ANALYTICAL APPROACH FOR DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SAXAGLIPTIN AND DAPAGLIFLOZIN IN PHARMACEUTICAL DOSAGE FORM

[1] Seju D Patel, [2] Dr. Pragnesh Patani
[1]Assistant Professor, [2]Professor
[1] Department of Quality Assurance,
[1]A-One Pharmacy College, Ahmedabad, India

Abstract: A simple, rapid, economical, precise and accurate stability indicting HPLC method for simultaneous estimation of Dapagliflozin and Saxagliptin in their combined dosage form has been developed. A stability indicting high performance liquid chromatographic method was developed for the simultaneous estimation of Dapagliflozin and Saxagliptin in their combined dosage form has been developed. The separation was achieved by LC- 20 AT C18 (250mm x 4.6 mm x 2.6 µm) column and Buffer (pH 6.0): Acetonitrile (70:30) at a flow rate of 1 ml/min. Detection was carried out at 275 nm. Retention time 3.593 min and 5.263 for Dapagliflozin and Saxagliptin respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Dapagliflozin 10-30 µg/ml and for Saxagliptin 5-15 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Dapagliflozin and Saxagliptin in their combined dosage form. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial combined dosage form.

Key Words- Dapagliflozin, Saxagliptin, RP-HPLC Method Development, Validation, Forced Degradation

I INTRODUCTION

Saxagliptin Saxagliptin (rINN) is an orally active hypoglycemic (anti-diabetic drug)of dipeptidyl peptidase-4 (DPP-4) inhibitor class which work by affecting the action of hormones called incretins.[1]

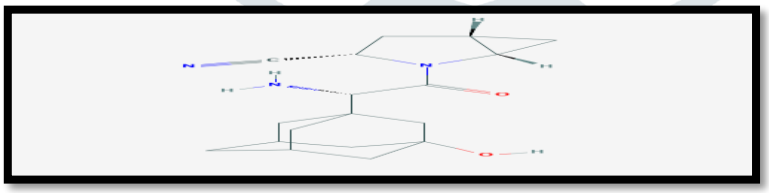

Fig.1 Chemical Structure of Sxagliptin

Dapagliflozin is a drug of thegliflozin class, used to treat type 2 diabetes Dapagliflozin inhibits subtype 2 of dSGLT2) which areresponsible for at least 90% of the glucose reabsorption in the kidney. Blocking this transportermechanism causes blood glucose to be eliminated through the urine. In clinical trials, dapagliflozinlowered HbA1c by0.6 versus placebo percentage points when added to metformin.[2,3]

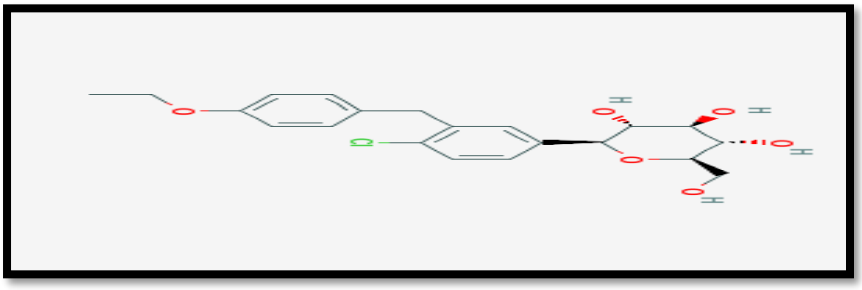

Fig.2 Chemical Structure of Dapagliflozin

Analytical method development is defined as development, revision and application of validated, standardized and official methods of analysis. Method validation is the process of documenting or proving that selected method provides analytical data for the intended use. Method is validated by using parameters like accuracy, precision, linearity, limit of detection, limit of quantitation, system suitability, selectivity and specificity [4,5]. The purpose of stability indicating method is to provide evidence on how the quality of a drug substance or product varies with time under the influence of a variety of environmental factor such as temperature, humidity and light and to establish retest period for the drug substance, or a shelf life for the drug product and recommended storage condition. According to ICH guidelines, in force degradation studies a variety of condition like pH, light, oxidation, dry heat etc. and separation of drug from degradation product is carried out [6,7].

