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Research

RP-HPLC Method Development Validation And Degradation Studies For Combined Tablet Dosage-Form Of Saxagliptin & Dapaglifozin

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Abstract

A new simple, rapid, economical reverse phase high performance liquid chromatographic method was developed for the determination of Dapagliflozin and Saxagliptin in bulk and dosage-form. The separation was carried out by using column as Hypersil ODS-C18 (250mm×4.6 mm i.d.2.5µm), mobile phase Methanol: Acetonitrile: acetate buffer (pH of4.0)40:40:20 v/v, at a flow rate of 1.0ml/min, diluent as 80:20v/v mixture of water & methanol used. The detection was made by UV-Vis. Spectrophotometer at 228nm. The retention times were 2.314min for Dapagliflozin and 2.904min for Saxagliptin. Calibration curve was linear over the concentration range of 2.04 to 12.05µg/ml for Dapagliflozin and 1.06 to 6.10µg/ml for Saxagliptin, mean recoveries obtained for Dapagliflozin and saxagliptin were 99.89-100.37% and 100.37-100.83% respectively, limit of detection and limit of quantification were found to be 0.257 & 0.780µg/ml and 0.439 & 1.33µg/ml respectively. The propose method was validated as per the ICH guidelines parameters. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug in the combined dosage form.

Keywords: Saxagliptin, Dapagliflozin, Tablet- Qtern*, degradation studies, ICH-guidelines.

INTRODUCTION

Saxagliptin (SAXA), chemically, known as (1s,3s,5s)-2-[(2s)-2-amino-2-(3-hydroxyl-tricyclo [3.3.1.1] dec-1-yl) acetyl] -2-azabicyclo[3.1.0] hexane-3-carbonitrile (Figure 1), is a potent, selective, long-acting, and reversible inhibitor of the enzyme di-peptidyl peptidase 4 (DPP-4) used for treatment of type 2 diabetes mellitus [1]. It is used as mono-therapy or in combination with other drugs. Dapagliflozin (DAPA) is chemically described as (1s)-1,5-anhydro-1-C-[4-chloro-3-[(4-ethoxy phenyl)methyl]phenyl]-D-glucitol (Figure 2). It belongs to a new class of oral anti-diabetic drugs called sodium glucose co-transporter 2 (SGLT2) inhibitors [2].

These sodium glucose co-transporters are responsible for glucose re-absorption in the kidney. DAPA is a first generation, selective SGLT inhibitor that blocks glucose transport with 100- fold selectivity for SGLT2 over SGLT1. The US FDA has approved a once-daily dose of Qtern (10 mg Dapagliflozin and 5 mg Saxagliptin) for the treatment of type-2 diabetes [3].

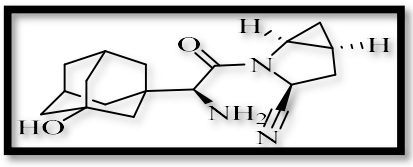

Fig 1: Structure for Saxagliptin

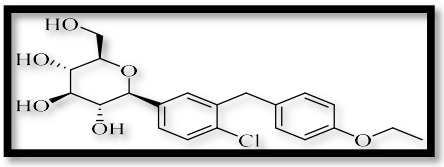

Fig 2: Structure for Dapagliflozin

A survey of literature revealed the availability of number of analytical methods for the quantitative determination of DAPA and SAXA alone or combination with other drugs. Thereported methods were mainly based on HPLC using UV-VIS, fluorescence, electrochemical or mass spectrometry detectors. The available methods are based on spectrophotometry, thin layer chromatography or high performance liquid chromatography [4-7]. However, in the present study, an attempt was made to develop a simple, precise and accurate method for the simultaneous estimation of these drugs in combined pharmaceutical dosage form and validate as per International Conference on Harmonization (ICH) guidelines [8-9].

MATERIALS AND METHODS

Chemical and Reagents

Pharmaceutical grade DAPA and SAXA were obtained as a gift samples from JaiLara Drugs Private Limited., Hyderabad, Telangana. Methanol used in analysis was of HPLC grade and potassium *di*-hydrogen *ortho*-phosphate and *ortho*-phosphoric acid used were of analytical reagent grade. All chemicals were purchased from Merck® India Ltd, Mumbai, and Rankem® Laboratory Chemicals. TabletsQTERN® Astra Zeneca Limited(10 mg DAPA and 5 mg SAXA) were purchased in local pharmacy.

