozin reported in Table No.4 and Table No.5. As resulted, the achieved drug recovery of both saxagliptin and dapagliflozin were in the range of 100.4-100.7 and 100-105, respectively. As recommended by International conferences of Harmonization (ICH) guidelines the drug recovery should be within the range of 90-110% and the RSD in percentage should be less than 2%.

Table No.4: Accuracy data of Saxagliptin

Level of Recovery	Concentration of Std added (µg/ml)	Peak area	Average peak area	% Recovery	SD	%RSD
80 %	80	5411491	5358651	101.55	52,205.78	0.97 %
	80	5307231				
	80	5357233				
100 %	100	6415386	6389267	100.42	25,146.17	0.39 %
	100	6365307				
	100	6387110				
120 %	120	8130423	8193454	102.25	58,603.06	0.72 %
	120	8246328				
	120	8203611				

Table No :5 Accuracy data of dapagliflozin

Level of Recovery	Concentration of Std added (µg/ml)	Peak area	Average peak area	% Recovery	SD	%RSD
80 %	8	152778	155933.5	100.45	3832.93	2.46 %
	8	160199				
	8	154824				
100 %	10	198330	200174.5	102.57	2012.14	1.01 %
	10	202319				
	10	199864				
120 %	12	231192	236990	101.40	5531.69	2.33 %
	12	242210				
	12	237568				

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTITATION (LOQ):

the limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope of the regression equation. As observed, the LOD and LOQ 3.37 µg/ml and 11.24 µg/ml for saxagliptin and 1.30 µg/ml 4.34 µg/ml were observed for dapagliflozin respectively.

ROBUSTNESS:

The method was found to be robust when minor changes were made in optimized chromatographic condition such as flow rate flow rate (1.0 ± 0.1 ml/minutes), organic modifier (70% ± 2% ml), and temperature (28°C ± 2°C) wavelength, injection volume on selected separation variables including capacity factor (k'), resolution (Rs), tailing factor (Tf), separation factor, theoretical plates (N)and peak area. The result is given in table no 6 and table no 7.

Table No: 6 Robustness data of Saxagliptin

Table No: 7 Robustness data of dapagliflozin

Variables	Saxagliptin			
	tR(min)	K '	T_f	N
Flowrate (+2)	6.34	3.23	1.36	15220
Flowrate (-2)	7.02	2.2	1.37	14476
ACN-MeOH 20% (+2%)	6.23	2.12	1.37	12145
ACN-MeOH 20% (-2%)	7	3.16	1.35	16705
Temperature (+2 c)	6.6	2.97	1.35	14597
Temperature (-2 c)	6.6	2.97	1.35	14597
Mean±S.D	6.63±0.33	2.78±0.49	1.36±0.01	

Variables	Dapagliflozin				
	tR(min)	K '	T_f	R_s	N
Flowrate (+2)	11..24	6.5	1.29	24.87	57169
Flowrate (-2)	12.04	4.48	1.33	22.74	53975
ACN-MeOH 20% (+2%)	11.3	4.66	1.31	23.95	53975
ACN-MeOH 20% (-2%)	11.88	6.07	1.31	23.68	59076
Temperature (+2 c)	11.57	5.95	1.3	24.01	56476
Temperature (-2 c)	11.57	5.95	1.3	24.01	56476
Mean± S.D	11.60± 0.31	5.60± 0.83	1.31± 0.01	23.88± 0.69	

