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Stability Indicating HPTLC Method Development and Validation for the Estimation of Saxagliptin in Bulk and its Dosage Form

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Abstract:

A simple, precise, accurate and specific HPTLC method was developed and validated for analysis of Saxagliptin in bulk and its dosage form with its stability indicating studies. The TLC plates used for chromatography as a stationary phase were aluminum plates precoated with silica gel 60F254. The development was done with a mobile phase Toluene: Methanol: Ammonia (6:4:0.2 v/v/v). The densitometric scanning was done at 222nm and separation occurred was found to be at Rf value of 0.50 ± 0.03. Good results were obtained for all the validation parameters which were performed in accordance with the ICH guidelines. This method for saxagliptin yielded a good linear relationship for 500-3000 ng/band concentration range. Regression equation obtained was y= 1999.3x + 529.36 and the R^2 was 0.995. The method was found to be precise as all the results of precision parameters were within the limits (%RSD Less than 2). Percentage recovery was found to be in the range of 98% to 102%. The method was found to be robust as indicated by its results obtained below 2.0% RSD. LOD and LOQ values of the method were 21 ng/band and 66 ng/band respectively. Assay of the tablet dosage form was found to be 99.50%. Forced degradation studies of Saxagliptin were performed at various stressed conditions indicated that the drug is sensitive to acid, alkali, neutral, thermal and photolytic stress conditions and highly sensitive to oxidation degradation. The developed method can be used for the analysis of Saxagliptin.

Keywords: HPTLC, Saxagliptin, Stability indicating, Validation.

INTRODUCTION:

Saxagliptin [SXG] is chemically, (1S,3S,5S)-2-[(2S)-2-amino-2-(3-hydroxy-1- adamantyl) acetyl]-2-azabicyclo [3.1.0] hexane-3-carbonitrile, previously identified as BMS- 477118 (Fig no.1). It is a new oral anti diabetic (hypoglycemic) drug of the dipeptidyl peptidase- 4 (DPP-4) inhibitor class of drugs. As it is a DPP-4 inhibitor type of drug, it acts by reducing the incretin breakdown and increasing insulin production, ultimately regulating blood glucose levels. Because incretin hormones are more active in response to higher blood sugar levels (and vice versa) the risk of dangerously low blood sugar levels (hypoglycemia) is low with SXG monotherapy [1]. Literature survey has reveals that there are few analytical methods such as UV visible spectroscopic, HPLC and HPTLC are available for SXG alone [2-6] and with other drugs such as metformin [7-15]. A stability indicating HPTLC method is also reported for estimation of SXG alone but this method does not provide any evidence of degradation under photolytic and thermal condition. Therefore, objective of the present investigation is to develop stability indicating method to study the degradation of the SXG under stress conditions and validate the developed method as per ICH guidelines.

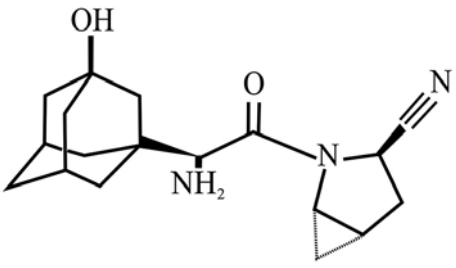

Figure 1- Chemical structure of Saxagliptin.

EXPERIMENTAL SECTION

Materials:

The standard sample of SXG (% purity 99.89% v/v) was kindly supplied by Glenmark pharmaceutical Ltd., Mumbai, India. Tablets of SXG, Onglyza (5mg) tablets manufactured by Bristol- Myers Squibb, s. r. l., Italy, were purchased from local market which are available under brand name. Other chemicals used were of AR grade (Make) and were procured from the local supplier.

Instruments Used:

Merck HPTLC silica gel precoated TLC plates 60F254 were used for chromatographic separation. The sample applicator used was CAMAG sample applicator Linomat 5. The syringe used for application was CAMAG 100µl (Hamilton). The CAMAG twin trough glass developing chamber (10×10 cm) was used for linear ascending development. Scanning of the plates was done using CAMAG TLC Scanner 3 (CHF47150) and the software used for interpretation was CAMAG Win cats (version 4.0.1). Weighing balance used was of Shimadzu (AX200). Ultra-sonic water bath of Wensar (WUC2L) was used for sonication. A hot air oven of Biotechnic (BTI 29) was also used. An electric water bath of Systronics (1601) was used for heating purpose and a UV cabinet of Labline BTI (49) was used for detection of spots and for photolytic degradation at 254nm.