Instrumentation and Chromatographic Conditions

HPLC system used in analysis consists of high pressure gradient pumps (LC-20AD) equipped with multichannel PDA detector (SPD-M20A), System controller (CBM-20A), make from Shimadzu Corporation, Japan. The sample introduction was performed with 20 mL sample loop injector (Rheodyne 7725*i*).

Other equipment used in analysis consists of digital analytical balance (ACS 100-4, Lab Friend), Ultrasonic Bath (LeelaSonic-200, India) and pH meter (HTLP-081).

All separations were performed on Hypersil ODS-C18; 4.6mm x 250mm, 2.5m molecule size at ambient temperature using mobile phase consisting of Methanol: Acetonitrile: acetate buffer with a pH of4.0(40:40:20 v/v) in isocratic mode at flow rate of 1 mL/min. The detection was performed at optimum wavelength of 228 nm.

EXPERIMENTAL

Preparation of Standard Stock Solution: Dapagliflozin & Saxagliptin were made into discrete stock arrangements by dissolving unequivocally weighed 10 mg of each medication in 100 mL of methanol, bringing about a 100µgml-1 stock arrangement of each medication. For linearity & other logical cycles, these stock arrangements were additionally weakened with a similar portable stage as expected to lay out working standard arrangements of 1-6 µg/ml & 2-12 µg/ml of SAXA & DAPA, individually. A Millipore layer channel with a 0.45 µm pore size was utilized to channel the last arrangement.

Preparation of DAPA and SAXA tablets solution: Weigh twenty tablets containing DAPA and SAXA (10mg and 5mg) and crushed into fine powder. Take equivalent weight of crushed powder of one tablet and dissolved in methanol in a 100 mL volumetric flask, and sonicat for 15 min. From the resulting solution, suitable aliquot was pipette out and transferred into a 10mL volumetric flask and the volume was made with mobile phase to obtain 20µg/mL of DAPA and 10µg/mL of SAXA, respectively. The prepared dilution was subjected to chromatographic analysis under mentioned conditions in triplicate and the corresponding concentrations were determined using the straight-line equation of DAPA and SAXA obtained in calibration curve experiments.

Calibration Curve (CC) standards: From the above prepared stock solutions, six dilutions were prepared and diluted with mobile phase to obtain CC standards with concentrations of 2, 4, 6, 8, 10 and 12 µg/mL of DAPA and 1, 2, 3, 4, 5, and 6 µg/mL of SAXA. Each CC standard was injected in triplicate and the peak area was plotted against the corresponding drug concentration. The straight-line equation was obtained and the regression coefficient (R^2) were determined for DAPA and SAXA, respectively.

Selection of UV wavelength: Saxagliptin λ maximum is at 235 nm, whereas dapagliflozin is at 224 nm in an 80:20 mixture of water & methanol. Both drugs were detected at 228 nm, either alone or together, the reaction

was sufficient.

METHOD VALIDATION

The developed method was validated as per ICH guidelines Q2(R1) with respect to system suitability, specificity, accuracy, precision, linearity, range, LOD&LOQ, and degradation studies.

System suitability: The framework reasonableness boundaries for following element, repeatability, number of hypothetical plates and goal among Dapagliflozin & Saxagliptin tops were tried by infusing a clear versatile stage and afterward rehashing the blend of Dapagliflozin (5µg/ml) &Saxagliptin (2.5µg/ ml) six times.

Specificity: The explicitness study expected to show the capacity to separate the title analyte tops from organic grid, fake treatment and any connected debasement tops. Each excipient that is regularly utilized in the making of measurements structures was available in the fake treatment arrangement. The specificity of the proposed method was established by the complete separation and resolution of DAPA and SAXA from its interfering excipients, if any. Blank tablets were chromatographed.