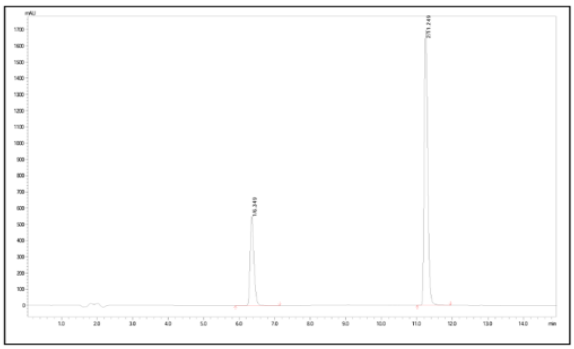

Fig.7. Effect of flow rate 1.1 ml/minute on saxagliptin and dapagliflozin

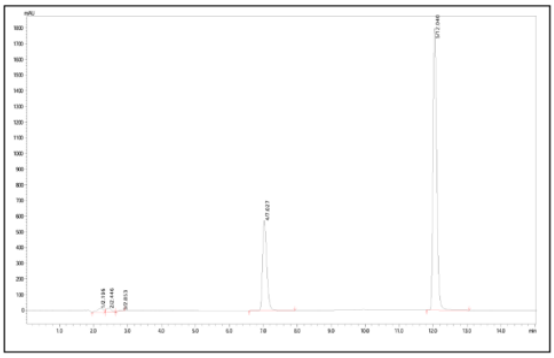

Fig. 8 Effect of flow rate 0.9 ml/minute on saxagliptin and dapagliflozin

FORCE DEGRADATION STUDY

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 (0.1N HCl), alkali hydrolysis (0.1N NaOH), oxidative degradation (6%H2O2) and thermal degradation (samples placed in an oven) and further results are shown in Table no.8.

Table No: 8 Force degradation data of saxagliptin

Stress Condition	T_r Degradation	t_R after degradation	No. of degradants	% degradation
Acid (0.1N/ M HCl) +45˚C +12 Hrs	6.83	6.61	1 degradant	0.19 %
Base (0.1N NaOHl) +60˚C +12 Hrs.	6.83	6.53	3degradants	4.40 %
Thermal (45˚C) + 12 Hrs.	6.83	6.6	0 degradant	0 %
Oxidation (6%H2O2) + Room Temp.	6.83	6.62	0 degradant	0 %

Table No: 9 Force degradation data of dapagliflozin

Stress Condition	T_r Degradation	t_R after degradation	No. of degradants	% degradation
Acid (0.1N/ M HCl) +45˚C +12 Hrs	11.59	11.63	4 Degradant	29.90 %
Base (0.1N NaOHl) +60˚C +12 Hrs.	11.59	11.63	2 Degradant	2.98 %
Thermal (45˚C) + 12 Hrs.	11.59	11.63	2 Degradant	0.35 %
Oxidation (6%H2O2) + Room Temp.	11.59	11.63	1 Degradant	0.34 %