Preparation of standard stock solution:

Accurately weighed about 5mg of SXG was transferred into a 10ml volumetric flask containing 5ml of methanol. The content was dissolved by shaking for 10min followed by 10 min sonication. Volume was made up to the mark with methanol to make the standard stock solution concentration 500 µg/ml. Further the solutions were diluted to get appropriately to get desired concentration.

Preparation of sample solution:
Twenty tablets of SXG were weighed (Onglyza-5mg) and average weight of these tablets was calculated. Tablets were finely powdered. Accurately weighed about 240mg of powdered tablet (equivalent to labelled claim 5mg of Saxagliptin) and was transferred to 10ml volumetric flask and 5ml of methanol was added, the content was dissolved by sonication for 20 min. Further it was diluted with methanol up to the mark to make the stock solution concentration 500 µg/ml and the content filtered through Whatman filter paper no. 41. Further, it was diluted appropriately to get the desired concentration.

Chromatographic Conditions:
The precoated silica gel chromatographic plates were used for analysis. Samples were applied on plates as bands of 5mm width at room temperature. The mobile phase optimised for analysis was Toluene: Methanol: Ammonia (6:4:0.2 v/v/v) mixture. Detection was done at 222nm. Twin trough glass chamber (10×10 cm) was used for mobile phase saturation and development of plates. Mobile phase saturation was done for 10min and 10ml of mobile phase was used for a single development. The run distance was kept at 8cm on plates. Mobile phase saturation and plate run was done at room temperature. Drying of developed plates was done at room temperature for 5 min.

Method Validation[16-18]**:**
The developed method was validated for the following parameters as per ICH guidelines.

Linearity:
Different concentrations of SXG 0.5-3µ/ml was applied on the plate. It was developed using mobile phase [Toluene: Methanol: Ammonia (6:4:0.2 v/v/v)], dried and scanned at 222nm. The peak areas were measured. The calibration curve was prepared by plotting the peak area versus concentration.

Precision:
Intraday & Inter day precision: Three replicates of preanlyzed sample of SXG (2000ng/band) at regular interval on the same day at 0hr, 3hr and 6hr for Intraday & on three different consecutive days for Inter day precision.
Repeatability- It was performed by applying same concentration (2000ng/band) of SXG at six points on a plate and the peak areas were measured. Results of these are reported in terms of %RSD.

Accuracy:
Accuracy was determined by calculating the recovery of SXG by the standard addition method at 80%, 100% and 120% of the labeled claim. A known amount of standard SXG (4.05mg, 5.01 mg, 6.1mg) were added to pre-analyzed sample of tablet powder. Each solution was applied in triplicate and recovery was calculated

Specificity:
Chromatographic run was carried out by applying standard solution (500ng/band) of SXG, Sample solution (500ng/band), methanol and mobile phase and densitogram was recorded.

Robustness:
It was performed by varying the saturation time (5min and 15 min), total amount of mobile phase (11.2ml and 9.2ml) and mobile phase ratio variation (5:5:0.2 and 7:3:0.2 v/v/v).

Limit of detection and Limit of quantitation (LoD and LoQ):
As per ICH guideline, the (LoD) and (LoQ) of the SXG were calculated using following equations-
$LOD=3.3(\sigma/S)$,
$LOQ=10\ (\sigma/S)$
Where, σ is the standard deviation of the response and S is the slope of the calibration curve.