Accuracy and Precision: The accuracy and precision were evaluated by fortifying a powder mixture of blank tablets with the amounts of 50, 100 and 150 % of label claimed of DAPA and SAXA. The resulting tablets were analyzed in triplicate for three successive days and the % recovery at each level for each day was determined. The amount found compared with the amount added and the % RSD was considered as an indication of precision. Further, to determine the intermediate precision, results from the intra-day and inter-day studies.

Linearity and Range: Aliquots of medication working arrangements were moved to 10 mL volumetric flask and weakened with the versatile stage to lay out six standard focuses. The least-squares relapse strategy in straight relapse examination was used to assess linearity. The data pairs were subjected to regression analysis and the corresponding slopes and intercepts were determined for DAPA and SAXA, respectively.

Robustness: Chromatographic parameters including wavelength, pH of the buffer component, mobile phase ratio & flow rate have all been purposefully adjusted to the smallest possible degree. We also looked at the percentage recovery, percent RSD & tailing factor of the peaks for saxagliptin & dapagliflozin.

LOD&LOQ: The recipe used the direct relapse condition alongside the standard deviation of the catch & slant to ascertain the LOD&LOQ for SAXA & DAPA.LOD=3.3 Q/S & LOQ=10 Q/S

Where Q: the standard deviation of the intercept, S: the slope of the calibration curve.

Forced degradation studies: Constrained corruption studies were led on dapagliflozin & saxagliptin to show their individual security, demonstrating a strategy characteristic. The corruption examination utilized warm & photolytic debasement, oxidation (3% H2O2), corrosive hydrolysis (0.5 N HCl) and base hydrolysis (0.5 N NaOH).

Acid & base degradation: In a 10 ml standard volumetric flask, 1 ml of Dapagliflozin and Saxagliptin from the sample stock solution were mixed with 0.5N hydrochloric acid stopper formacid and base degradation experiments. To promote degradation in basic media, 0.5 N sodium hydroxide was utilized. After both samples were neutralized, the final solution was injected in triplicate under ideal chromatographic conditions.

Oxidative degradation: In the same way, 3% H2O2 was used to induce deterioration by oxidation reaction. The final solution was injected in triplicate under optimum chromatographic conditions.

Thermal degradation: To test for warm pressure, 10ml standard volumetric cups containing 1 ml aliquots of Saxagliptin&Dapagliflozin test arrangements were set inside & warmed to 80°C ± 1 °C for four hours utilizing a temperature-controlled broiler. This arrangement was additionally weakened with portable stage to get the chromatogram & the last arrangement was infused in three-fold.

Photolytic degradation: To test for warm pressure, 10ml standard volumetric flask containing 1 ml aliquots of Saxagliptin&Dapagliflozin test arrangements were set inside & warmed to 80°C ± 1 °C for four hours utilizing a temperature-controlled broiler. This arrangement was additionally weakened with portable stage to get the chromatogram & the last arrangement was infused in three-fold.

Photolytic degradation: Transferring 1ml aliquots of Dapagliflozin and Saxagliptin sample solutions into a 10ml standard volumetric flask and subjecting "exposed to visible light providing an overall illumination of minimum of a of 1.2 million lux hours and 200-watt hours/square meter with spectral distribution of 320-400 nm" for four hours resulted in photolytic deterioration. This solution was diluted further with mobile phase before being injected in triplicates under optimal chromatographic conditions.

RESULTS AND DISCUSSION

This study's essential objective is to create and approve an enhanced technique for the accomplished by fluctuating various boundaries.Different mobile phases were tried to achieve the separation and resolution of DAPA and SAXA. Initially, when methanol and acetonitrile were tried with water as an aqueous phase, inadequate separation with unaccepted peak shape were obtained for DAPA and SAXA which suggests replacing the water with buffer of appropriate strength as an aqueous phase. When mobile phase comprising of Methanol: Acetonitrile: acetate buffer with a pH of4.0(40:40:20 v/v) was tried with Hypersil ODS-C18; 4.6mm x 250mm, 2.5µm column, at flow rate of 1 mL/min, adequate retention of DAPA and SAXA (2.30 min and 2.72min, respectively) at 228 nm. Good peak shape with acceptable system suitability parameters (theoretical plates: DAPA: 5832, SAXA: 4392; asymmetry: DAPA: 1.34, SAXA: 1.43) were obtained. The chromatogram of DAPA and SAXA is presented in Figure 3.

