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Stability Indicating RP-HPLC Method for Determination of Saxagliptin and Dapagliflozin in Bulk and Tablet Dosage Forms

Md. Rageeb Md. Usman[*], Sufiyan Ahmad[1], Chandrakant Deore[1], Tanvir Shaikh[2], Bharat Jain[2] Md. Imran[3]

[*]*Department of Pharmacognosy, Smt. S. S. Patil Chopda College of Pharmacy, Chopda, Maharashtra, India*
[1]*Department of Quality Assurance, Gangamai College of Pharmacy, Nagaon, Dist. Dhule (M.S.), India.*
[2]*Department of Pharmaceutics, Smt. S. S. Patil Chopda College of Pharmacy, Chopda, Maharashtra, India*
[3]*Department of Pharmaceutical Chemistry, KBHSS Trusts Institute of Pharmacy, Malegaon, Dist. Nasik*

Abstract

In the present work, An accurate, precise and reproducible high performance liquid chromatographic method wasdeveloped for quantitative estimation of Saxagliptin (SAXA) and Dapagliflozin (DAPI)simultaneously intablet dosageforms. Agilent (S. K.) gradient system UV Detectorand RP C18 (Thermo)with 250mm x4.6 mm i.d. and 5µm particle size.Methanol 0.1 % o- phosphoric acid(60:40) was used as the mobile phase for the method. The detection wavelength was 220 nm and flow rate were1ml/min. In the developed method, the retention time of Saxagliptin and Dapagliflozinwere found to be 5.41min and 7.30minrespectively. The drug was subjected to oxidation, acid hydrolysis, alkaline hydrolysis and heat to apply stress condition for degradation. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. So the proposed methods can be used for the routine quality control analysis of Saxagliptin and Dapagliflozinin bulk drug as well as in formulations.

Keywords: Saxagliptin, Dapagliflozin, RP- HPLC, stress condition, degradation, stability

INTRODUCTION

Stability testing and stress degradation studies play a very crucial role in drug development. Stability is fundamental to all product characteristics, and the term "Stability indicating assay" has been used to describe a procedure which affords specific determination of drug substance in the presence of its degradation products. The prime goal of studying the stability of a drug is to determine the shelf life of the drug. The various conditions specified for stress degradation studies include acidic, alkaline, oxidation, photolytic and thermal.[1]

Type 2 diabetes mellitus (T2DM) is a chronic progressive metabolic disorder characterized by absolute or relative insulin deficiency.[2] Expected rise in prevalence of diabetes is mainly due to increased life span because of better healthcare facilities and increasein 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. [3]

Dapagliflozin (Figure 1) 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 g/mol.[4] 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.[5]

Saxagliptin(Figure 2) is chemically known as (1S, 3 S, 5S)-2[(2S)-2- amino- 2- (3- hydroxy- 1- adamantyl) acetyl]-2azabicyclo hexane-3-carbonitrile) with molecular formula of C18H25N3O2 and molecular weight of 315.41 g/mol.[6]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 sustainedinhibition of plasma DPP-4 activity and reduction of postprandial hyperglycaemia, including after dinner, associated with an increase in plasma glucagon-like peptide-1 levels.[7-9] Combination of Dapagliflozin and Saxagliptin is marketed as a Tablet (Qtern) containing 10 mg of Dapagliflozin, 5 mg of Saxagliptin.

Combination of these two drugs is indicated for the treatment of type-2 Diabetes. Using Dapagliflozin leads to heavy glycosuria (glucose excretion in theurine), which can lead to weight loss and tiredness. The purpose of this study was to develop a stability-indicating method for the simultaneous determination of Saxagliptin and Dapagliflozinin bulk drugs and to apply the developed method for the quantitative determination of these drugs from tablets. The HPLC technique was chosen because of its previously mentioned advantages. The proposed method was able to separate the compounds of interest and their degradation products within 10min. Thereafter, this method was validated as per International Conference on Harmonization (ICH) guidelines .[10, 11]

Literature survey revealed a variety of analytical methods viz. HPLC, LC-MS and, GC has been reported for estimation of Dapagliflozin and Saxagliptin individually or in combination with other drugs. The reported methods are Spectrohotometric[12-18], HPLC[19-38], LC-MS [39-40]and GC[41]method are reported for the simultaneous estimation of DAPI and SAX in combined pharmaceutical formulation.

