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Research Article

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A Validated Stability-Indicating High Performance Thin Layer Chromatographic Method for the Analysis of Saxagliptin in Bulk Drug and Tablet Formulation

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ABSTRACT

The objective of the present research work is developing a simple, precise, and accurate stability-indicating normal-phase HPTLC method for estimation of Saxagliptine in the bulk drug and tablet formulation. Chromatography was performed on the plates precoated with silica gel 60F_{254} using 1% methanolic ammonium acetate: toluene 5:5 (v/v) as a mobile phase. Densitometric quantification was performed at 215 nm by reflectance scanning. The retardation factor of Saxagliptine was 0.48 ± 0.02. Validation of the method in accordance with ICH guidelines yielded good results for range, linearity, precision, accuracy, specificity and robustness. The data of linear regression analysis indicated a good linear relationship over the range of 300–1100 ng/band concentrations with correlation coefficient 0.997. The limits of detection and quantitation were found to be 0.25 and 0.77 resp. Results from analysis of a commercial tablet formulation was 99.12 ± 1.58%. The method precision for the determination of assay was below 2.0 %RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 99.97% to 101.11%. Stress testing of Saxagliptin was carried out according to the international conference of harmonization (ICH) guideline Q1A (R2). The drug was subjected to acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis which enabled separation and detection of degradation products from acidic, basic, neutral and oxidation stress. No degradation products were obtained after photo and dry heat stress condition.

Keywords: HPTLC- densitometeric; Saxagliptin; Stress degradation; Stability indicating

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INTODUCTION

Saxagliptine is chemically [1S,3S,5S-2-2S-2-amino-2-3hydroxy-1adamantyl)acetyl]-2-azabicyclo[3.1.0]hexane-3-carbonitrile,is a new oral hypoglycemic(antidiabetic drug) of the n dipeptidyl peptidase -4[DPP-4] inhibitor class of drugs [1]. DPP-4 is an enzyme that breaks down incretin hormones. As a DPP-4 inhibitor [2], Saxagliptin slows down the breakdown of incretin hormones, increasing the level of these hormones in the body which is responsible for the beneficial actions of Saxagliptin, including increasing insulin production in response to meals and decreasing the amount of glucose that the liver produces. Because incretin hormones are more active in response to higher blood sugar levels, the risk of dangerously low blood sugar (hypoglycemia) is low with Saxagliptin. Saxagliptin is available as tablets at the dose of 5 mg in the market under the brand name of Onglyza by Bristol-Myers Squibb and Astra Zeneca. Various methods in the literatures involve determination of Saxagliptin in human plasma by LCMS/MS [3], estimation of saxagliptin by UV-spectroscopy [4,5], HPLC [6-8]. However no method is available for stability indicating method for assay of Saxagliptin in bulk drug and pharmaceutical dosage form. The international conference on Harmonization (ICH) guideline Q1A (R2) for parent drug stability-indicating test suggests that stress testing on the drug be performed to establish the stability characteristics and to support the suitability of proposed analytical method [9-11]. It has been suggested that stress testing should include the effect of temperature, light and oxidizing agents as well as susceptibility across a wide range of PH values. Therefore, one aim of this work was to develop a validated stability indicating HPTLC method that can be used successfully to determine saxagliptine in tablet dosage form without

interference from degradation product due to stress. In the present work we have developed a new, simple, precise and stability indicating method for determination of Saxagliptin in bulk drug and in tablet formulation.

EXPERIMENTAL SECTION

Standard, Sample and Chemicals

A gratis sample of saxagliptin was received from Mylan Laboratories Ltd., Hyderabad. All other reagents used for experimentation were of analytical reagent (AR) grade. Methanol (Loba chemise, Mumbai, India), toluene (AR) grade, ammonium acetate was purchased from Merck specialities private limited, India. NaOH (Finar chemicals Ahmadabad India, HCL (finar chemicals, Ahmadabad India), and H_2O_2 (Chedyes Corporation, Ahmadabad, India) were used. Film coated tablets of saxagliptin (Onglyza, Bristol-Myers Squibb, S.R.L Anagni Frosinone, Italy) were procured from local market.

