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A Validated High Performance Liquid Chromatography Method for the Determination of Saxagliptin and Metformin in Bulk and Pharmaceutical Dosage Form, a Stability Indicating Study

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Abstract: *A Stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Saxagliptin (SAX) and Metformin (MET) in bulk and pharmaceutical dosage form. The separation was carried on kromasil- C_{18} column (4.5 x 250 mm; 5 µm) column with mobile phase consisting of 50mM sodium dihydrogen phosphate buffer (pH adjusted to 2.7 using orthophosphoric acid): methanol in the ratio of 80:20 v/v with a flow rate of 0.9 ml/min and PDA detection at 242nm. The linearity was found to be in range of 0.5-3 µg/mL (R_2 = 0.9995) and 50-300 µg/mL (R_2 = 0.9995) for SAX and MET respectively. The method has shown good, consistent recoveries for SAX 98.39-101.53% and MET 100.46 -101.59% respectively. The method was found to be accurate, precise, specific, robust and linear for the determination of SAX and MET in bulk and pharmaceutical dosage form.*
Keywords: *ICH guidelines, Metformin, Method validation, RP-HPLC, Saxagliptin.*

I. Introduction

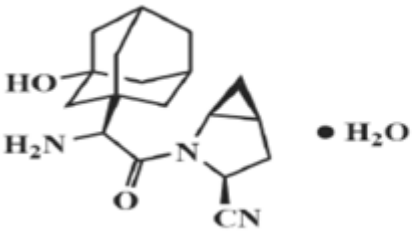

Figure 1: Structure of Saxagliptin

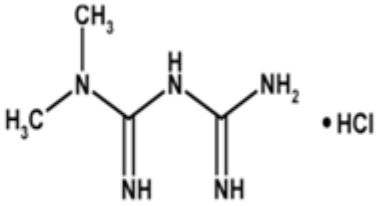

Figure 2: Structure of Metformin HCl

Saxagliptin is a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Chemically, it is (1S, 3S, 5S)-2-[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile. SAX is part of a class of diabetes medications called dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 is an enzyme that breaks down incretion hormones. As a DPP-4 inhibitor, SAX slows down the breakdown of incretion hormones, increasing the level of these hormones in the body. Type 2 diabetes mellitus is a common chronic disease that causes significant morbidity and mortality worldwide. The primary goal of treatment is to target glycemic control by maintaining the glycosylated hemoglobin (HbAlc) level near 6% to 7% without predisposing patients to hypoglycemia. Currently available anti-diabetic agents work by different mechanisms to lower blood glucose levels. The usual adult dose is 2.5 to 5 mg once daily regardless of meals. A daily dose 2.5 mg is recommended for patients with moderate to severe renal impairment or those who are taking potent CYP 3A4 inhibitors. In randomized clinical trials, SAX alone lowered HbAlc levels by about 0.5%; with better efficacy seen when combined with other agents.

Metformin is anti-diabetic drug in biguanide class. Chemically, it is (I, N, N-dimethyldiguanide). MET used in the treatment of Type 2 diabetes. MET decreases hepatic gluconeogenesis by interfering with respiratory oxidation in mitochondria. It suppresses gluconeogenesis from several substrates, including lactate, pyruvate, glycerol and amino acids. In addition MET increases intra-mitochondrial levels of calcium (Ca++), a modulator of mitochondrial respiration. It is a biguanide developed from galegine, a guanidine derivative found in Galege officinalis (French lilac). Chemically, it is a hydrophilic base which exists at physiological pH as the cationic species (>99.9%). Consequently, its passive diffusion through cell membranes should be very limited. MET is excreted unchanged in urine. The elimination half-life ($t_{1/2}$) of MET during multiple dosages in patients with good renal function is approximately 5 hours. Lactic acidosis is the feared adverse effect of the biguanide drugs but its incidence is very low in patients treated with MET. We suggest that the mean plasma

concentrations of MET over a dosage interval be maintained below 2.5 mg/mL in order to minimize the development of this adverse effect.Literature survey reveals RP-HPLC [1-8] methods were reported for the estimation of Saxagliptin and Metformin To the best of our knowledge there is no stability indicating RP-HPLC method reported for the simultaneous estimation of SAX and MET in bulk and pharmaceutical dosage form. Therefore, attempts were made in this study to develop a rapid, sensitive and selective stability indicating RP-HPLC method for the simultaneous determination of SAX and MET as per ICH guidelines [9-11]. The chemical structure of SAX was shown in **Fig 1**. The chemical structure of MET was shown in **Fig 2**.