The results obtained for system suitability, accuracy and precision (system & method) studies of DAPA and SAXA are presented in Table 1, 2 and 3 respectively. The mean values of amount found were close to the amount added and the low %RSD values indicates the acceptable accuracy and precision of the developed method. To determine the intermediate precision, which concludes no significant difference between intra- and inter-day precision and good intermediate precision given in Table 4.

In calibration curve experiments, DAPA was found linear in the range of 2–12 µg/mL and SAXA 1–6 µg/mL, respectively. The straight-line equations and regression coefficients obtained for DAPA was y=15108x+1385.7, R^2=0.9998 and for SAXA *y*=17071x+550.47, R^2=0.9997, respectively in Figure 4 & 5 data was given in Table 5&6.

When the blank tablets,Figure 8,and tablets containing the DAPA and SAXA were subjected to chromatographic analysis, no interfering peaks were observed at the retention times of the DAPA and SAXA suggesting the specificity of the developed method.

When tablets prepared on lab scale were analyzed, the results obtained were in good agreement with the nominal amounts of the drugs. DAPA was found 99.23% and SAXA was found 100.23%, respectively Table 7, Figure 7.

It was found that the constraints of quantification &detection for dapagliflozin&saxagliptin, separately, were 0.257 & 0.780 µg/ml & 0.439 & 1.33 µg/ml. In Figures 9 &10, the chromatograms are shown.

Forced degradation studies: Samples of dapagliflozin&saxagliptin subjected to different forced degradation settings showed distinct active & degradation product peaks at different retention times in their chromatograms. The peaks of the degradation products were identified and contrasted with those of the reference solution, indicating their stability. Acid degradation studies revealed the presence of one extra peak at 3.012 minutes, while base degradation studies revealed the presence of two additional peaks at 1.842 and 1.982 minutes, oxidative stress conditions, a considerable drop was seen in the area containing the extra peak single further peak was noted at 3.107 minutes, in temperature conditions, the peak area shrank by a non-significant amount and an additional brief peak appeared at 3.120 minutes and finally the photolytic circumstances caused the peak area to decrease non-significantly and no new peak was found the data was listed in Table 8.

Table 1: System suitability of Dapagliflozin & Saxagliptin

InjectionNumber	Dapagliflozin		Saxagliptin	
	Retention Time	Peak Area	RetentionTime	Peak Area
1	2.273	74271	2.754	54553
2	2.321	74528	2.732	54330
3	2.287	75429	2.683	53224
4	2.278	74472	2.711	54132
5	2.345	76929	2.783	52320
6	2.273	76293	2.683	53465
Mean	2.30	75320.33	2.72	53670.67
SD(±)	0.03	1094.92	0.040	835.72
%RSD	1.30	1.45	1.47	1.56
USPTailingFactor	1.34		1.43	
USP PlateCount	5832		4392	

Table 2: Accuracy of Dapagliflozin & Saxagliptin

Name of	%LevelSpiking	Amountof	Amount	TotalDrug(µg)	Total	%RSD	%

thedrug		drug (Tablet) µg	ofdrug(Standard) µg		Found(µg) Mean ±SD (n=3)		Recovery
Dapagliflozin	50%	4	2	6	6.02 ± 0.102	1.70	100.33
	100%	4	4	8	8.03 ± 0.071	0.89	100.37
	150%	4	6	10	9.98 ± 0.06	0.60	99.87
Saxagliptin	50%	2	1	3	3.02 ± 0.058	1.92	100.67
	100%	2	2	4	4.03 ± 0.074	1.858	100.83
	150%	2	3	5	5.01 ± 0.09	1.80	100.37

Table 3: System & method precision of Dapagliflozin & Saxagliptin At 5 &2.5 µg/ml

