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A Stability Indicating HPTLC Method Development and Validation for Analysis of Sitagliptin As Bulk Drug And In Formulation

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ABSTRACT

Sitagliptin chemically is (3R) -3-amino-1-[3- (trifluoromethyl)-6,8-dihydro-5h- [1,2,4] triazolo [3,4-c] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-one (Fig. 1), an oral anti-diabetic agent that blocks Dipeptidyl peptidase-4 (DPP-4) activity. Sitagliptin increased incretin levels (GLP-1 and GIP) which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion. The present work describes development and validation of a new simple, accurate, precise and stability indicating HPTLC method for the determination of sitagliptin in tablet dosage form. The chromatographic separation was achieved by using Toluene: Ethyl acetate: Methanol (3: 6: 1 v/v/v) as mobile phase and UV detection at 238 nm. The developed method was validated with respect to linearity, accuracy, precision, limit of detection, limit of quantitation and robustness as per ICH guidelines. The drug was subjected to stress condition of acid hydrolysis, alkali hydrolysis, photolysis, thermal degradation. Results found to be linear in concentration range of 100-500 ng/band. The developed method can be used for the quantification of bulk drug as well as in formulation.

Keywords: Sitagliptin, HPTLC, Degradation Studies

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INTRODUCTION

Sitagliptin chemically is (3R) -3-amino-1-[3- (trifluoromethyl)-6,8-dihydro-5h- [1,2,4] triazolo [3,4-c] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl) butan-1-one (Fig. 1), an oral anti-diabetic agent that blocks Dipeptidyl peptidase-4 (DPP-4) activity. Sitagliptin increased incretin levels (GLP-1 and GIP) which inhibit glucagon release, in turn decreases blood glucose, but more significantly increases insulin secretion¹⁻³.

Literature survey revealed that few analytical methods such as spectrophotometric⁴⁻⁵, HPLC⁶⁻⁷ and LC-MS⁸ methods have been reported for the estimation of sitagliptin. The objective of present study was to develop and validate a new, simple, precise, accurate and selective stability indicating HPTLC method for estimation of sitagliptin as per International Conference on Harmonization (ICH) guidelines

MATERIALS AND METHOD

Chemicals and reagents

Sitagliptin Phosphate was obtained as a gift sample. The pharmaceutical dosage form used in this study was JANUVIA tablet labeled to contain 64.25 mg of Sitagliptin Phosphate equivalent to 50 mg of Sitagliptin was purchased from the market. Toulene, Ethyl acetate, Methanol and other chemicals (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic separation of drug was performed on Merck TLC plates precoated with silica gel 60 F₂₅₄ (10 cm × 10 cm with 250 μm layer thickness) from E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). Samples were applied on the plate as a band with 6 mm width using 100 μL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Toluene: Ethyl acetate: Methanol (3: 6: 1 v/v/v) as mobile phase. The mobile phase was saturated in chamber for 15 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner operated by WINCATS software. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Experimental, Result and Discussion:

Selection of Mobile Phase and Chromatographic Conditions:

Chromatographic separation studies were carried out on the working standard solution of Sitagliptin (50 μg/ml). Initially, trials were carried out using various solvents in various

proportions on normal TLC plates, to obtain the desired R_f and shape for drug peak. After few trials Toluene: Ethyl acetate: Methanol (3: 6: 1 v/v/v) was chosen as the mobile phase, which gave acceptable peak parameters. Other chromatographic conditions like chamber saturation time, run length, sample application volume were optimized.

Preparation of Standard Stock Solution:

Standard stock solution of Sitagliptin was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 $\mu\text{g/ml}$. From the standard stock solution, working standard solution was prepared containing 50 $\mu\text{g/ml}$ of Sitagliptin.

Preparation of Sample Solution:

A tablet containing 64.25 mg of Sitagliptin Phosphate equivalent to 50 mg of Sitagliptin (Januvia 50 mg) was weighed and powdered. Powder equivalent to 10 mg of drug was transferred to 10 ml volumetric flask and volume was made up with methanol to get concentration (1000 $\mu\text{g/ml}$) and was sonicated for 10 min. Solution was filtered and diluted to get 50 $\mu\text{g/ml}$. 4 μl of the resultant solution was applied on TLC plate to get concentration of 200 ng/band.