II. Experimental Details

2.1. Chemicals and reagents

Saxagliptin was collected as a gift sample from Aurobindo Pharmaceuticals Limited, Hyderabad, India. Metformin HCl was collected as a gift sample from Ranbaxy Laboratories Limited, Gurgaon, India. Methanol required for the experiment was of HPLC grade and was purchased from E - Merck Specialties Pvt. Ltd, Mumbai, Potassium dihydrogen phosphate (KH_2PO_4) was purchased from Qualigens Fine Chemicals Ltd, Mumbai and Orthophosphoric acid was purchased from SD Fine Chemicals Ltd, Gujarat.

2.2. Instrument and chromatographic conditions

Chromatography was performed on Waters HPLC 2695 equipped with quaternary pumps with PDA detector. The chromatographic separation was performed using Kromasil- C_{18} column (4.5 x 250mmx5µm particle size). Separation was achieved using a mobile phase consisting of 50mM sodium dihydrogen phosphate buffer (pH adjusted to 2.7 using orthophosphoric acid): methanol (80:20 v/v), pumped at a flow rate of 0.9 ml/min. The eluent was monitored using PDA detector at a wavelength of 242nm. The mobile phase was vacuum filtered through 0.22µm nylon membrane filter followed by degassing in an ultrasonic bath prior to use. Data acquisition and integration was performed using Empower 2 software.

2.3. Preparation of Buffer Solution

The buffer solution was prepared by dissolving 6.24 g of sodium dihydrogen phosphate ($NaH_2P_4.2H_2O$) in 1000 ml of water and the pH was adjusted to 2.7 by adding orthophosphoric acid drop wise.

2.4. Preparation of Mobile phase

A mixture of 80 volumes of 50mM sodium dihydrogen phosphate buffer previously adjusted to pH 2.7 with orthophosphoric acid and 20 volumes of methanol.

2.5. Preparation of standard solution

Weighed accurately about 5 mg of Saxagliptin working standard and 500 mg of Metformin working standard in 100 ml volumetric flask, 30 ml of methanol was added and sonicated for 15 minutes and the volume was made up to the mark with methanol. Further dilution was made to get the final concentration of 2 µg/ml and 200 µg/ml of SAX and MET respectively.

2.6. Sample preparation for calibration curve of SAX and MET

Aliquots were taken from working standard solution and diluted with methanol to give final concentration of 0.5-3 µg/ml and 50-300 µg/ml for SAX and MET. Separately injected the linearity solution in increasing concentration levels into the chromatograph and recorded the peak response. Calibration graph was constructed by plotting peak area versus concentration.

2.7. Precision

2.7.1. Repeatability

Solution containing 2 µg/ml and 200 µg/ml of SAX and MET was prepared. Prepared solution was analyzed six times in same day as per the proposed method.

2.7.2. Intermediate precision

2.7.2.1. Intraday precision

Solution containing 2 µg/ml and 200 µg/ml of SAX and MET was prepared from their respective standard stock solution. Analysis was replicated for 3 different times within same day.

2.7.2.2. Intraday precision

Solution containing 2 µg/ml and 200 µg/ml of SAX and MET was prepared from their respective standard stock solution. Analysis was replicated for 3 different days.

2.8. Linearity

The calibration curves constructed for SAX and MET were checked for linearity over the concentration range of 0.5-3 µg/ml and 50-300 µg/ml for SAX and MET. Calculated the correlation coefficient.

2.9. Accuracy

Accuracy was calculated by addition of standard drugs to preanalyzed sample at 3 different concentration levels and computing percentage recoveries. Standard limit of % recovery study is 98 - 102 % as per ICH guideline. From the studies it was concluded that % recovery study of SAX and MET complies with standard limit of ICH guideline.

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

The Limit of Detection and Limit of Quantification of the developed method were calculated from the standard deviation of the y-intercepts and slop of the calibration curve of SAX and MET using the formula as given below.

Limit of Detection = 3.3 α / S

Limit of Quantitation = 10 α / S

Where α is the standard deviation of the y – intercepts and S is the slop of the calibration curve.