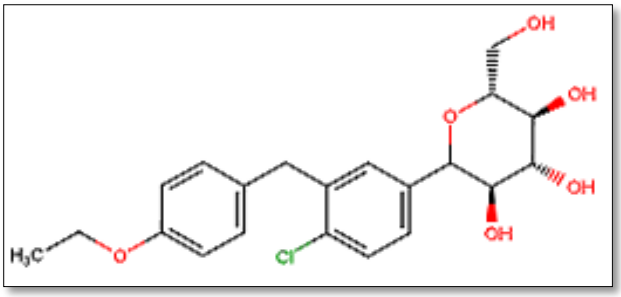

Fig. 1: Structure ofDapagliflozin

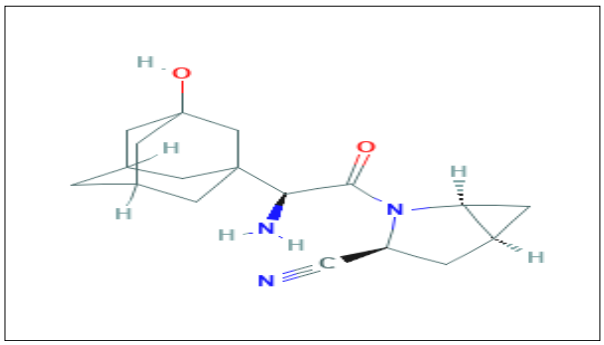

Fig. 2: Structure ofSaxagliptin

MATERIALS AND METHODS

Instruments

The analysis of the drug was carried out on Agilent (S.K.) gradient system UV detector. Equipped with RP C_{18} (Thermo),(4.6mm x 250mm; 5µm), SP940 D pump, a 20µl injection loop and UV740D Absorbance detector and running chemstation software.

Materials and Reagents

Dapagliflozin andSaxagliptinwere obtained as gift samples from R.S.I.T.C Jalgaon. O-phosphoric acid were procred from Avantor Performance material India Ltd. Thane, Maharashtra and Methanol were HPLC grade procured from Merck specialities Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai. The pharmaceutical preparations of binary combination of Dapagliflozin andSaxagliptinthat is **(AstrazenecaAB) QTERN**. The commercial formulation of Dapagliflozin andSaxagliptinis available in ratio of (10:5 mg) in tablet.

Chromatographic Conditions

RP C_{18} (Thermo), (4.6mm x 250mm) particle size packing 5µm ; detection wavelength 220 nm; flow rate 1 ml/min; temperature ambient; sample size 20 µl; mobile phasemethanol: water (OPA 0.1%) (40:60).

Preparation of standard stock solution

Saxagliptinsolution: (Stock I)

From the freshly prepared standard stock solution (1000µg/ml), 0.1ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 10 µg/ml.

Dapagliflozin solution: (Stock II)

From the freshly prepared standard stock solution (2000µg/ml), 0.1 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10 µg/ml.

Preparation of std. Saxagliptin and Dapagliflozin solution :(Stock III)

From the freshly prepared standard stock solution (1000µg/ml), 0.1 ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10 µg/ml (Figure 3 and Table 1).

FORCED DEGRADATION STUDY

According to ICH guideline, the limit of degradation approximately 0 to 30%. The amount of degradation given in percentage (Table 2).