Instrumentation

Chromatographic separation of the drugs was performed on Merck HPTLC plates precoated with silica gel 60F254 (10 cm × 10 cm with 200 mm layer thickness, E. Merck, Germany). The sample was applied on to the plates as a band with 5 mm width using a CAMAG 100µl sample syringe (Hamilton, Switzerland) with a Linomat-IV TLC applicator. Linear ascending development was carried out in a twin-trough glass chamber (10 cm × 10 cm). Densitometric scanning was performed using a CAMAG TLC-Scanner 3 (version 4.0.1) supported with CAMAG Win cats® software (version 4.0.1). An electronic balance (ACCULAB Model ALC-210.4 Huntington valley, PA) a sonicator (EN 30 US, Entertech Fast-clean, Mumbai, India), and photo stability chamber (Desaga, SarstedtGrouppe) were also used.

Preparation of Standard Solution

Accurately weighed saxagliptin standard (10 mg) was transferred in to a 10 ml volumetric flask and dissolved in and diluted to the mark with methanol to obtain the standard stock solution 1 mg/ml.

Preparation of Sample Solution

Twenty tablet containing saxagliptin were accurately weighed. Tablets were finely powdered. Accurately weighed amount of powder equivalent to 10 mg of saxagliptin was transferred to 10 ml volumetric flask and 5 ml of methanol was added. The mixture was sonicated for 20 min, diluted to mark with methanol and filtered through whatman filter paper no. 41. An aliquot 1 ml was transferred in to a 10ml volumetric flask and to the mark with methanol to obtain the saxagliptin sample solution 100 µg/ml.

Chromatographic Conditions

Methanolic ammonium acetate (1%)-toluene mixture in the ratio of 5:5 v/v was optimized for thin- layer chromatography plate development and the chamber was saturated with the mobile phase at room temperature for 15 min. The run distance was kept at ~70 mm and 10 ml of the mobile phase was used for a single development. The dosing speed of nitrogen applicator was kept 150 nL with a pre-dosage volume of 5 ml. Sample were applied as bands of 5 mm width with gaps of 5 mm in between. The developed plates were dried at room temperature for 5 min. detection was done at 215 nm using a deuterium lamp in absorption/reflectance mode. The slit dimension of the detector was kept at 4 mm × 0.45 mm.

Forced Degradation Study of Saxagliptin

The degradation samples were prepared by transferring 10 mg of saxagliptin in to a 10 ml volumetric flask and diluted to the mark with 0.1 M HCL, 0.1 M NaOH, water, and 3% H_2O_2 for acidic, basic, neutral and oxidative degradation studies, respectively. Hydrolytic reaction were carried out in 0.1 M HCL, 0.1 M NaOH, water and 3% H_2O_2 at 60°C for 30 min respectively. Pure solid drug (1 mm thick layer in a dish) was exposed to dry heat at 60°C in an oven for 48 h. Pure solid drug (1 mm thick layer in a Petri dish) was kept in the photo stability chamber for 6 days. The samples were taken out and examined by HPTLC after suitable dilution.

Method Validation

The method was validated for the parameters listed below as per ICH guidelines.

Linearity:

Different concentrations of saxagliptin (300-1100 ng/band) were applied on to the HPTLC plate and the peak areas were measured in the densitometer. The calibration curve was constructed by plotting the peak area versus concentration, and the regression equation was constructed. Each response was an average of five determinations.

Precision:
Interday and intraday precision were evaluated by determining three corresponding responses, three times on same day and on three different days for saxagliptin and the results were reported in the terms of %RSD. Repeatability was carried out by applying a saxagliptin solution (200µg/ml) five times on to the TLC plate and result were reported in terms of %RSD.

Accuracy:
Accuracy was determined by calculating the recovery of saxagliptin by the standard addition at different levels (70, 85, 100, 115 and 130%) of the labelled claim. A known amounts of a standard of saxagliptin were added to preanalyzed sample of tablet powder. Each solution was applied in triplicate and recovery was calculated by measuring the peak areas and fitting these values in to the regression equation of the calibration curve.