2.11. Robustness

As per ICH, the prepared solution was analyzed as per proposed method with small but deliberate change in chromatographic conditions as listed below:

- Change in flow rate
- Change in mobile phase composition
- Change in nanometer
- Change in temperature
- Change in pH

2.12. Stability of solution

Three different concentration of SAX and MET were prepared from sample solution and stored at room temperature for 6 hours and 24 hours at room temperature. They were then injected into HPLC system.

2.13. Analysis of marketed formulation of SAX and MET

To determine the content of SAX and MET in formulations, tablet powder equivalent to 5 mg of SAX and 500 mg of MET were weighed and transferred in to 100 ml volumetric flask, 30 ml of methanol was added and sonicated for 15 minutes and the volume was made up to the mark with methanol. Further dilution was made to get the final concentration of 2 µg/ml and 200 µg/ml of SAX and MET respectively. The analysis was repeated for six times.

III. Results and Discussion

Validation of developed RP-HPLC method was carried out as per ICH guidelines Q2 (R1). To develop an effective method for the analysis of the drugs, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection wavelength, ideal mobile phase and its combination, optimum pH and concentration of the standard solutions were studied. The mobile phase consisted of 50mM sodium dihydrogen phosphate buffer (pH adjusted to 2.7 using orthophosphoric acid): methanol (80:20 v/v) with a flow rate of 0.9 ml/min was selected for analysis after preliminary tests the optimized chromatographic condition represented in **Table 1**. The retention time of SAX and MET were found to be 2.285 min and 3.157 min, respectively. A representative chromatogram of standard and sample were shown in **Fig 3(a) and (b).** The system suitability tests were carried out on freshly prepared standard solutions and the %RSD values were within the limit (<2.0) and represented in **Table 2**. The linearity was found in the concentration of 0.5-3 µg/ml and 50-300 µg/ml for SAX and MET. The correlation coefficient was found to be 0.9995 and 0.9995 for SAX and MET for both the drugs respectively. The results are presented in **Table 3** and **Fig 4 and 5**. The % accuracy was found between the range of 98.39-101.53 for SAX and 100.46-101.59 for MET and represented in **Table 4** and **5**. By performing system precision and method precision studies, the % RSD values were within the limits (<2.0) and the method was found to be highly precise and represented in **Table 6**. The results of intra-day and inter-day precision studies were shown in **Table 7** and **8**. They revealed that the %RSD of intra-day and inter-day were within the permissible limits of 2%. Robustness of the method was studied by changing the chromatographic conditions slightly and the results are presented in **Table 9**. Specificity was evaluated by injecting the blank, placebo and sample. There was no other interfering peak around the retention time of SAX and MET. So the proposed method was found to be simple, accurate and specific, hence it could be used for

routine analysis of SAX and MET in combined dosage form. The results of LOD and LOQ for SAX and MET obtained were presented in **Table 10.**

Table 1 Optimized Chromatographic Condition for the Estimation of Saxagliptin and Metformin

Parameter	**Condition**
Mobile phase	50 mM sodium dihydrogen phosphate buffer (pH adjusted to 2.7 using orthophosphoric acid): methanol (80:20 v/v)
Diluent	Methanol
Column	Kromasil- C_{18} column (4.5 X 250mm; 5µm)
Column temperature	30^0C
Detection wavelength	242nm
Injection volume	10µl
Flow rate	0.9 ml/min
Run time	6 min

Table 2 System Suitability Parameters of Saxagliptin and Metformin

S.No	**Parameters**	**Name of Drug**		**Acceptance Criteria**
		Saxagliptin	**Metformin**	
1	Retention time	2.258 min	3.157 min	---
2	Theoretical plates (N)	7041	6288	> 2000
3	Tailing factor	1.52	1.50	< 2
4	Asymmetry factor	1.91	1.92	< 2
5	Capacity factor	1.14	2.01	> 1 < 10
6	Resolution	2.71		> 2
7	% RSD of Peak area	0.9292	0.6664	< 2
8	% RSD of Retention time	0.4147	0.8913	< 2

Table 3 Linearity Data of Saxagliptin and Metformin

S.No	**Saxagliptin**		**Metformin**	
	Concentration (µg/ml)	**Mean Peak Area (n = 6)**	**Concentration (µg/ml)**	**Mean Peak Area (n = 6)**
1	0.5	489493	50	3455863
2	1.0	1023721	100	7036198
3	1.5	1581503	150	11251752
4	2.0	2056856	200	14180815
5	2.5	2554863	250	17730520
6	3.0	2994392	300	21439436
Slope		**1010503.88**	**Slope**	**71437.31**
y- intercept		**12934.29**	**y- intercept**	**12201.04**
Correlation coefficient		**0.9995**	**Correlation coefficient**	**0.9995**