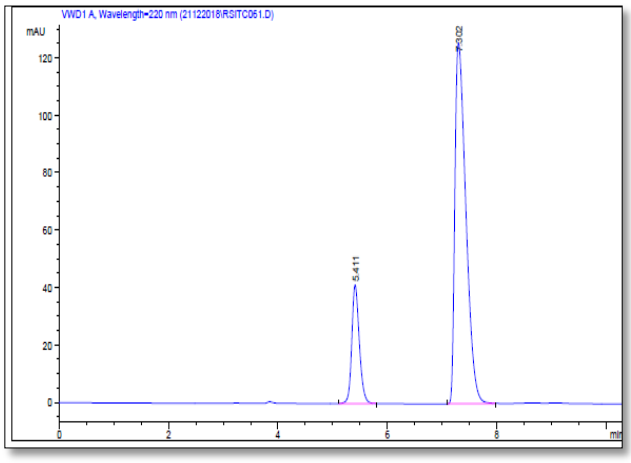

Figure 3:Chromatogram of standard combination of SAXA and DAPI

Table 1:Chromatogram of standard combination containing SAX and DAPI

Ret time (min)	K'	Area mAU	Height mAU	symm	Width (min)	Plates	Resolution	Selectivity
5.411	-	396.90527	41.37801	0.76	0.1407	8187	-	-
7.302	-	175550500	125.63819	0.46	0.2160	6334	6.23	1.35

Table 2: Stress Studies (amount of degradation in percentage)

Stress condition	Amount of degradation (%)
Acid hydrolysis	24
Alkaline hydrolysis	28-17
Oxidation	5-10

1. Acid hydrolysis:

These samples were prepared by weighing the drugs 10mg each and transfer in 10ml volumetric flask. Then addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add 0.1 N HCl and adjust volume 10ml with it. After complete preparation of solution, store it at 80^0c about 3 hours in water bath (Figure 4).

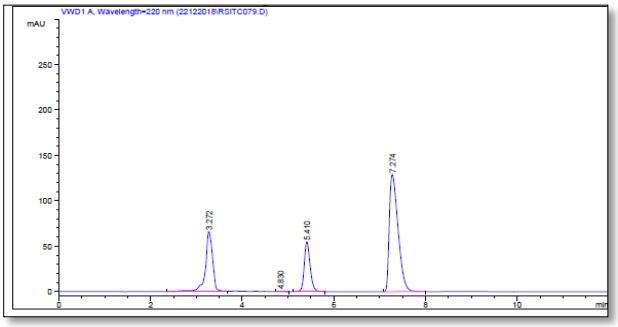

Figure4: Acid Degradation Study

2. Alkaline hydrolysis

These samples were prepared by weighing the samples 10mg each and transfer in 10ml volumetric flask. Then addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add sodium hydroxide (0.1 N) and adjust volume 10ml with it. After complete preparation of solution, store it at 80^0c about 3 hours in water bath (Figure 5).

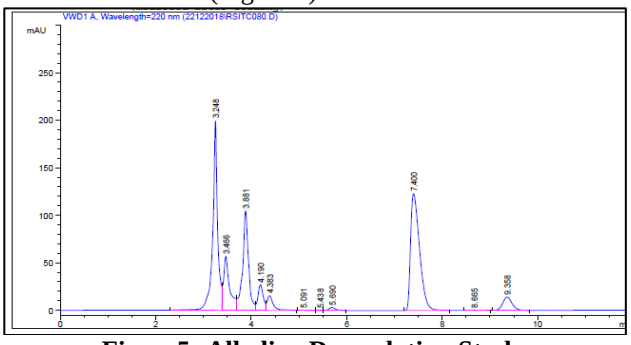

Figure5: Alkaline Degradation Study

3. Oxidation:

These samples were prepared by weighing the samples 10mg each and transfer in 10ml volumetric flask. Then addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add hydrogen peroxide (3%) and adjust volume 10ml with it. After complete preparation of solution, store it at 80^0c about 3 hours in water bath (Figure 6).

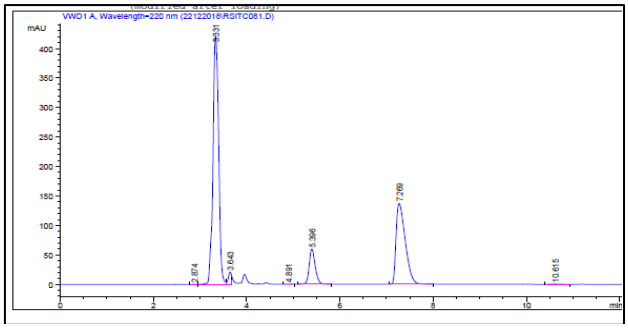

Figure6: Oxidative Degradation Study

Method validation [42-46]