Selection of Analytical Wavelength

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200 – 400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 238 nm.

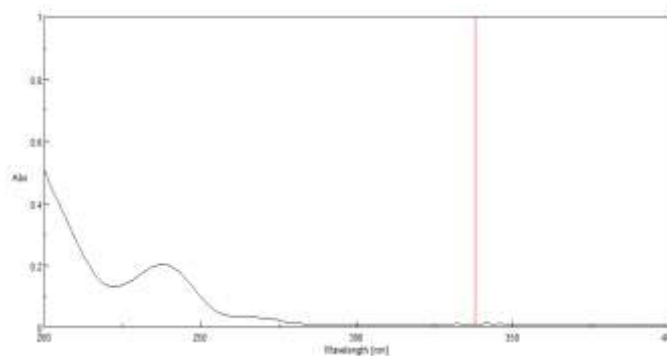


Figure 1: UV Spectrum of Sitagliptin (10 $\mu\text{g/ml}$)

Densitogram:

Solution of Sitagliptin (50 $\mu\text{g/ml}$) was prepared. 4 μl (200 ng/band) of solution was applied on pre-activated TLC plate with the help of Hamilton syringe (100 μl), using Linomat V sample applicator. The development chamber was saturated with mobile phase for 15 min. The spotted plate was placed in the saturated chamber and developed up to 90 mm distance. The plate was dried and was scanned at 238 nm. The retention factor was found to be 0.38 ± 0.03 respectively.

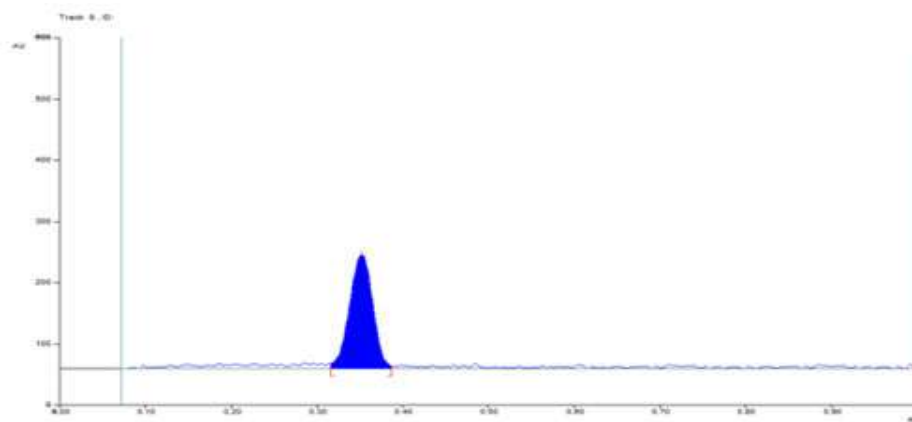


Figure 2: Densitogram of standard solution of Sitagliptin (200 ng/band)

Summary of chromatographic parameters selected:

Chromatographic parameters are summarized in Table 5.6.2

Table 1: Chromatographic parameters:

Sr. No.	Parameter	Conditions used for Analysis
1	Stationary phase	TLC aluminum plate pre-coated with silica gel 60 F ₂₅₄
2.	Mobile phase	Toluene: Ethyl acetate: Methanol (3:6:1 v/v/v)
3.	Detection Wavelength	238 nm
4.	Saturation time	15 min
5.	Band width	6 mm

Stress degradation studies of bulk drug

Stress degradation studies were carried under condition of acid, base, neutral hydrolysis, oxidation, dry heat and photolysis. For each working standard solution of Sitagliptin subjected to stress condition. Dry heat and photolytic degradation were carried out in solid state.

Degradation under acid catalyzed hydrolytic condition

To 1 ml of 1000 µg/ml solution of Sitagliptin, 1 ml of 0.5 N HCl was added. The volume was made up to 10 ml with methanol. The above solution was kept for reflux for half hour at 25°C and then cooled. 5 µl of the resultant solution was then applied on TLC plate and densitogram was developed. Average 76.48 % of Sitagliptin was recovered with no peak of degradation.