Table 4 Accuracy Data of Saxagliptin

Parameters	**Amount Present (µg/ml)**	**Amount Added (µg/ml)**	**Peak Area**	**Amount Found (µg/ml)**	**Amount Recovered (µg/ml)**	**% Amount Recovered**
80%	2	1.6	36331133	3.53	1.53	100.87
			3643482	3.54	1.54	101.53
			3632797	3.53	1.53	100.85
100%	2	2	4068379	3.97	1.97	99.49
			4059363	3.94	1.94	98.39
			4066238	3.95	1.95	98.48
120%	2	2.4	4547306	4.42	2.42	100.41
			4536981	4.41	2.41	100.09
			4541736	4.41	2.41	100.28
Average						**100.04**
SD						**1.074**
%RSD						**1.073**
SE						**0.3579**
CI (Confidence Interval 99%)						**98.83 – 101.24**

Table 5 Accuracy Data of Metformin

Parameters	**Amount Present (µg/ml)**	**Amount Added (µg/ml)**	**Peak Area**	**Amount Found (µg/ml)**	**Amount Recovered (µg/ml)**	**% Amount Recovered**
80%	200	160	25703850	362.62	162.62	101.50
			25664161	362.06	162.06	101.15
			25691815	362.45	162.45	101.59
100%	200	200	28582840	403.23	203.23	101.55
			28585670	403.27	203.27	101.57

			28505953	402.15	202.15	101.01
120%	200	240	31289902	441.43	241.43	100.79
			31307641	441.68	241.68	100.90
			31268462	441.12	241.12	100.46
Average						**101.16**
SD						**0.4088**
%RSD						**0.4041**
SE						**0.1363**
CI (Confidence Interval 99%)						**100.70 – 101.61**

Table 6 Precision Data of Saxagliptin and Metformin

Saxagliptin				**Metformin**		
Sample Solution	**Concentration (µg/ml)**	**Peak Area**	**% Amount Found**	**Concentration (µg/ml)**	**Peak Area**	**% Amount Found**
Sample solution-1	2	2055197	99.93	200	14234579	100.40
Sample solution-2		2068037	100.55		14140298	99.74
Sample solution-3		2023033	98.37		14365576	101.33
Sample solution-4		2082166	101.24		14241358	100.45
Sample solution-5		2070306	100.67		14243949	100.47
Sample solution-6		2069453	100.62		14292596	100.81
Average			**100.23**	**Average**		**100.53**
SD			**0.9144**	**SD**		**0.4777**
% RSD			**0.9123**	**% RSD**		**0.4751**

Table 7 Intra-day Precision Data of Saxagliptin and Metformin

Parameter	**Saxagliptin**			**Metformin**		
	Concentration (µg/ml)	**Peak Area***	**% Amount Found***	**Concentration (µg/ml)**	**Peak Area***	**% Amount Found***
0 Hours	2	2053762	99.89	200	14190078	100.23
3 Hours		2053988	99.97		14204003	100.30
6 Hours		2053751	99.92		14195302	100.27
SD			**0.1161**	**SD**		**0.3228**
%RSD			**0.1159**	**%RSD**		**0.3218**

* Mean of six determinations

Table 8 Inter -day Precision Data of Saxagliptin and Metformin

Parameter	**Saxagliptin**			**Metformin**		
	Concentration (µg/ml)	**Peak Area***	**% Amount Found***	**Concentration (µg/ml)**	**Peak Area***	**% Amount Found***
Day – I	2	2054039	99.89	200	14195512	100.18
Day – II		2053932	99.92		14191394	100.21
Day – III		2053532	99.91		14202477	100.23
SD			**0.1116**	**SD**		**0.2219**
%RSD			**0.1114**	**%RSD**		**0.2214**