1. Linearity

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 10-50 µg/mL for Saxagliptin and 20-100 µg/ml for Dapagliflozin was prepared and injected in HPLC and response was measured at 220 nm(Table 3 and 4).The respective linear equation for Saxagliptinwasy = 22.658 x + 26.217 and Dapagliflozin equation y = 39.908 x + 82.621 where x is the concentration and y is area of peak. The correlation coefficient was 0.999 and 0.999. The calibration curve of Saxagliptin andDapagliflozin is depicted in (Figure 7and 8).

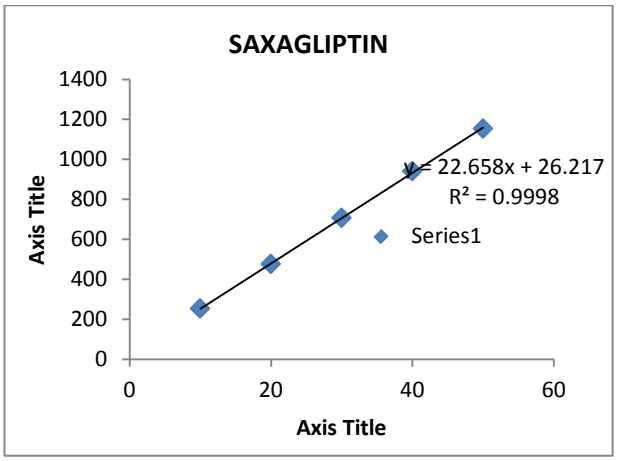

Figure 7: Calibration curve of Saxagliptin

Table 3: Linearity data for Saxagliptin

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	10	252.21	253	252.605	0.56	0.22
	20	475.39	476.54	475.965	0.81	0.17
	30	707.83	706.58	707.205	0.88	0.12
	40	941.56	940.58	941.07	0.69	0.07
	50	1152.6	1153.29	1152.945	0.49	0.04
	Equation		y = 22.658 x + 26.217			
	R^2		0.9998			

Table 4: Linearity data for Dapagliflozin

Method	Conc. µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of peak area
		1	2			
RP-HPLC Method	20	874.54	873.52	874.03	0.72	0.08
	40	1672.32	1673.11	1672.715	0.56	0.03
	60	2484.4	2485.22	2484.81	0.58	0.02
	80	3305.58	3304.11	3304.845	1.04	0.03
	100	4047.98	4048.87	4048.425	0.63	0.02
	Equation		y = 39.908 x + 82.621			
	R^2		0.9997			

Table 5: Result of Recovery data for Saxagliptin andDapagliflozin

Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean*± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean*± S.D.
SAXA	80%	10	8	17.98 ±0.05	7.98 ±0.05	99.77 ±0.62
	100%	10	10	20.07± 0.07	20.58 ±0.07	100.67± 0.64
	120%	10	12	22.09± 0.04	12.09± 0.04	99.97± 0.36
DAPI	80%	20	16	35.82± 0.03	15.82± 0.03	99.76± 1.06
	100%	20	20	39.74± 0.16	20.58± 0.16	98.72 ±0.80
	120%	20	24	22.22 ±0.04	24.22± 0.04	99.97 ±0.16
	120%	8	9.6	17.53± 0.14	9.53 ±0.14	99.30± 1.44

***mean of each 3 reading for RP-HPLC method**

Table 6: Statistical Validation of Recovery Studies Saxagliptin andDapagliflozin

Level of Recovery (%)	Drug	% RSD	Standard Deviation*	Mean % Recovery
80%	SAXA	0.62	0.62	99.77
	DAPI	1.06	1.06	99.76
100%	SAXA	0.63	0.64	100.67
	DAPI	0.82	0.81	98.72
120%	SAXA	0.36	0.36	99.97
	DAPI	0.16	0.16	99.97

***Denotes average of three determinations for RP-HPLC method**

Table 7: Intraday and Inter day Precision studies on RP-HPLC method for SAXA&DAPI