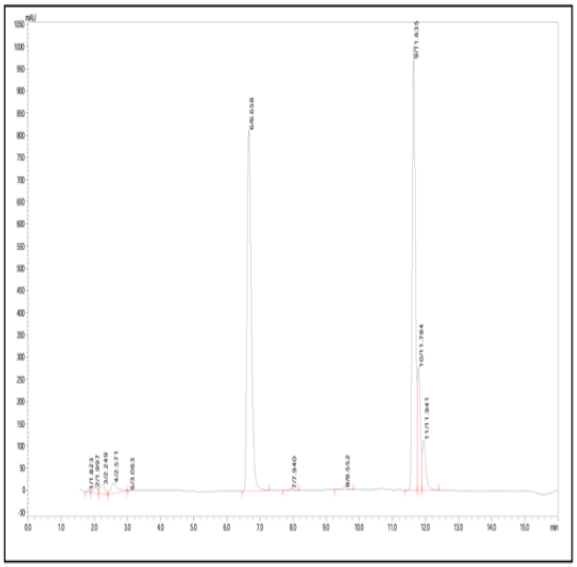

Fig.9 Effect of Acid Hydrolysis on stability of saxagliptin and dapagliflozin

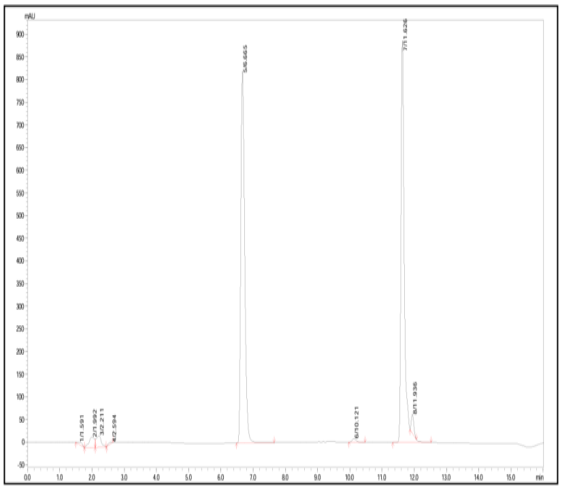

Fig.10 Effect of Alkali Hydrolysis on stability of saxagliptin and dapagliflozin

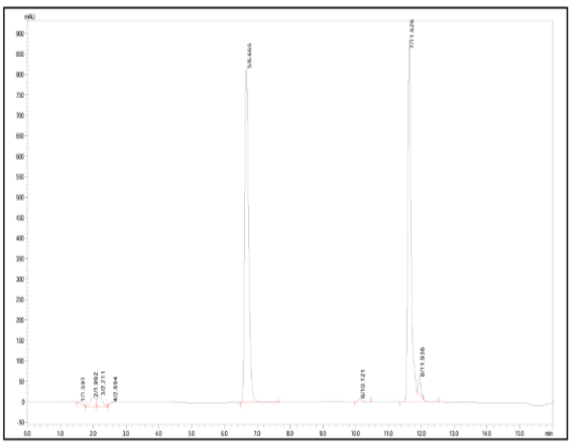

Fig 11. Effect of temperature on stability of saxagliptin and Dapagliflozin

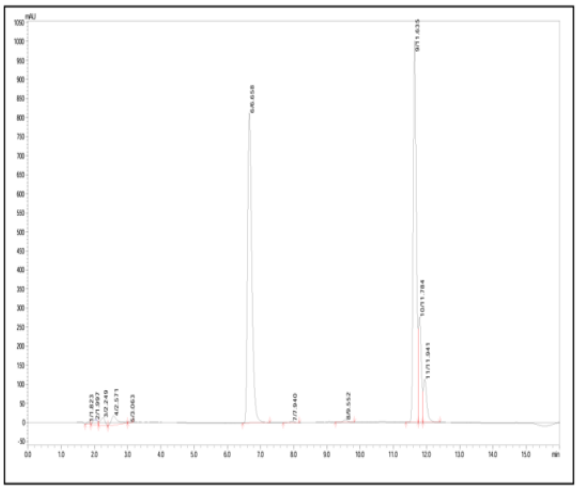

Fig. 12. Thermal effect on stability of saxagliptin and dapagliflozin

VI. CONCLUSION

A New UHPLC method for the simultaneous estimation of Saxagliptine and Dapagliflozin in their combine dosage form was developed and validated as per the ICH guidelines. % RSD of the Saxagliptin and Dapagliflozin were found to be less than 2%. respectively. % Recovery was obtained as 100.42%-102.25% and 100.45%- 102.57% for Saxagliptin and Dapagliflozin respectively. the LOD and LOQ 3.37 µg/ml and 11.24 µg/ml for Saxagliptin and 1.30 µg/ml 4.34 µg/ml were observed for dapagliflozin respectively. The developed method is simple, specific, sensitive, precise and accurate and was found suitable for estimation of Saxagliptine and Dapagliflozin in pharmaceutical dosage forms. A new stability indicating Ultra-High performance liquid chromatographic (UHPLC) approach was developed.The developed UHPLC method was also found suitable for application in pharmacokinetic studies for the estimation of Saxagliptine and Dapagliflozin in plasma samples. The good agreement of the absorption and elimination parameters estimated using UHPLC method developed with those of literature values indicated that newly developed UHPLC method is suitable and could be used in pharmacokinetic studies.

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