* Mean of six determinations

Table 9 Robustness Data of Saxagliptin and Metformin

Parameters	**Saxagliptin**			**Metformin**		
	R.T	**% Amount Found**	**%RSD**	**R.T**	**% Amount Found**	**%RSD**
Flow minus (0.7 ml/min)	3.180	100.61	**0.2348**	2.300	101.30	**0.3754**
Flow plus (1.1 ml/min)	3.195	99.43	**0.3225**	2.310	100.64	**0.4408**
pH minus (- 0.2)	3.199	99.06	**0.5702**	2.310	99.63	**0.7150**
pH plus (+ 0.2)	3.185	100.09	**0.6774**	2.311	100.94	**0.2434**
nm plus (244)	3.183	99.31	**0.4186**	2.312	99.59	**0.3794**
nm minus (240)	3.134	98.91	**0.5205**	2.314	100.29	**0.5540**
Temperature plus (32^0C)	3.195	101.33	**0.2928**	2.308	100.65	**0.7085**
Temperature minus (28^0C)	3.199	98.44	**0.2351**	2.307	100.13	**0.7623**
Methanol (25)	3.334	98.23	**0.1899**	2.310	101.15	**0.4632**
Methanol (15)	3.334	98.33	**0.1550**	2.310	98.48	**0.1755**

*Mean of six determinations, R.T = retention time

Table 10 LOD and LOQ Data of Saxagliptin and Metformin

S.No	Saxagliptin		Metformin	
	Slope	**Y-Intercept**	**Slope**	**Y-Intercept**
1	1011688.35	12048.32	71424.61	12494
2	1009206.64	13581.89	71438.86	13209.10
3	1010988.92	12659.32	71430.98	13595.42
4	1010047.57	13228.07	71453.48	10663.96
5	1011479.57	12268.07	71420.20	12350.53
6	1009612.21	13820.10	71455.73	10893.25
Average	**1010503.88**		**71437.31**	
SD		**720.3838**		**1195.12**
	LOD (µg/ml)	**0.0023**	**LOD (µg/ml)**	**0.0552**
	LOQ (µg/ml)	**0.0071**	**LOQ (µg/ml)**	**0.1672**

Stability in sample solutions

No additional peak was found in chromatogram indicating the stability of SAX and MET in the sample solution.

Analysis of marketed formulation of SAX and MET

The validated RP-HPLC method was successfully applied for the assay of SAX and MET in marketed formulations. Assay results are represented in **Table 11**.

Table 11 Analysis of Saxagliptin and Metformin in Marketed Formulation

Formulation	**Saxagliptin**			**Metformin**		
	Label Claim (mg)	**Amount Found (mg)**	**% Assay**	**Label Claim (mg)**	**Amount Found (mg)**	**% Assay**
Kombiglyze (Saxagliptin 5 mg and Metformin 500 mg)	5	4.97	99.49	500	506.82	101.36
		4.95	99.03		503.47	100.69
		4.98	99.61		507.91	101.58
		4.98	99.78		506.43	101.28
		4.97	99.47		503.64	100.72
		4.93	98.75		501.84	100.36
Average			**99.35**	**Average**		**100.99**
SD			**0.3870**	**SD**		**0.4751**
%RSD			**0.3895**	**%RSD**		**0.4704**
SE			**0.1580**	**SE**		**0.1939**
CI (Confidence Interval 99%)			**98.71 – 99.98**	**CI (Confidence Interval 99%)**		**100.20 – 101.77**

IV. Forced Degradation Study

Forced degradation studies were performed to evaluate the stability indicating properties (Specificity) of the proposed method. SAX and MET was subjected to neutral, acid, base, oxidation, thermal and photo degradation to ensure the effective separation of degradation peaks and main peak. From the degradation of these solutions under the stress condition gives us an idea about the origin of degrading products. Degradants did not show any interference with the elution of drug peaks. Hence, the method is stability indicating. The results of degradation studies were shown in **Table 12**.

4.1. Control Sample

A quantity tablet powder equivalent to 5 mg of SAX and 500 mg of MET were accurately weighed and transferred to 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent. Further pipette 0.4 ml of the above stock solution and transferred to 10 ml volumetric flask and made up to 10 ml with diluent to get the final concentration of 2 µg/ml of SAX and 200 µg/ml of MET and 10 µl of the final solutions were injected in to the system and the chromatograms were recorded.

4.2. Neutral Degradation Studies

A quantity tablet powder equivalent to 5 mg of SAX and 500 mg of MET was accurately weighed and transferred to 100 ml volumetric flask and it is dissolved in30 ml of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent. Further pipette 0.4 ml of the above stock solution and transferred to 10 ml volumetric flask and made up to 10 ml with diluent to get the final concentration of 2 µg/ml of SAX and 200 µg/ml of MET and the solution was refluxed in water bath for 30 min at 80^0C and 10 µl of the refluxed solutions were injected in to the system and the chromatograms were recorded.