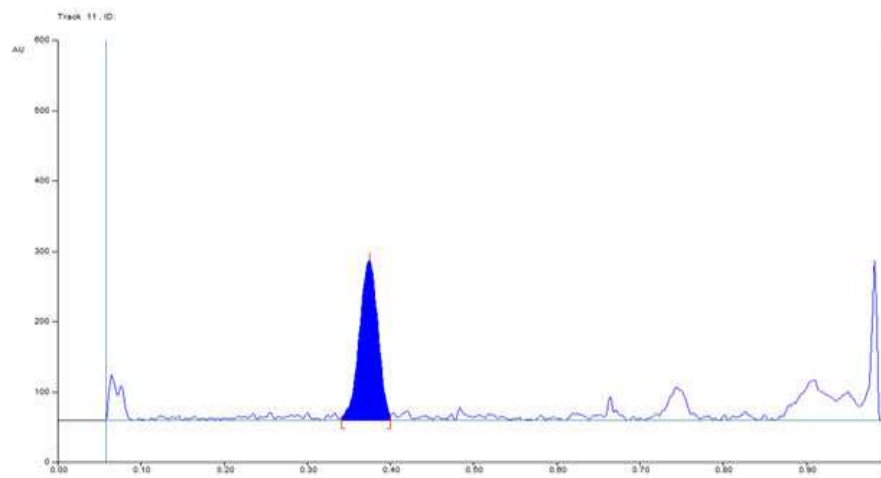


Figure 3: Representative Densitogram of acid induced degradation of Sitagliptin (500 ng/band)

Degradation under alkali catalyzed hydrolytic condition:

To 1 ml of 1000 $\mu\text{g/ml}$ solution of Sitagliptin, 1 ml of 1 N NaOH was added. The volume was made up to 10 ml with methanol. The above solution was kept for overnight at room temperature. 5 μl of the resultant solution was then applied on TLC plate and densitogram was developed. Average 69.41 % of Sitagliptin was recovered with no peak of degradation.

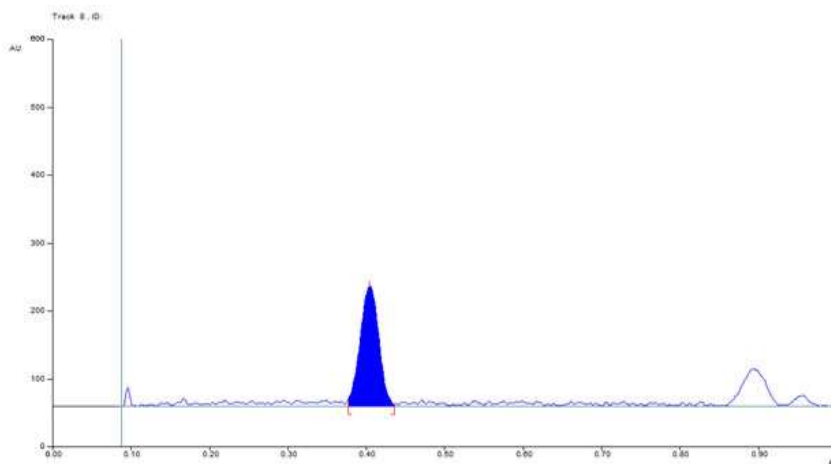


Figure 4: Representative Densitogram of alkali induced degradation of Sitagliptin (500 ng/band)

Degradation under neutral hydrolytic condition:

To 1 ml of 1000 $\mu\text{g/ml}$ solution of Sitagliptin, 1 ml of distilled water was added. The volume was made upto 10 ml with methanol. The above solution was kept for reflux for 2 hours at 25°C and then cooled. 5 μl of the resultant solution was then applied on TLC plate and densitogram was developed. Average 91.48 % of Sitagliptin was recovered with no peak of degradation.