Combination of Saxagliptin and Dapagliflozin is available in tablet dosage form of 5:10 mg respectively. Two anti-hyperglycaemic agents with complementary mechanisms of action in a once-daily tablet: Dapagliflozin, a sodium-glucose cotransporter 2 (SGLT-2) inhibitor; and saxagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor. The FDA approved ***Qtern*** based on data from a 24-week, Phase III, multi-centre, randomised, double-blind, placebo-controlled trial (n=315) designed to evaluate the efficacy and safety of saxagliptin added to dapagliflozin in adult patients with type-2 diabetes who experienced inadequate glycaemic control (HbA1c ≥7% to ≤10.5%) with metformin (≥1,500mg per day). The safety of combined use of dapagliflozin and saxagliptin has been evaluated in a pooled safety analysis (N=1,169; 492 treated with *Qtern*) of three Phase III placebo-controlled clinical trials for up to 52 weeks.

Various methods are reported for the analysis of individual drug and in combination with other drugs for Saxagliptin and Dapagliflozin but no Stability indicating HPLC method reported for these drugs in combined dosage form. So, that need was felt, to develop stability indicating RP-HPLC Method for the Simultaneous Estimation of Saxagliptin and Dapagliflozin in their Combined Dosage form.[8-11].

1.1 Materials and Methods

1.1.1Chemicals and Solvents

Saxagliptin and Dapagliflozin were procured from Rivan Pharmaceuticals, India as a gift sample. HPLC grade solvents: Water, Methanol, Acetonitrile were obtained from Merck India Ltd., Mumbai. ***Qtern*** Tablet (Saxagliptin 5mg and Dapagliflozin 10mg) was procured from local market.

1.1.2 HPLC instrumentation and chromatographic conditions

Separation and estimation was carried out using HPLC system a Hypersil BDS C_{18} column (250 × 4.6 mm i.d., 5µm particle size) was used. Samples were injected using Rheodyne injector with 20 µL loop and detection was carried out using PDA detector. Data was analyzed by using Thermo separation Product UV 2000

1.1.3 Selection of Mobile Phase

Trail contains various mobile phase which are considered of Methanol, Water and Acetonitrile in different proportions and different volumes at different flow rate were tried. On the basis of various trails the mixture of Buffer (Potassium Phosphate) at pH 6.0: Acetonitrile (70:30), at 1.0 mL/min flow rate, proved to be better than the other mixture in terms of peak shape, theoretical plate and asymmetry

1.1.4 Preparation of standard solution

Take 10 mg of Saxagliptin and 20 mg of Dapagliflozin was weighed and transferred to a 100 mL volumetric flask individually than volume was made up to the mark with mobile phase and mix it well.

1.1.5 Preparation sample solution

1.1.5.1 Sample Stock Solution (Saxagliptin 100 µg/mL, and Dapagliflozin 200 µg/mL)

Take synthetic mixture equivalent to 10mg Saxagliptin and 20mg Dapagliflozin was transferred to a 100 mL volumetric flask, Add 60 ml Mobile Phase and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through What man filter paper no. 42.

1.1.5.2 Working Sample Preparation (Saxagliptin 10 µg/mL, and Dapagliflozin 20 µg/mL):

Take 1 mL from standard stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase

1.1.6 Mobile phase preparation [Buffer 0.05M Potassium Phosphate, pH 6.0: Acetonitrile (70:30)]
Potassium Phosphate: Weigh accurately about 6.8 gm and dissolve it in to 1000ml of water adjust the pH with 0.1N NaOH up to pH 6.
Take 700ml of Buffer and 300ml of Acetonitrile, Mix it well.

1.1.7 Selection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study drug solutions of Dapagliflozin (20 ppm) and Saxagliptin (10 ppm) were prepared in Methanol. These drug solutions were than scanned in UV region of 200-400 nm and overlay spectrums were recorded.