4.3. Acid Degradation Studies

A quantity tablet powder equivalent to 5 mg of SAX and 500 mg of MET was accurately weighed and transferred to 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent, 0.4 ml of the above stock solution was transferred to 10 ml volumetric flask to that 0.4 ml of 2 N hydrochloric acid was added and it was diluted to get the final concentration of 2 µg/ml of SAX and 200 µg/ml of MET and refluxed for 30 min at 80^0C, 10 µl of the refluxed solutions were injected in to the system and the chromatograms were recorded.

4.4. Alkaline Degradation Studies

A quantity tablet powder equivalent to 5 mg of SAX and 500 mg of MET was accurately weighed and transferred to 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent, 0.4 ml of the above stock solution was transferred to 10 ml volumetric flask to that 0.4 ml of 2 N sodium hydroxide was added it was diluted to get the final concentration of 2 µg/ml of SAX and 200 µg/ml of MET and refluxed for 30 min at 80^0C, 10 µl of the refluxed solutions were injected in to the system and the chromatograms were recorded.

4.5. Oxidative Degradation Studies

A quantity tablet powder equivalent to 5 mg of SAX and 500 mg of MET was accurately weighed and transferred to 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent, 0.4 ml of the above stock solution was transferred to 10 ml volumetric flask to that 0.4 ml of 3 % hydrogen peroxide (H_2O_2) was added it was diluted to get the final concentration of 2 µg/ml of SAX and 200 µg/ml of MET and refluxed for 30 min at 80^0C, 10 µl of the refluxed solutions were injected in to the system and the chromatograms were recorded.

4.6. Thermal Degradation Studies

A quantity tablet powder equivalent to 5 mg of SAX and 500 mg of MET was accurately weighed and transferred to 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent, 0.4 ml of the above stock solution was transferred to 10 ml volumetric flask and made up to 10 ml with diluent to get the final concentration of 2 µg/ml of SAX and 200 µg/ml of MET and the solution was placed in oven at 80^0C for 48 hrs, 10 µl of the solutions were injected in to the system and the chromatograms were recorded.

4.7. Photolytic Degradation Studies

A quantity tablet powder equivalent to 5 mg of SAX and 500 mg of MET was accurately weighed and transferred to 100 ml volumetric flask and it is dissolved in 30 ml of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 ml with diluent, 0.4 ml of the above stock solution was transferred to 10 ml volumetric flask and made up to 10 ml with diluent to get the final concentration of 2 µg/ml of SAX and 200 µg/ml of MET and the solution was exposed to UV light by keeping the volumetric flask in UV chamber for 7 days, 10 µl of the solutions were injected in to the system and the chromatograms were recorded.

Table 12 Forced Degradation Study Data of Saxagliptin and Metformin

Parameters	**Degradation Time**	**Peak Area***		**% Degradation**		**% of Active Drug Present After Degradation**	
		SAX	**MET**	**SAX**	**MET**	**SAX**	**MET**
Control sample	-	2068037	14292596		-	-	
Neutral sample	30 min	2025622	14168544	2.06	0.87	99.48	99.91
Acidic degradation	30 min	1954663	13411457	5.51	6.21	95.03	94.57
Alkaline degradation	30 min	1971758	13652694	4.68	4.50	95.86	96.27
Oxidative degradation	30 min	1989815	14121574	3.80	8.09	96.74	92.69
Thermal degradation	48 hrs	2013075	14156781	2.67	1.20	97.87	99.58
Photolytic degradation	7 days	2020472	14168544	2.31	0.85	98.23	99.83

* Mean of six determinations

V. Conclusion

A stability indicating RP-HPLC method has been developed and validated for the determination of Saxagliptin and Metformin in combined pharmaceutical dosage forms. The developed method was validated as per ICH guidelines and was found to be accurate, precise, robust, specific and less time consuming. No interference from any components of pharmaceutical dosage form observed, and the method has been successfully used to perform rapid and accurate analysis of Saxagliptin and Metformin in their combined pharmaceutical dosage form.