Limit of detection and limit of quantitation:
The limit of detection (LOD) and limit of quantitation (LOQ) of the drug were calculated using following equations as per ICH guideline.

$$LOD=3.3(\sigma/s),\ LOQ=10\ (\sigma/s)$$

Where σ is the standard deviation of the response and S is the slope of the calibration curve.

Specificity:
It is performed using the typical constituted placebo, blank solvent used for the analysis of active pharmaceutical ingredient (API), and placebo degraded under same conditions as applied for API.

Robustness:
It was performed by total amount of mobile phase, and chamber saturation time.

RESULTS AND DISCUSSION

Optimization of the Mobile Phase
The stressed samples were initially analyzed by HPTLC using the mobile phase as chloroform – methanol (2:8 v/v) broad peak was observed, after that methanol- toluene was used but tailing was observed. Then acetic acid was added to the mobile phase, but saxagliptin and the degradation product were not separated. Subsequent separations were attempted by using methanolic ammonium acetate with toluene in ratio (5:5 v/v). Eventually, the mobile phase consisting of methanolic ammonium acetate – toluene (5:5) gave the best results. The retardation factor of saxagliptin was about 0.48 ± 0.02 (Figure 1).

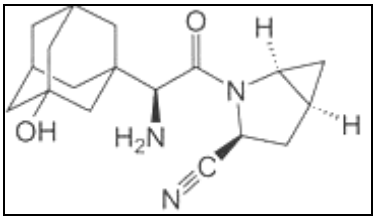

Figure 1: Structure of saxagliptin

Method Validation
The response for the drug was found to be linear in the concentration range 300-1500 ng/band for saxagliptin with correlation coefficient of 0.997. The regression equation obtained was y= -323.8+1.915x, where y is the peak area and x is the concentration (ng/band) (Table 1). The results of intraday and interday precision data are listed in Table 2. The %RSD values were 1.35-1.50% and 1.57-1.81% for intraday and interday precision, respectively, and the repeatability was found to be 1.26%, which confirms that the method is precise. The results of the accuracy study are shown in Table 3. The recovery was in the range 99.97-101.11%, indicating the method accuracy. The LOD and LOQ were found to be 0.25 and 0.77ng/band, respectively. The specificity of the method was ascertained by absence of any other peak of the placebo, degradation products of the placebo, and the solvent. The method was found to be robust (Table 4). The summary of the validation parameters is given in Table 5.

Analysis of Formulation
In Onglyza tablet the mean content of saxagliptin was found to be 99.11% with a %RSD of 1.43%, which indicated that the method is suitable for routine analysis of saxagliptin in its formulation.

Table 1: Calibration data for linearity

Amount (ng/band)	Peak area ± %RSD at 215 nm (n=3)
300	211.23 ± 1.60
500	621.58 ± 1.92
700	992.03 ± 1.88
900	1459.86 ± 1.31
1100	1748.12 ± 1.41

%RSD = percent relative standard deviation; n = no. of replicates

Table 2: Precision of the analytical method

Amount (ng/band)	Peak area ± %RSD (n=3 intraday)	Peak area ± %RSD (n=3) interday
1000	1628.27 ± 1.43	1638.44 ± 1.81
1000	1631.30 ± 1.50	1677.02 ± 1.67
1000	1641.44 ± 1.35	1663.46 ± 1.57

Table 3: Recovery study of analytical method

Level (%)	wt.of sample(mg)	Pure drug added (mg)	Amt. recovered (mg)	% Recovery ±%RSD
70	168.8	0	0.1	101.11 ± 1.50
85	169	0.75	0.76	100.12 ± 1.17
100	168.5	1.5	1.4	99.98 ± 1.81
115	168.3	2.25	2.23	99.97 ± 1.21
130	168.9	3	3.01	100.17 ± 1.43