	System Precision		Method Precision	
InjectionNumber	Peak areas of Dapagliflozin	Peak areas of Saxagliptin	% Assay of Dapagliflozin	% Assay of Saxagliptin
1	75433	55393	100.55	101.98
2	76393	54930	101.45	101.03
3	74598	55593	99.34	100.45
4	75587	56093	100.02	102.4
5	75093	54832	100.11	99.56
6	76930	56204	102.31	102.1
Mean	**75672.33**	**55507.50**	**100.63**	**101.25**
SD(±)	**855.04**	**572.39**	**1.08**	**1.11**
RSD(%)	**1.13**	**1.03**	**1.07**	**1.09**
Acceptance criteria:	%RSD should not be more than 2			

Table 4a: Intra-day precision of Dapagliflozin & Saxagliptin

	Dapagliflozin			Saxagliptin		
S.No.	Concentration (µg/ml)	Peak Area (n=3)	%RSD	Concentration (µg/ml)	Peak Area (n=3)	%RSD
1	4.0	61329.67 ± 873.55	1.42	2.0	44477.67 ± 402.05	0.90
2	5.0	75713.33 ± 701.81	0.93	2.5	55899 ± 1006.83	1.80
3	6.0	9098.133 ± 1178.59	1.29	3.0	68851.33 ± 425.49	0.62

Table 4b: Inter-day precision of Dapagliflozin & Saxagliptin

Day	Conc.	Area of Dapagliflozin	Area of Saxagliptin
Day1	Dapagliflozin 4.0(µg/ml) & Saxagliptin 2.0 (µg/ml)	61325.00	45244.5
Day2		61556.16	45207.67
Day3		61971.83	45523.33
	Mean	1743889	61617.67
	SD(±)	10074.39	327.77
	%RSD	0.58	0.53
Day1	Dapagliflozin 5.0(µg/ml) & Saxagliptin 2.5 (µg/ml)	75587.83	55402.33
Day2		75953.33	55686.33
Day3		77193	56342.33
	Mean	2175454.67	76244.72
	SD(±)	9196.19	841.32
	%RSD	0.42	1.10
Day1	Dapagliflozin 6.0 (µg/ml) & Saxagliptin 3.0 (µg/ml)	90420.17	67639.83
Day2		93098.50	66911.67
Day3		93411.33	66790
	Mean	2596807	92310
	SD(±)	7229.83	1644.10
	% RSD	0.28	1.78

Table 5: Linearity table of Dapagliflozin

Standard Concentration(µg/ml)	Peak Area	Found Concentration(µg/ml)	Recovery%
2	32183	2.04	101.93
4	61382	3.97	99.28
6	91483	5.96	99.39
8	123034	8.05	100.65
10	151382	9.93	99.28
12	183384	12.05	100.39
Mean(n=6)			**100.15**
SD(±)			**1.05**
%RSD			**1.05**

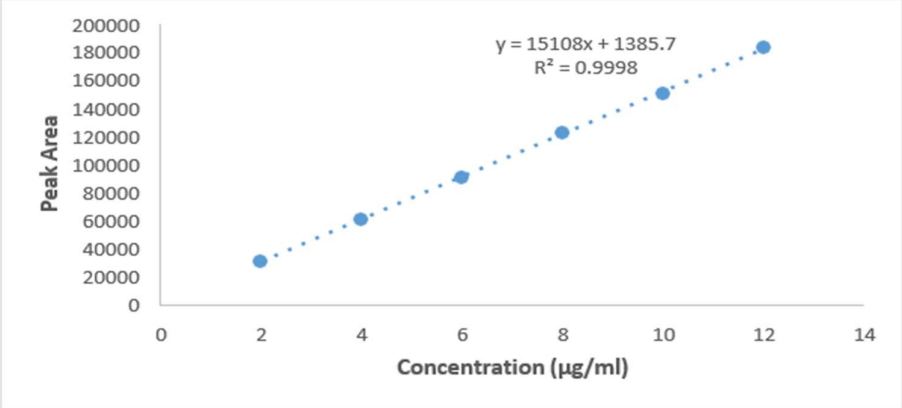

Fig 3: Calibration Curve for Dapagliflozin

Table 6: Linearity table of Saxagliptin

Standard Concentration(µg/ml)	Peak Area	Found Concentration(µg/ml)	Recovery%
1	17543	1.06	105.99
2	34557	2.06	102.83
3	52457	3.10	103.50
4	68648	4.05	101.34
5	85134	5.02	100.39
6	103456	6.10	101.54
Mean(n=6)			**102.60**
SD(±)			**1.99**
%RSD			**1.95**