Forced degradation study of SXG:
The samples of SXG were prepared for acidic, alkaline, neutral and oxidative degradation studies. For acid hydrolysis, quantity equivalent to 5mg of SXG of tablet powder (average weight 240mg) was accurately weighed and transferred into a 10ml volumetric flask in which 3ml of 0.1 N HCl was added, which was further diluted with water up to the mark. The same procedure was followed for 0.1 N NaOH, 3% $H_2O_2$and 3ml distilled water for basic, oxidative and neutral degradation respectively. These reactions were carried out at 60°C for 1hr. For photo stability study, 240mg of tablet powder in volumetric flask was kept in UV cabinet under UV light (254nm) for 24hr. Thermal degradation was carried out at 60^0C for 10min (dry heat). The samples were examined by HPTLC after appropriate dilutions and result was compared with Control.

RESULTS & DISCUSSION

Optimization of mobile phase:
Aliquot portions of standard stock solution (500µg/mL) 0.4µL (2000ng/band), was applied on different TLC plates in the form of band (band size: 6 mm) and the plates were run in different solvent systems. In an attempt to achieve the desired R_f value range with a compact band, several trials were made by using different solvent systems containing non-polar solvents and relatively polar like Methanol: Toluene, Toluene : Methanol : Triethylamine, Methanol: Toluene : Ethyl Acetate, Toluene : Methanol : Ammonia; at different concentration levels were tried in order to determine the best conditions for the effective separation of Saxagliptin. Among the different mobile phase combinations tested, Toluene: Methanol: Ammonia (6:4:0.2 v/v/v) gave compact bands which showed symmetrical peak on chromatogram and R_f value that is 0.50 ± 0.03 respectively.

Analysis of marketed formulation:
The estimation of SXG in tablet (Onglyza tablets 5mg) was carried out by applying the 0.4 µl (2000 ng/band) of the prepared sample was applied and the peak area was noted in five replicates. The average % assay was found to be 99.50%, w/w with %RSD of 1.48%. It indicates that the method is suitable for routine analysis of SXG in its formulation.

Method validation:
The response for the drug was found to be linear in the concentration range of 500-3000 ng/band (Table 1) at 222nm. The calibration curve was constructed by plotting the peak areas versus concentration. The regression equation obtained was $Y= 1999.3x+529.36$ and the R^2 was

0.995 (figure-2). In precision study the intraday, inter day precision and repeatability were performed by applying 2000 ng/band. The mean peak area, S.D. and %RSD for intraday, inter day precision and repeatability were tabulated in table 2. The %RSD was in the range of 0.83 – 1.94 % which was well within the acceptable limits showed that the method is precise (acceptance criteria- % RSD should be less than 2.0 %). The accuracy was determined by performing the recovery study at three different levels 80%, 100% and 120%. Percentage recovery, S.D. and % RSD (Table 3) was found to be in the range 98% to 102%, which showed that the developed method is accurate. In specificity study the absence of any other peak at the retention time of SXG in the blank and the diluents showed that the method is specific for its determination. The percentage RSD of area of the SXG by the change in saturation time, total amount of mobile phase and mobile phase ratio variation was found well within the acceptable limit (Table 4) and showed that the method is robust. The LOD and LOQ were found to be 21 ng/band and 66 ng/band respectively.

Forced degradation studies:

In forced degradation studies, SXG was subjected to acidic, alkaline, neutral, oxidative, photolytic and thermal stress conditions. The percentage assay and percentage degradation compared with the control sample and is given in table 5. In acidic, alkaline, neutral, oxidative, photolytic and thermal the % degradation was found to be 8.09%, 10.68%, 6.46%, 38.64%, 15.59% and 10.53% respectively. The drug was found to be more degradable under oxidative degradation as compared to other stress conditions. The resultant chromatograms of various stress conditions are shown in respective figures (figure- 4-8).

Table 1: Linearity of SXG

Sr. No	Concentration (ng/band)	Peak area with ± SD at 222nm
1	500	1409.87±33.50
2	1000	2511.73± 25.28
3	1500	3684.31±71.69
4	2000	4588.28± 42.62
5	2500	5601.7±31.83
6	3000	6372.51±46.01

Table 2: Precision study

	Intraday			Inter-day			Repeatability
	0hr	3hr	6hr	Day 1	Day 2	Day 3	
Mean peak area*	3966.88	4199.04	4773.47	4586.94	4426.58	4341.3	4544.47**
S.D.	43.521	75.50	62.16	89.245	48.762	35.198	66.73
%RSD	1.097	1.79	1.30	1.94	1.10	0.81	1.46