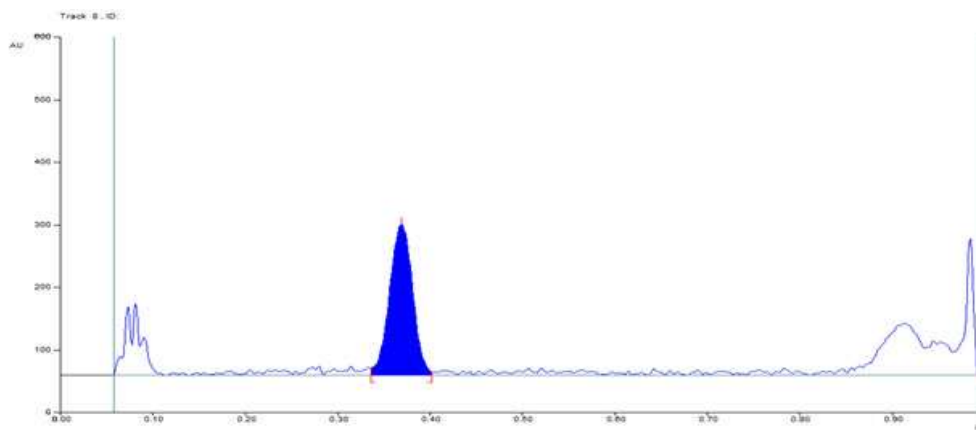


Figure 5: Representative Densitogram of neutral degradation of Sitagliptin (500 ng/band)

Degradation under oxidative condition:

To 1 ml of 1000 µg/ml solution of Sitagliptin, 1 ml of 30% H₂O₂ was added. The volume was made upto 10 ml with methanol. The above solution was kept for half hour at room temperature. 5 µl of the resultant solution was then applied on TLC plate and densitogram was developed.

Average 95.36 % of Sitagliptin was recovered with no peak of degradation.

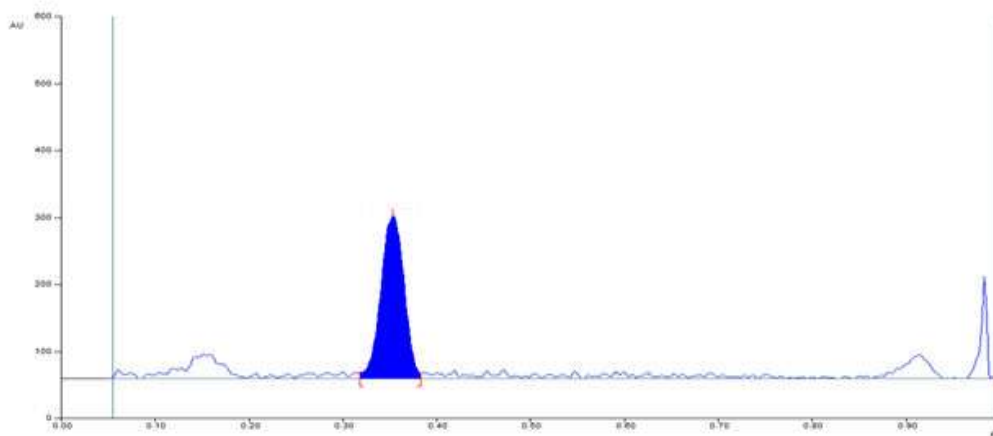


Figure 6: Representative Densitogram of oxidative degradation of Sitagliptin (500 ng/band)

Degradation under dry heat:

Dry heat studies were performed by keeping drug sample in oven (80°C) for 8 hours. Sample was withdrawn, dissolved in methanol and diluted to get 100 µg/ml. 5 µl of the resultant solution was then applied on TLC plate and densitogram was developed. Average 77.20 % of Sitagliptin was recovered with no peak of degradation.

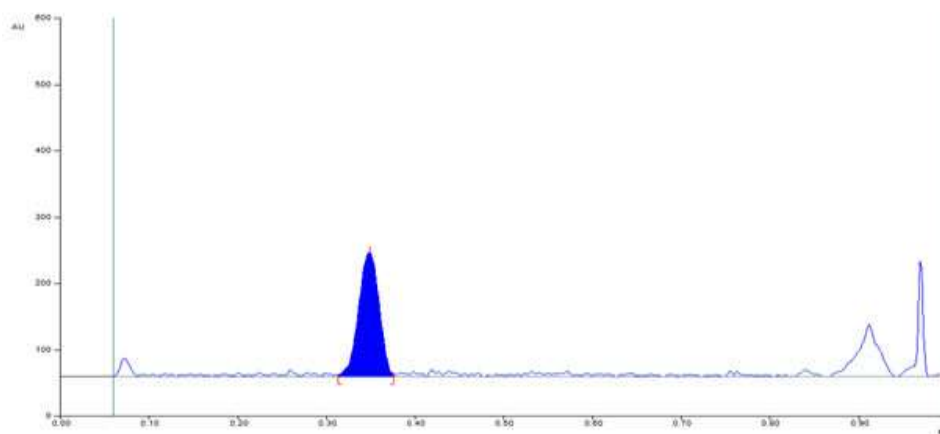


Figure 7: Representative Densitogram of dry heat degradation of Sitagliptin (500 ng/band)