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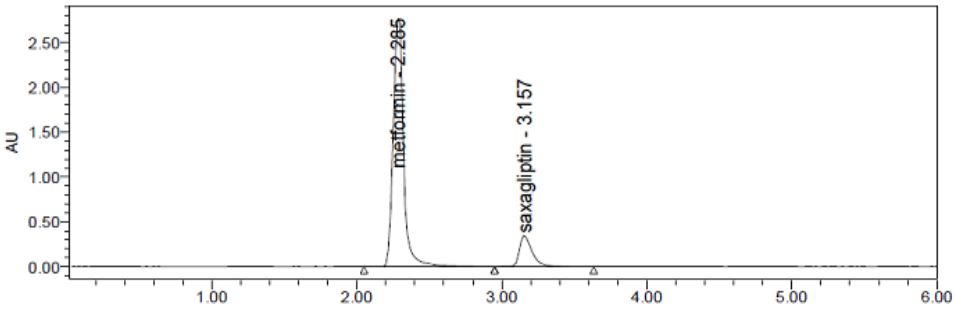

Figure 3 (a) Typical HPLC Chromatogram of Saxagliptin and Metformin Standard Drug

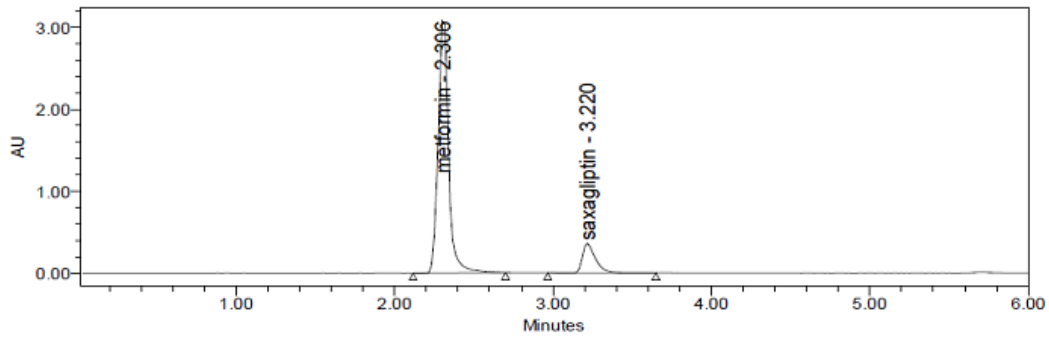

Figure 3 (b) Typical HPLC Chromatogram of Saxagliptin and Metformin Sample Drug

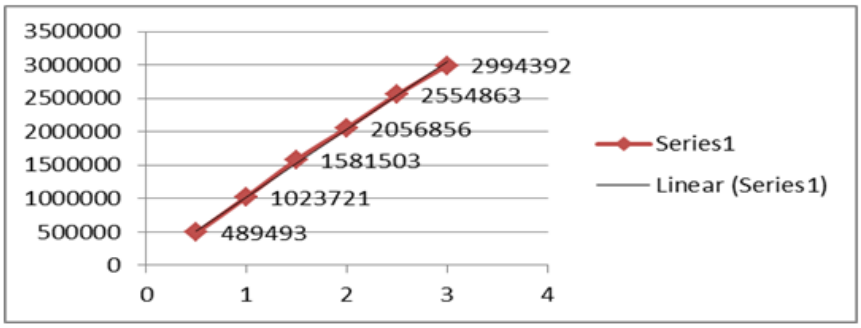

Figure 4 Linearity Plot of Saxagliptin

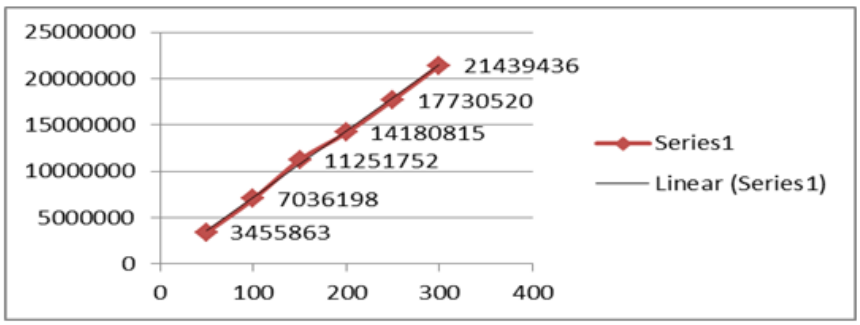

Figure 5 Linearity Plot of Metformin