Drug	Conc (µg/ml)	Intraday Precision		Interday Precision	
		Mean± SD	%Amt Found	Mean± SD	%Amt Found
SAXA	20	474.64 0.96	0.16	473.45 0.96	0.20
	30	709.45 1.08	0.15	710.61 1.48	0.21
	40	940.40 1.25	0.13	940.93 0.91	0.10
DAPA	40	1676.01± 0.96	0.16	1678.25 ±0.96	0.06
	60	2485.08 ±0.87	0.035	2486.08 ±0.87	0.03
	80	3299.09 ±0.24	0.01	3296.11 ±0.34	0.01

***Mean of each 3 reading for RP-HPLC method**

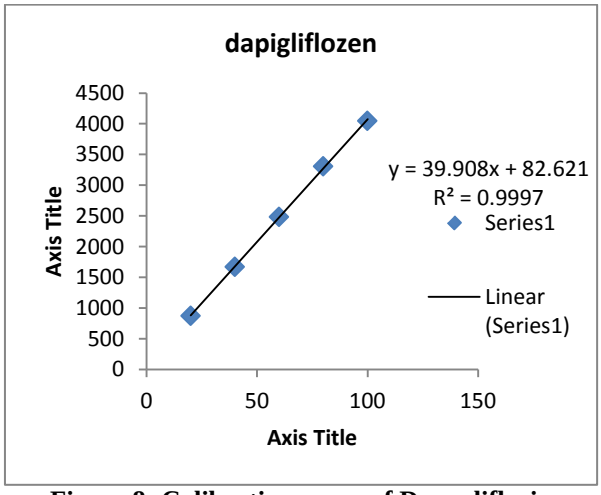

Figure 8: Calibration curve of Dapagliflozin

2. Accuracy

Accuracy was performed with the help of recovery method by standard addition of standard solution in pre-analysed tablet solution in different levels (80%, 100%, and 120%). Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD at each level was calculated and presented in following table. The result indicates that the proposed method was accurate (Table 5 and 6).

3. Precision

The precision was performed by intraday (repeatability) and interday (reproducibility) study. The detailed observation shown in (Table 7).

4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions (Table 8).

Table 8: Studies for Saxaand Dapi

Parameters	Drug	
	Saxagliptin	**Dapagliflozin**
S.D.	**3.3**	10
SLOPE	21.94	19.23
LOD	0.040	0.1230
LOQ	0.01230	0.5460

5. Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The results indicate that less variability in retention time and tailing factor were observed (Table 9).

6. System Suitability:

The system suitability parameter like capacity factor, asymmetry factor, tailing factor, HETP and number of theoretical plates were also calculated. It was observed that all the values are within the limits and the results are shown in Table. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of SAXA and DAPI in tablet formulation. The results are furnished in (Table 10).

Table 9: Result of Robustness study of SaxagliptinandDapagliflozin

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	%RSD	Amount of detected (mean ±SD)	% RSD
		Saxagliptin		Dapagliflozin	
Chromatogram of flow change 0.9 ml	20+40	569.04 ±0.45	0.35	1484.02±5.13	2.6
Chromatogram of flow change 1.1 ml	20+40	422.99±1.47	0.08	2045.5±2.86	1.43
Chromatogram of comp change wavelength change 219nm	20+40	506.0±1.37	0.27	1635.8±2.65	0.16
Chromatogram of comp change wavelength change 221nm	20+40	457.12±0.28	0.062	1726.21±1.042	0.604
Chromatogram of mobile phase change 59+41 ml	20+40	459.8±0.16	0.08	1693.9±1.82	0..11
Chromatogram of mobile phase change 61+39 ml	20+40	481.28 ±0.4	0.04	1695.33±1.56	0.09

Table 10: Repeatability studies on RP-HPLC forSaxagliptin andDapagliflozin

Method	Concentration of SAXA and DAPI(mg/ml)	Peak area	Amount found (mg)	% Amount found
SAXA	30	705.670	30.03	100.10
	30	707.350		
		Mean	706.51	
		SD	1.19	
		%RSD	0.17	
DAPI	60	2480.430	60.11	100.18
	60	2482.120		
		Mean	2481.28	
		SD	1.20	
		%RSD	0.05	