** Mean of three replicates, **Mean of six replicates*

Table 3: Recovery Study

Level of recovery (%)	Amount of sample present (µg/ml)	Amount of standard drug added (µg/ml)	Amount recovered* (µg/ml)	% recovery*	±S.D.	%RSD
80	2	1.6	1.62	101.25	±55.59	0.74
100	2	2.0	2.01	100.5	±71.28	0.85
120	2	2.4	2.41	100.4	±43.78	0.48

** Mean of three replicates*

Table 4: Robustness Study

Condition	Saturation time		Total amount of mobile phase		Mobile phase ratio variation (v/v/v)	
Variation	5min	15min	11.2ml	9.2ml	5:5:0.2	7:3:0.2
Mean peak area*	4552.18	4630.95	4666.09	4748.36	4071.87	4963.11
±S.D.	19.32	22.07	37.97	38.89	26.26	39.68
%RSD	0.42	0.47	0.813	0.819	0.64	0.79

** Mean of three replicates*

Table 5: Forced Degradation Studies

Sr. No.	Stressed conditions	Percentage Assay	Degradation (%)
1	Control sample	99.5%	--
2	0.1 N HCl	91.41%	8.09%
3	0.1N NaOH	88.82%	10.68%
4	H_2O	93.04%	6.46%
5	H_2O_2	60.86%	38.64
6	Photolytic	83.91%	15. 59%
7	Thermal	88.97%	10.53%

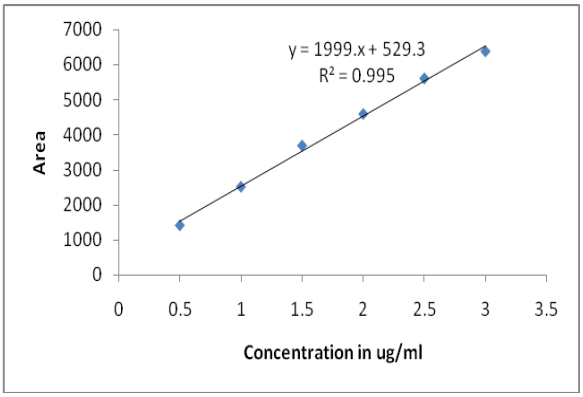

Figure 2: Calibration curve of SXG

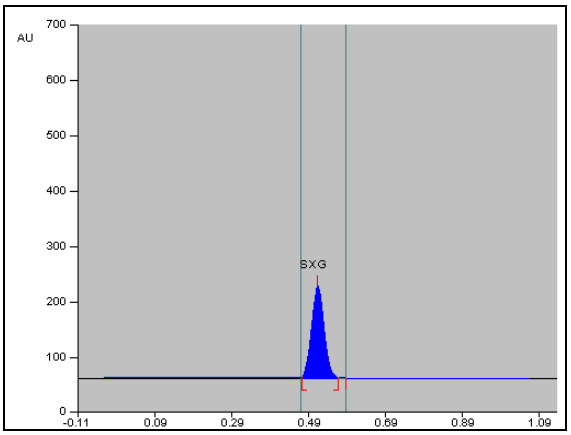

Figure 3: Chromatogram of SXG (API)