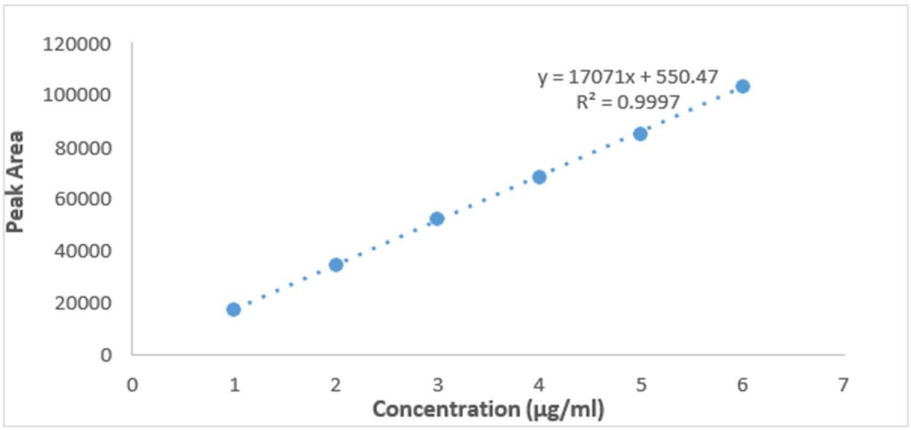

Fig 4: Calibration Curve for Saxagliptin

Table 7: Assay of Dapagliflozin & Saxagliptin

Marketed Formulation (Qtern) Tab	*Retention Time (min)	#*Peak Area	*%Assay
Dapagliflozin10mg	2.314	763628	99.23
Saxagliptin5mg	2.904	542920	100.23