Assay preparation for marketed formulation

For analysis of the tablet dosage form, 20 tablets were weighed individually and their average weight was determined. After that they were crushed to fine powders and powder equivalent to 0.70 mg Saxagliptin and Dapagliflozin into 10 ml volumetric flask and diluted with 10 ml methanol, and sonicate to dissolve it completely and make volume up to the mark with diluent. The solutions were shaken vigorously for 10 min and filtered through 0.45 µm membrane filters. Further pipette 0.1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents (10 µg/ml). The simple chromatogram of test Saxagliptinand Dapagliflozinshown in (Figure9). The amounts of SAXA and DAPIper tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation Analysis of marketed formulation were also % label claim was found to be 99-101% satisfactory were concluded(Table 11).

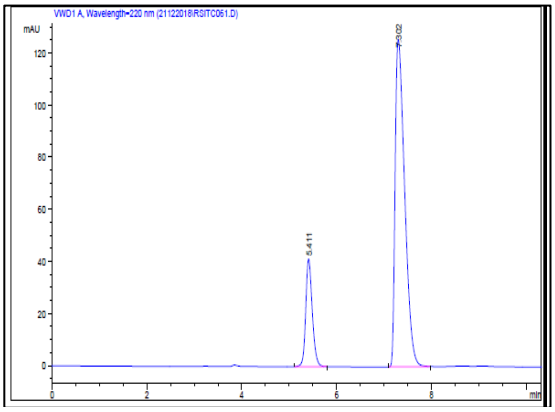

Figure 9: Chromatogram for marketed formulation

Table 11: Analysis of marketed formulation

Assay	Drug	Amount found	% label claim	S.D.	% RSD
RP-HPLC Method	Saxa	40.30	100.77	1.07	0.03
	Dapi	80.71	100.89	0.72	0.14
	Saxa	40.35	100.88	0.03	0.11
	Dapi	80.67	100.84	0.04	0.08

RESULTS AND DISCUSSION

The present study was aimed at stability indicating RP-HPLC method development and validation for simultaneous estimation of Saxagliptin, Dapagliflozinand their degradation products. A non-polar C-18 analytical chromatographic column was chosen as the stationary phase for the separation and simultaneous determination of Saxagliptin, Dapagliflozinand their degradation products. Mixtures of commonly used solvents like water and methanol in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of methanol and 0.1 % o-phosphoric acid in the ratio of 40:60 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention times of the Saxagliptin and Dapagliflozinwere found to be 5.41 min and 7.30 min respectively. The forced degradation study was conducted for determining the stability indicating power of an analytical procedure. The result of the stress studies shown in (Table 2).

Therefore, the proposed method was simple, specific and sensitive and can be used for simultaneous analysis of Saxagliptin, Dapagliflozinand their degradation products in bulk samples and its tablet dosage forms.

CONCLUSION

A new analytical method was developed to be routinely applied to simultaneous determination of Saxagliptin and Dapagliflozinin pharmaceutical dosage form. In this study, the stability of Saxagliptin and Dapagliflozinin present dosage forms was established through employment of ICH recommended stress conditions. The developed procedure was evaluated for linearity, accuracy, precision and robustness to ascertain the stability of the analytical method. The method was proved to be specific, linear, precise, accurate, robust and stability-indicating. Hence, the method is recommended for routine quality control analysis and stability sample analysis.

Abbreviation Used

UV: Ultraviolet; **HPLC:** High Performance Liquid Chromatography;**SAXA:**Saxagliptin; **DAPI:** Dapagliflozin;**T2DM:**Type 2 diabetes mellitus;**DPP:**Dipeptidyl Peptidase (DPP); LC-MS: Liquid Chromatography Mass Spectroscopy;**GC:** Gas Chromatography; **ICH:**International Conference on Harmonization; **RSD:** Relative Standard Deviation; **RT:** Retention Time; **SD:** Standard Deviation.

Conflict Of Interest

Authors have no conflicts of interest to declare.

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