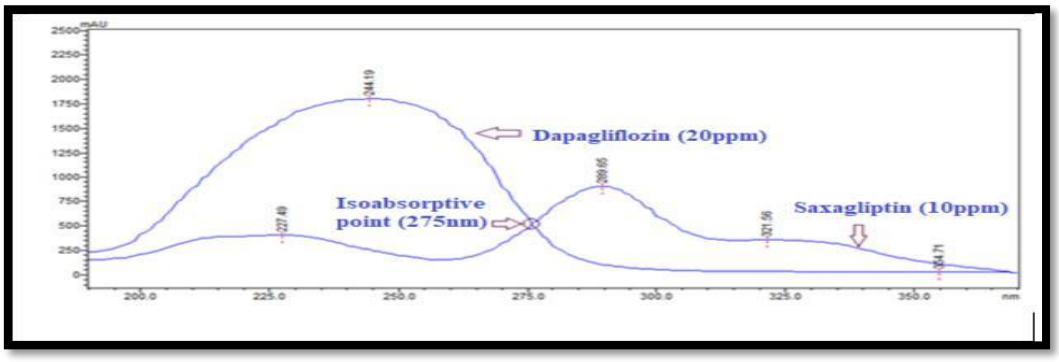

Fig.3 Selection of detection wavelength (275nm)

1.1.8 Optimization of HPLC method

The pure drug solution of Saxagliptin and Dapagliflozin were injected individually into HPLC system and allow to run in different mobile phases like methanol, water, acetonitrile and phosphate buffer in different proposition to find the optimum conditions for the separation of Saxagliptin and Dapagliflozin. It was found that mobile phase containing phosphate buffer (pH 6.0): Acetonitrile (70:30v/v) at a flow rate of 1.0 ml/min with detection wavelength 275nm gave satisfactory results with sharp, well defined and resolved peaks with minimum tailing as compared to other mobile phase. Under this conditions the retention time were typically 3.687 min for Saxagliptin and 5.880 min for Dapagliflozin (fig. 4)

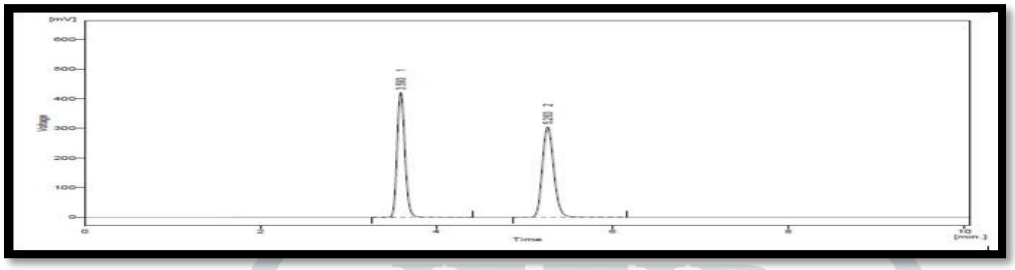

Fig.4 Chromatogram of Saxagliptin and Dapagliflozin for optimized method Method Validation

The developed method was validated as per ICH Q2 (R1) guidelines for following parameters:
Linearity, Precision, Accuracy, Sensitivity, Robustness.

2.1 Force degradation

2.1.1. Acid degradation

Acid degradation were performed by transferring 1ml of stock solution to 10 ml volumetric flask. Then take Two ml of 0.1 N HCl solution ,.and mixed well and allow it for 4 hrs at RT. After that the volume was adjusted with the diluent to get 10µg/ml for Saxagliptin and 20µg/ml for Dapagliflozin.