Photo-degradation studies:

Photolytic degradation studies were carried out by exposing drug to UV light up to 200 watt hours /square meter. Sample was weighed, dissolved in methanol to get concentration of 1000 µg/ml and further dilutions were made with methanol to get final concentration (100 µg/ml). 5 µl of the resultant solution was then applied on TLC plate and densitogram was developed. Average 98.78 % of Sitagliptin was recovered with no peak of degradant. Representative densitogram is shown in Figure 8.

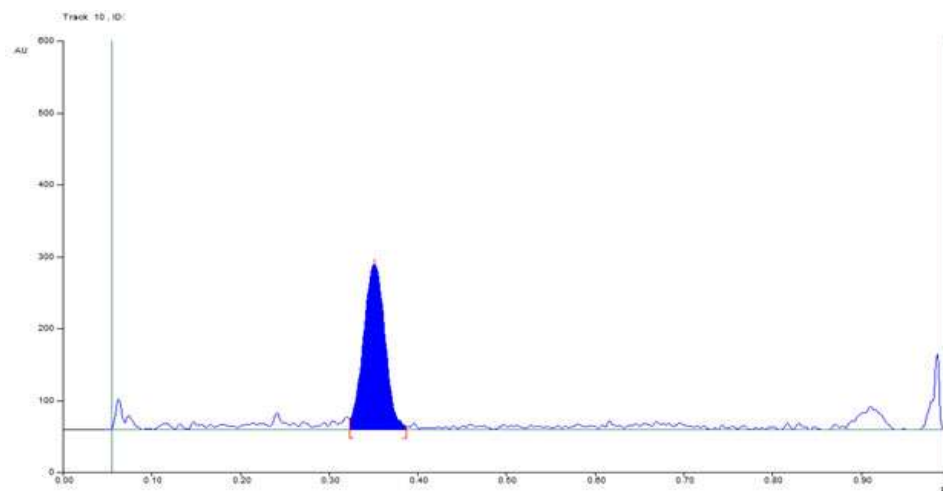


Figure 8: Representative Densitogram of Sitagliptin Photolytic degradation (500 ng/band)

Table 2: Summary of stress degradation study of Sitagliptin

Stress condition/Duration	% Assay of active substance	Rf values of degraded products
Acid (0.5 N HCl, reflux for half hrs)	76.48	-
Base (1 N NaOH, kept for overnight)	69.41	-

Water (reflux for 2 hrs)	91.48	-
30% V/V H ₂ O ₂ (30 min)	95.36	-
Heat dry (80 ⁰ C)	77.20	-
UV light(200 Watt hours /Square meter)	98.78	-

Validation of Analytical Method

The method was validated as per ICH Q2 (R₁) guidelines.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.99, indicating the non-interference of any other peak of degradation product or impurity.

Linearity

From the standard stock solution (1000 µg/ml) of Sitagliptin , solution was prepared containing 50 µg/ml of Sitagliptin. This solution was further used for spotting. Five replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analyzing five concentrations over concentration range of 100-500 ng/band for Sitagliptin Figure. 9. The results obtained are shown in Table 3, the peak area were plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig.10 for Sitagliptin.

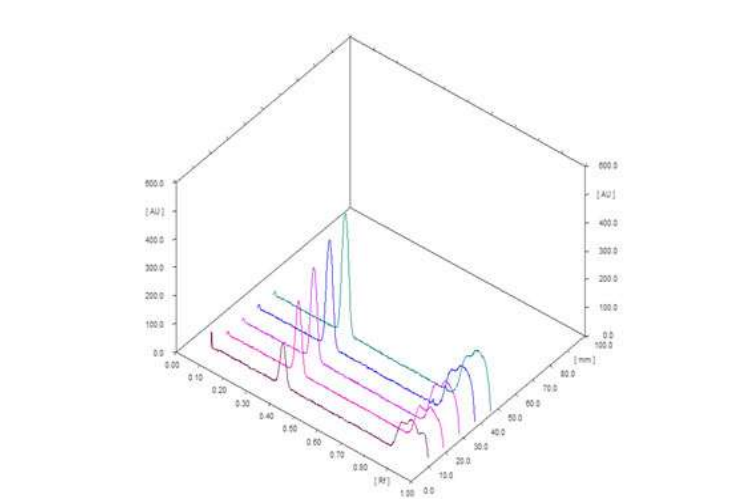


Figure: 9: Densitogram of linearity of Sitagliptin (100-500 ng/band)