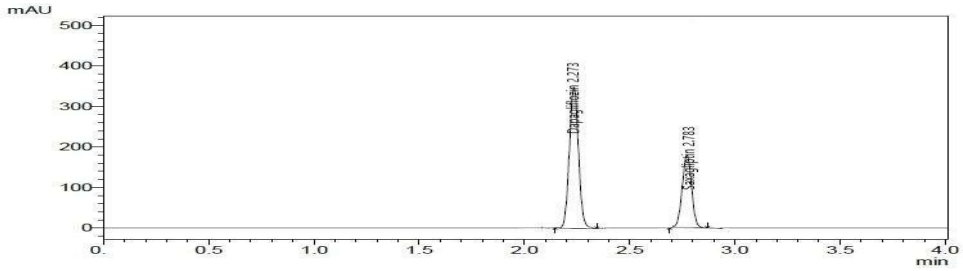

Fig 5: Chromatogram of standard Dapagliflozin & Saxagliptin

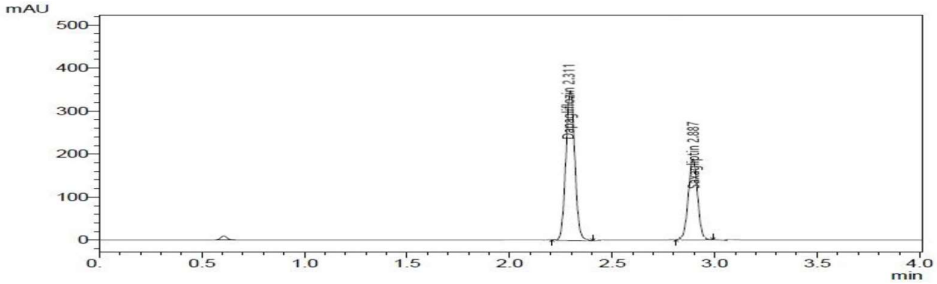

Fig6: Assay Chromatogram of (Sample)Tablet

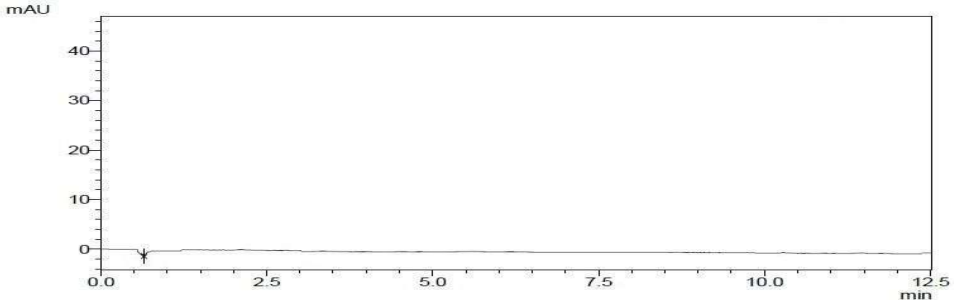

Fig7: Blank Chromatogram

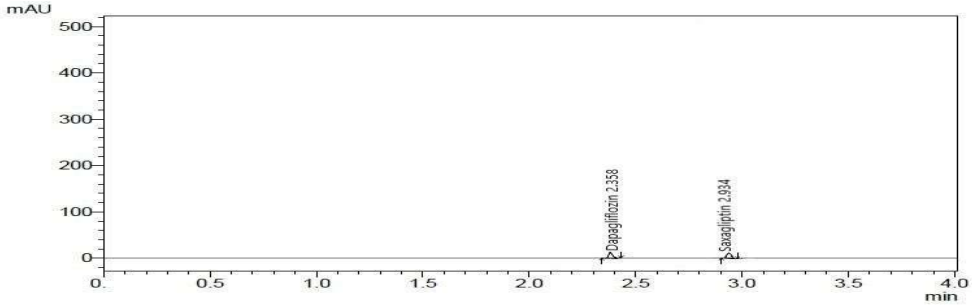

Fig 8: LOD Chromatogram of Dapagliflozin & Saxagliptin

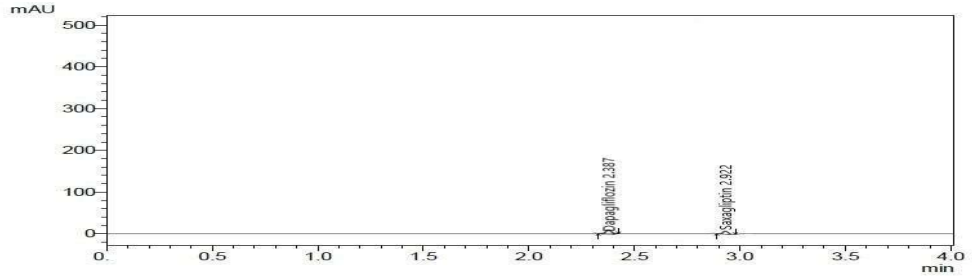

Fig 9:LOQ Chromatogram of Dapagliflozin & Saxagliptin

Table 8: Degradation studies of Dapagliflozin & Saxagliptin

Condition	Rt(min)		Purity Angle		Purity threshold		%Drug degraded	
	DAPA	SAXA	DAPA	SAXA	DAPA	SAXA	DAPA	SAXA
Acid	2.26	2.77	0.745	0.549	0.521	0.634	9.22	12.21
Base	2.45	3.02	0.646	0.434	0.527	0.534	6.33	9.45
Oxidative	2.26	2.76	0.454	0.250	0.594	0.367	7.22	5.67
Thermal	2.32	2.83	0.391	0.231	0.557	0.304	1.56	1.65
Photolytic	2.31	2.86	0.332	0.173	0.483	0.235	0.92	1.13

CONCLUSION

The propose method was validated as per the ICH guidelines parameters. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug in the combined dosage form. The ongoing review, which is introduced in this proposition, tried to explore the turn of events and approval of new scientific strategies involving RP-HPLC for advertised mix dose structures and the related dynamic drug parts, chromatography was utilized to isolate the picked drug and the cycle was approved as per the ICH rule for scientific strategy approval. The consequences of the different chromatographic boundaries used to isolate saxagliptin & dapagliflozin in Programming interface & business details are canvassed.

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