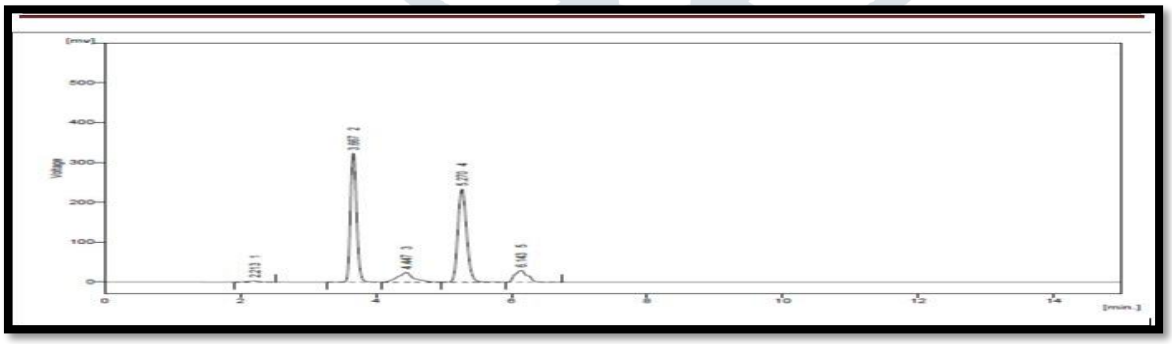

Fig.5 Chromatogram of combined Saxagliptin and Dapagliflozin mixture in acid degradation

2.1.2. Base degradation

Base degradation are performed by transferring 1ml of stock solution to 10 ml volumetric flask. Then take Two ml of 0.1 N NaOH solutions is added and mix for certain time and then put for 6hrs at RT. Then the final volume was adjusted with diluent to get 10µg/ml for Saxagliptin and 20µg/ml for Dapagliflozin. Respectively.

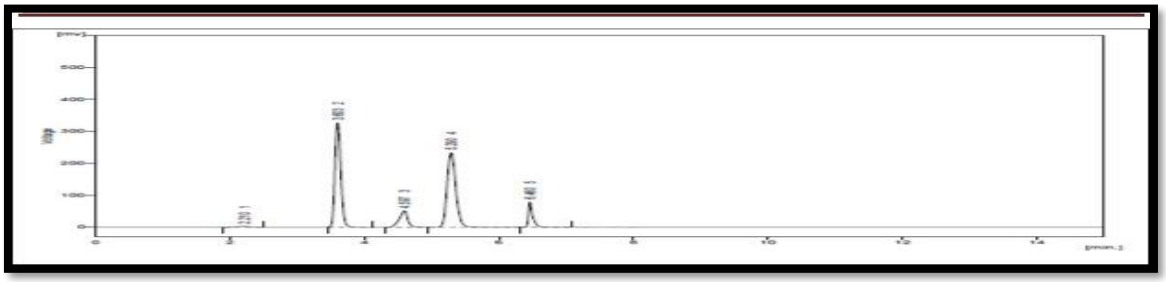

Fig. 6 Chromatogram of combined Saxagliptin and Dapagliflozin mixture in Base degradation

2.1.3. Oxidative degradation

Oxidation degradation studies are performing by transferring 1ml of stock solution to the 10 ml of volumetric flask. Then add Two ml of 3% H_2O_2 solutions and mixed well and put for 3 hrs at RT. Then the volume was adjusted with diluent to get final 10µg/ml for Saxagliptin and 20µg/ml for Dapagliflozin. Respectively.

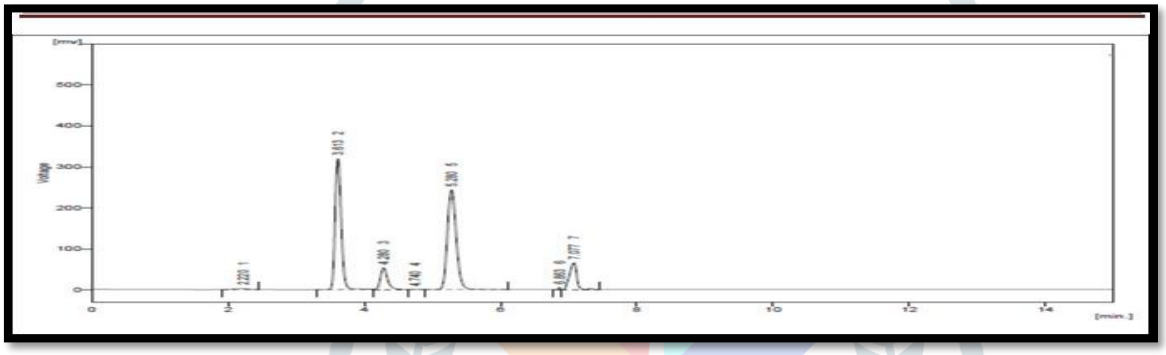

Fig. 7 Chromatogram of combined Saxagliptin and Dapagliflozin mixture in Oxidative degradation

2.1.4. Photo degradation

Photo degradation studies were performed by taking 1ml of stock solution to 10 ml of volumetric flask. Then the flask is kept for 10 hrs in UV Chamberas required. Then the volume was adjusted with diluent to get final concentration of 10µg/ml for Saxagliptin and 20µg/ml for Dapagliflozin respectively.