Table 3: Linearity study of Sitagliptin

Replicate	Amount of Sitagliptin (ng/band)				
	100	200	300	400	500
1	3158	5420.5	6785.9	7805.8	9475
2	3105	5343	6523.8	8179.6	9552.9
3	3138.3	5343.5	6605.3	7969	9349.5
4	3023	5481.6	6574.2	7812.5	9140

5	3135.2	5475.3	6586.7	7924.5	9359
Average	3111.9	5412.78	6615.18	7938.28	9375.28
SD	53.18	67.77	100.10	152.26	156.34
% RSD	1.70	1.25	1.51	1.91	1.66

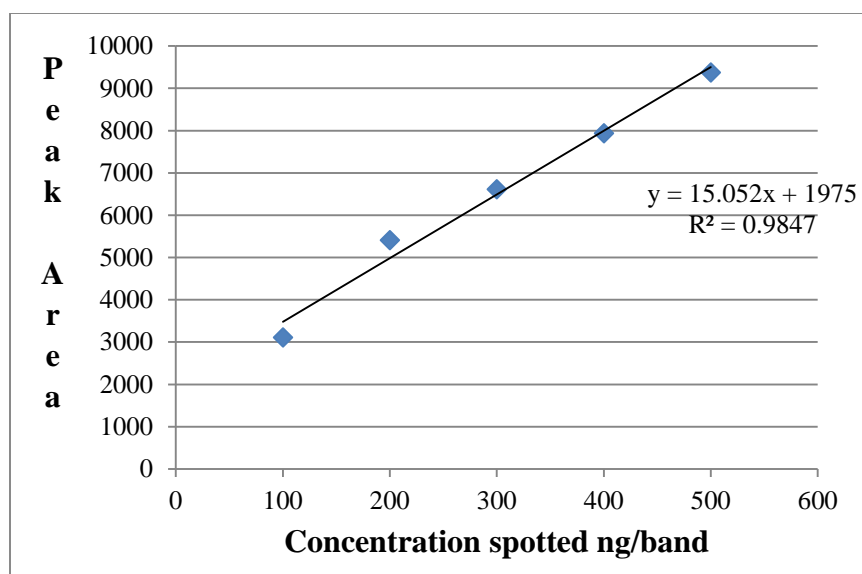


Figure 10: Calibration curve for Sitagliptin

Range

Sitagliptin = 100-500 ng/band

Precision

The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies, 3 replicates of 3 concentrations were analyzed on the same day, and percentage RSD was calculated. For the Inter-day variation studies, 3 replicates of 3 concentrations were analyzed on 3 consecutive days and percentage RSD were calculated. For Intra-day precision % RSD found to be 0.97-1.27 % and for Inter-day precision % RSD found to be 0.86-1.30 % (Table No.4 & 5).

Table 4: Intra-day precision of Sitagliptin

Conc.(ng/band)	Area	Mean Area	SD	% RSD
100	3078	3120.07	39.8298	1.27
	3125			
	3157.2			
200	5416.5	5409.03	56.6701	1.04
	5349			
	5461.6			
300	6645.9	6629.97	64.7865	0.97
	6685.3			
	6558.7			

Table 5: Inter-day precision of Sitagliptin

Conc.(ng/band)	Area	Mean Area	SD	% RSD
100	3147.2	3128.73	40.7678	1.30
	3082			
	3157			
200	5461.6	5443.3	49.7422	0.91
	5481.3			
	5387			
300	6543.3	6534.03	56.3741	0.86
	6585.2			
	6473.6			

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ are calculated from the formula: -

$$\text{LOD} = 3.3 \sigma / S$$

$$\text{LOQ} = 10 \sigma / S$$

Where, σ = standard deviation of lowest response, $\sigma = 53.1881$

S = slope of calibration curve, S = 15.0523

LOD of Sitagliptin = 11.66 ng/band

LOQ of Sitagliptin = 35.33 ng/band

Assay

Januvia 50 mg tablet formulation analysis was carried out as mentioned under preparation of sample solution. Procedure was repeated for six times. Sample solution was injected and area was recorded. Concentration and % recovery was determined from linear equation (Table 6).