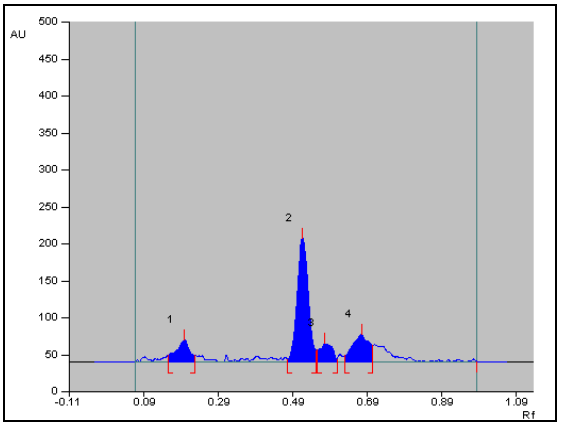

Figure 4: Chromatogram of Acidic Degradation

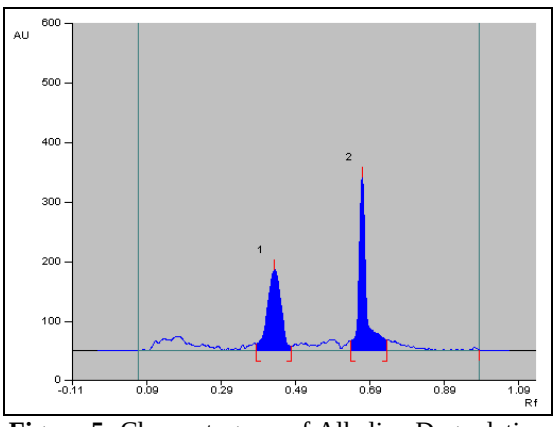

Figure 5: Chromatogram of Alkaline Degradation

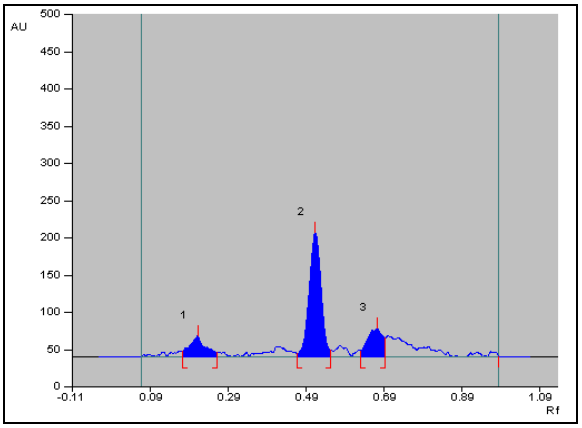

Figure 6: Chromatogram of Neutral Degradation

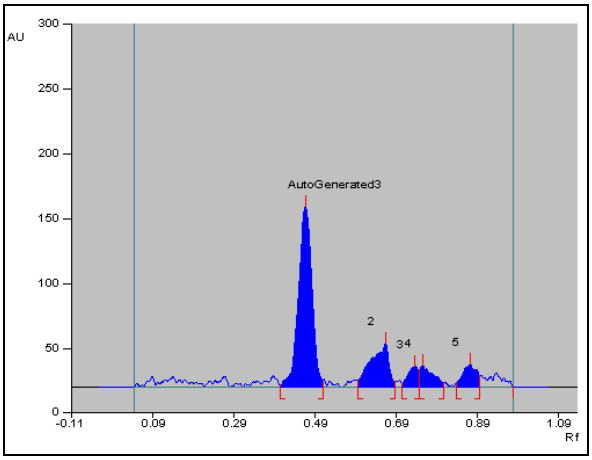

Figure 7: Chromatogram of Photolytic Degradation

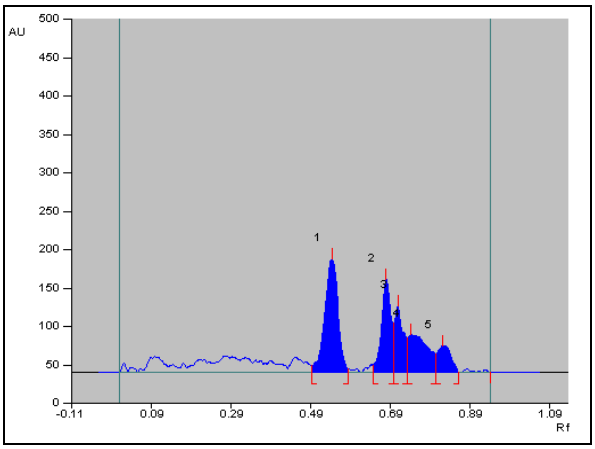

Figure 8: Chromatogram of Thermal Degradation

CONCLUSION

A simple, precise, accurate, specific stability indicating HPTLC method has been developed for the estimation of SXG in its bulk and pharmaceutical dosage form. The method was validated according to the ICH guidelines which gave excellent statistical results which proved the efficiency of the method. The developed method can be employed for the routine analysis of the SXG.

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