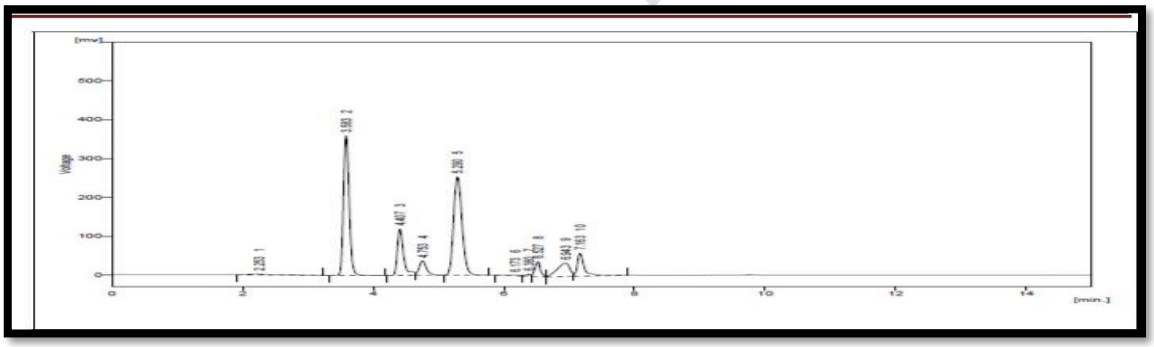

Fig. 8 Chromatogram of combined Saxagliptin and Dapagliflozin mixture in Photo degradation

2.1.5. Thermal degradation

Thermal degradation were performed by taking 1ml of stock solution to 10 ml of volumetric flask. And after that the flask was kept for 12 hrs in oven at 80^0C. Then the volume was adjusted with diluent to get final concentration of 10µg/ml for Saxagliptin and 20µg/ml for Dapagliflozin respectively.

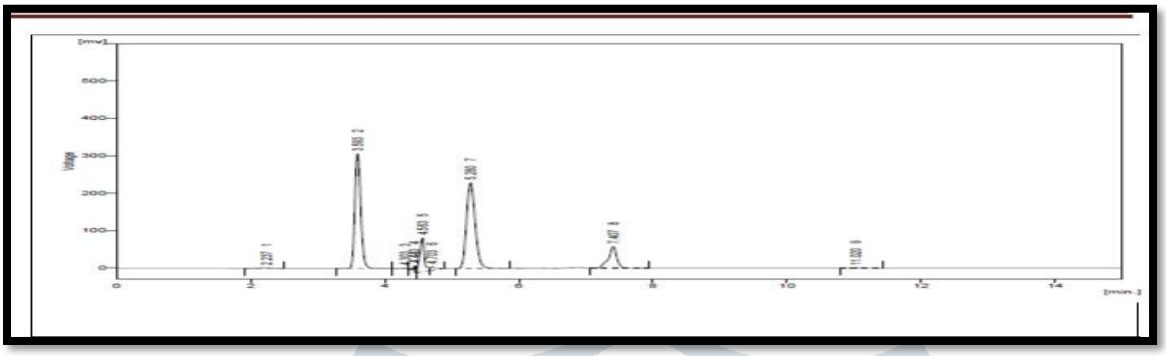

Fig. 9 Chromatogram of combined Saxagliptin and Dapagliflozin mixture in Thermal degradation

II RESULTS AND DISCUSSION

3.1 Linearity

The drug response was linear (r^2 = 0.995 for Saxagliptin and 0.996 for Dapagliptin) over the concentration range between 5-15 µg/ml for Saxagliptin and 10-30 µg/ml for Dapagliptin. The result is shown in (Table 1)