Table 6: Assay of formulation

Sr. No.	Peak area of Sitagliptin (200 ng/band)	Amount Recovered (ng/band)	% Recovery
1	5013.6	201.9	100.95
2	4980.2	199.681	99.8405
3	5035.4	203.349	101.674
4	5004.9	201.322	100.661
5	5038.7	203.568	101.784
6	5021.5	202.425	101.213
Mean	5015.72	202.04	101.02
% RSD	0.430	0.710	0.710

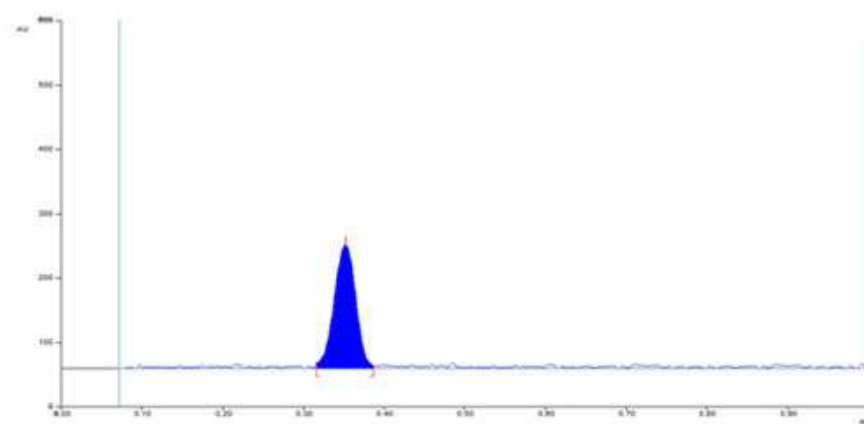


Figure 11: Densitogram of sample solution of Sitagliptin (200 ng/band)

Accuracy

Recovery studies were carried out by addition of standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 % to check the accuracy of the method. Basic concentration of sample chosen was 200 ng/band. % recovery was determined from linearity equation. The results obtained are shown in Table 7.

Table 7: Recovery studies of Sitagliptin

Level	Amount of sample Taken (ng/band)	Amount of std. added (ng/band)	Area	% Recovery	Avg. of % Recovery	% RSD
80	200	160	7489	101.772	100.42	1.31
			7345.6	99.125		
			7413.1	100.371		
100	200	200	8034.1	100.650	100.73	1.05
			7978.5	99.726		
			8105.9	101.842		
120	200	240	8499.2	98.523	99.51	0.86
			8595.8	99.982		
			8599.3	100.035		

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength, chamber saturation time were altered, Time was also changed from spotting to development and development to scanning and the effects on the area were noted. It was found that method is robust. The results obtained are shown in Table 8.

Table 8: Robustness study

Sr. No.	Parameters	Robust condition Mean of 3 (200 ng/band)	% RSD
1.	Time from application to development	(0, 30, 60, 90 min.)	1.652 0.68

		1.109
2.	Time from development (0, 30, 60, 90 min.) to scanning	1.168 1.193 1.769
3.	Scanning wavelength 238 ± 1 nm	1.747 1.452 1.882

Summary of validation study

The summary of validation parameters are summarized in Table 9

Table 9: Summary of validation parameters

Sr. No.	Validation parameters	Sitagliptin
1.	Linearity Equation (R ²)	y = 15.05x + 1975 R ² = 0.984
	Range	100-500 ng/band
2.	Precision (% RSD)	
	Intraday	0.97-1.27
	Inter-day	0.86-1.30
3.	Assay	101.02 ± 0.7
4.	Accuracy	
	80%	100.42 ± 1.31
	100%	100.73 ± 1.05
	120%	99.51 ± 0.86
5.	LOD	11.66 ng/band
6.	LOQ	35.33 ng/band
7.	Specificity	Specific
8.	Robustness	Robust

CONCLUSION:

The proposed stability indicating method was simple, precise, accurate, reproducible, and sensitive; and can be used for the determination of Sitagliptin in bulk samples and in tablet dosage form.

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