Table 4: Result of robustness study

Conditions	Modification (toluene-methanolic ammonium acetate)	Mean area* ± %RSD
Amount of mobile phase	10.5 ml	1679.51 ± 1.67
	10.2 ml	1655.17 ± 1.41
	9.7 ml	1624.12 ± 1.59
Chamber saturation time	20 min	1661.13 ± 1.70
	30 min	1613.20 ± 1.64
	40 min	1603.50 ± 1.74

*Average of three replicate

Table 5: Summary of validation parameters

Validation parameter	Saxagliptine
Regression equation	Y=1.915x-323.8
Correlation coefficient	0.997
Linearity	300-1100 ng/band
Precision (intraday)	1.35-1.50%RSD
Precision (interday)	1.57-1.81%RSD
Recovery	99.97-101.11%
LOD	0.25
LOQ	0.77
Specificity	Specific
Robustness	Method is robust
% Assay	99.11 ±1.43%
Rf	0.48 ±0.02

Degradation Studies

Forced degradation studies of saxagliptin were carried out under various stress conditions and the resultant chromatogram is shown in Figures 2 and 3. The percentage degradation was calculated, which is listed in Table 6. The drug content was found to be degrading up to 8%, 17%, and 10% in acidic, alkaline and oxidative condition respectively. In thermal and photolytic conditions the drug was relatively stable.

Table 6: Degradation behavior

Stress condition	% Degradation
0.1M NaOH (60°C for 30min)	17%
0.1M HCl (60°C for 30min)	8%
3% H_2O_2 (R.T.for 24hrs)	10%
H_2O (60°C for 30min)	Negligible
Thermal (In oven at 60°C for 24h)	Negligible
Photolysis (Under UV for 6 days)	Negligible

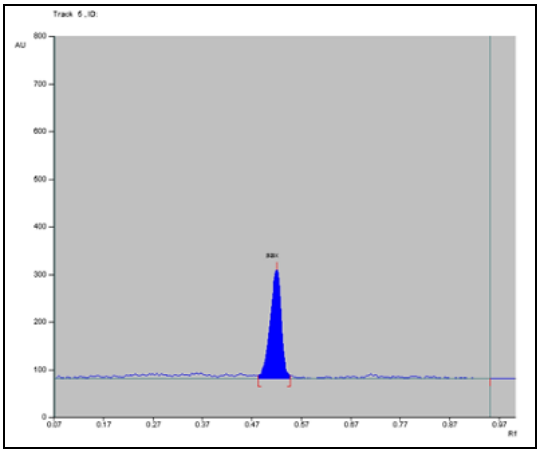

Figure 2: Densitogram of saxagliptin (2000ng/spot)

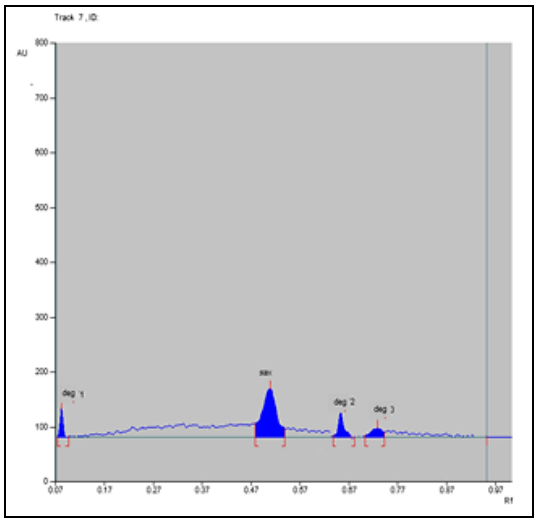

Figure 3: Densitogram of degradation behaviour of saxagliptin, showing alkali deg1, oxidative deg2, and acidic deg3

CONCLUSION

A new analytical method has been developed to determine Saxagliptine in a pharmaceutical dosage form. In this study, stability of Saxagliptin was established according to ICH-recommended stress condition. The developed method was proven to be linear, precise, accurate, and specific. There was no interference from any degradation products or excipients in the determination of Saxagliptin.

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