Table 1 linearity data of Saxagliptin and Dapagliptin

Parameters	Saxagliptin	Dapagliptin
Concentration Range (µg/ml)	5-15	10-30
Regression equation (y= mx + c)	y = 128.26x - 13.923	y = 278.78x - 33.37
Slope (m)	128.26	278.78
Intercept (c)	13.92	33.37
Correlation Coefficient ($r^{2)}$	0.995	0.996
LOD(µg/ml)	0.352	1.817
LOQ(µg/ml)	1.06	5.50

3.2 Precision

3.2.1 Repeatability

The data for repeatability of peak area measurement for Dapagliflozin and Saxagliptin based on six measurements of same solution of Dapagliflozin (20 µg/ml) and Saxagliptin (10 µg/ml).The % RSD for Dapagliflozin and Saxagliptin was found to be 0.548 and 0.431 respectively

Table 2 Repeatability data of Saxagliptin and Dapagliptin

Concentration	**Saxagliptin** (10 µg/ml)	**Dapagliptin** (20 µg/ml)
Area* (NMT-2%)	2767.210	2582.006
± SD	11.922	14.138
%RSD	0.431	0.548

*Average of six determinations, SD - standard deviation and RSD- Relative standard deviation

Table 3 Intra-day and Inter-day precision of Saxagliptin and Dapagliflozin

Drug	**Concentration (µg/ml)**	**Intra-day area* ± SD**	**%RSD**	**Inter-day area* ± SD**	**%RSD**
Saxagliptin	5	1360.157 ± 16.055	1.180	1354.395 ± 22.100	1.632
	10	2751.935 ± 25.746	0.936	2750.066± 20.819	0.757
	15	4127.806± 33.945	0.822	4121.045± 33.041	0.802
Dapagliflozin	10	1270.066 ± 12.365	0.974	1264.626 ± 18.356	1.452
	20	2569.238 ± 27.825	1.083	2567.497± 23.978	0.934
	30	3856.138 ± 34.620	0.898	3856.301 ± 24.087	0.625

*Average of three determinations

3.3 .Accuracy

Recovery studies:

As shown in Table-4, 5 good recoveries of the Saxagliptin and Dapagliflozin in the range from 98% to 102 % were obtained at various added concentrations.

Table 4 Determination of Accuracy for Saxagliptin

% Level of Recovery	Conc. of sample solution (µg/ml)	Conc. of standard Solution (µg/ml)	Total conc. (µg/ml)	Conc. recovered (µg/ml)	% Recovery

80	5	4	9	4.017	99.805
100	5	5	10	4.985	99.543
120	5	6	11	5.962	99.682

Table 5 Determination of Accuracy for Dapagliflozin

% Level of Recovery	Conc. of sample solution (µg/ml)	Conc. of standard Solution (µg/ml)	Total conc. (µg/ml)	Conc. recovered (µg/ml)	% Recovery
80	10	8	18	8.086	100.904
100	10	10	20	10.040	100.485
120	10	12	22	12.141	100.361

*Recovery should be 98-102%

3.4 Robustness:

The standard deviation of the peak areas was calculated for each parameter and the %RSD was found to be less than 2 %. Results shows low values of % RSD, as shown in Table 6 signify the robustness of the method.

Table 6 Robustness data of Saxagliptin and dapagliflozin

Parameters	Normal Condition	Change in Condition	Drug	Area*	% RSD
Mobile phase ratio (phosphate buffer: methanol (70:30w/v) (±2.0)	70 :30	68:22	Saxagliptin	2839.933	0.769
			Dapagliflozin	2667.013	0.685
		72:18	Saxagliptin	2715.545	0.392
			Dapagliflozin	2503.915	0.824
Change in flow rate (±0.2)	1.0 ml/min	0.8 ml/min	Saxagliptin	2875.120	0.598
			Dapagliflozin	2679.691	0.298
		1.2 ml/min	Saxagliptin	2712.666	0.383
			Dapagliflozin	2534.448	0.286
Change in pH (±0.2)	6.0	5.8	Saxagliptin	2654.498	0.442
			Dapagliflozin	2482.484	0.830
		6.2	Saxagliptin	2834.237	0.799
			Dapagliflozin	2661.675	1.347

*Average of three determinations, RSD- Relative standard deviation

III FORCED DEGRADATION STUDY

Results for stress degradation studies of Saxagliptin and Dapagliflozin are shown in the table 7 and 8 respectively. The results of the methods lie within the prescribed limit, showing that method is free from interference from excipient.

Table 7 Results of forced degradation study of Saxagliptin

Stress conditions	Time (min)	Area	Degradants (% area)
0.1 N HCl	60	2110.669	24.050
0.1 N NaOH	60	2139.015	23.030
3% H_2O_2	60	2198.981	20.873
Heat exposure	30	2293.998	17.454
Sunlight	60 120	2071.648	Absent 25.455

Table 8 Results of forced degradation study of Dapagliflozin

Stress conditions	Time (min)	Area	Degradants (% area)
0.1 N HCl	60	2032.436	21.948
0.1 N NaOH	60	2016.063	22.577
3% H_2O_2	60	2005.205	22.994
Heat exposure	30	2210.771	15.100
Sunlight	60 120	1895.103	Absent 27.222

IV CONCLUSION

The proposed stability- indicating RP-HPLC method is suitable for simultaneous estimation of Saxagliptin and Dapagliflozin in pharmaceutical dosage form without any interference from each other. All the parameters for both the drugs met the criteria of ICH guidelines for method validation. The results show that the developed method was accurate, precise, simple, specific, robust and found to be stability indicating under stress conditions.

V ACKNOWLEDGEMENTS

The authors are grateful thank to Rivan Pharmaceuticals, India for providing a gift sample of Saxagliptin and Dapagliflozin.

VI REFERENCES

1. "Drug profile for Saxagliptin", August-2017, Available from : https://www.drugbank.ca/drugs/DB06335
2. "Drug profile for Dapagliflozin, August-2017 Available from : http://www.drugbank.ca/drugs/DB06292
3. "Drug profile for Dapagliflozin, August-2017 Available from : https://www.trc-canada.com/product-detail/?CatNum=D185370
4. ICH Q2B (2005) Validation of Analytical Procedure: Methodology, International conference on Harmonization, IFPMA, Geneva, Switzerland.
5. ICH Q2 (R1) (2005) Validation of Analytical Procedure: Text & Methodology, International conference on Harmonizati on, IFPMA, Geneva, Switzerland.
6. Hotha K, Reddy S, (2013) Force Degradation Study: Practical Approach Overview Regulatory guidance and literature f or drug products and drug substance *Inter Res J Pharm*, , 4(5), 78.
7. ICH, Q1A (R2) (2003) Stability Testing of New Drug Substance and Drug Product, International conference on Harmoni zation, IFPMA, Geneva, Switzerland
8. Thangabalan B, Srosowmya P, Babu SM, (2014)"Method development and validation for the simultaneous estimation of saxagliptin and metformin in tablet dosage form by RP-HPLC method" *Int. j. pharm. And anal. Res.*, 3(4), 363-369

9. Pednekar S, Lokhande R, Sutar R, Kolhal S, Surve S, Gudekar S,(2014) "Simultaneous Determination of Metformin, Sitagliptin, Saxagliptin, Linagliptin and Vildagliptin in Multicomponent Pharmaceutical Preparations by RP-HPLC" *Int. j. pharm. Sci. rev. sci.*, 28(1), 128-133
10. Manasa S, Dhanlaxmi K, Reddy NG,Sreenivasa S. "Method Development and Validation of Dapagliflozin in API by RP-HPLC and UV-Spectroscopy" *int. J. Of Pharma. Sci and Drug Res.*, 2014, 6(3),250-252
11. Jani BR, Shah KV and Kapupara PP., (2015) "Development and Validation of UV Spectroscopic First Derivative Method for Simultaneous Estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture" *J. of Bioequv. study*, 1(1),102