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A STABILITY INDICATING HPTLC METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF VILDAGLIPTIN AS BULK DRUG AND FROM ITS PHARMACEUTICAL DOSAGE FORM

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Keywords:

Vildagliptin, HPTLC, Degradation Studies, Tablet dosage form

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ABSTRACT: Vildagliptin chemically (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile, is a potent dipeptidyl peptidase IV (dip-IV) inhibitor, a drug for the treatment of diabetes. DPP-IV inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes. The Present work describes the development and validation of a new simple, accurate, precise and stability-indicating HPTLC method for the determination of Vildagliptin in the tablet dosage form. The chromatographic separation was achieved by using Chloroform: n-Butanol: Methanol (5:2:3 v/v/v) as mobile phase and UV detection at 227nm. The developed method was validated with respect to linearity, accuracy, precision, the limit of detection, the limit of quantitation and robustness as per ICH guidelines. The described method was linear over a concentration range of 2000-20000 ng/ml for the assay of Vildagliptin. The assay was found to be 99.8%. The limit of detection (LOD) and the limit of quantification (LOQ) for Vildagliptin was found to be 357.31 ng/band and 1082.76 ng/band respectively. The drug was subjected to stress conditions of acid hydrolysis, alkali hydrolysis, photolysis, thermal degradation. Results found to be linear in the concentration range of 2000-20,000 ng/band. The proposed stability-indicating method can be used for the determination of vildagliptin in bulk samples and in the pharmaceutical dosage form.

INTRODUCTION: Vildagliptin chemically (S)-1-[N-(3-hydroxy-1- adamantyl) glycyl] pyrrolidine-2-carbonitrile, is a potent dipeptidyl peptidase IV (dip-IV) inhibitor, a drug for the treatment of diabetes. DPP-IV inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes.

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DPP IV inhibitors improve fasting and postprandial glycemic control without hypoglycemia or weight gain. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP IV, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the Islets of Langerhans in the pancreas [1-4].

A literature survey revealed that few analytical methods such as spectrophotometric [5-7], HPLC [8-11] and LC-MS [12-13] methods have been reported for the estimation of Vildagliptin in alone or in combination with other drugs. The less amount of literature provides the need for developing a new method.

So, an attempt was made in this study to develop a stability-indicating HPTLC method for estimation of vildagliptin in bulk and pharmaceutical formulation as per the International Conference on Harmonisation (ICH) guidelines. The proposed method is rapid, simple, accurate, and reproducible, and can be successfully employed in the routine analysis of vildagliptin in bulk samples and in the pharmaceutical dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Vildagliptin was obtained as a gift sample. The pharmaceutical dosage form used in this study was GALVUS tablets labeled to contain 50 mg of Vildagliptin was procured from the market. Chloroform, Methanol, and n-Butanol (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and Chromatographic Conditions: Chromatographic separation of the drug was performed on Merck TLC plates precoated with silica gel 60 F_{254} (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 Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using Chloroform: n-Butanol: Methanol (5:2:3 v/v/v) as mobile phase. The mobile phase was saturated in the chamber for 20 min. After development, TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on a CAMAG thin layer chromatography scanner at 227 nm for all developments operated by WINCATS software. The source of radiation utilized was a 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 Vildagliptin 100 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 a few trials, Chloroform: n-Butanol: Methanol (5:2:3 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 was optimized.

Preparation of Standard Stock Solution: Standard stock solution of Vildagliptin was prepared by dissolving 10 mg of drug in 10 ml of methanol to get a concentration of 1000 µg/ml. From the standard stock solution, the working standard solution was prepared to contain 100 µg/ml of Vildagliptin.

Preparation of Sample Solution: A tablet containing 50 mg of Vildagliptin (Galvus 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 µg/ml) and was sonicated for 10 min. The solution was filtered and 4 µl of the resultant solution was applied on a TLC plate to get a concentration of 4000 ng/band.

Selection of Analytical Wavelength: From the standard stock solution further dilutions were made using methanol and scanned over the range of 200 – 400 nm and the spectra were obtained. It was observed that the drug showed considerable absorbance at 227 nm.

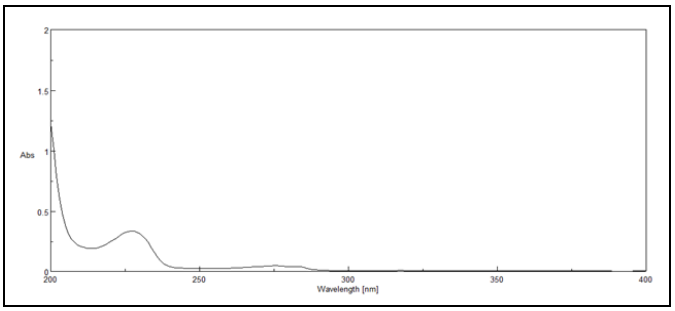

FIG. 1: UV SPECTRUM OF VILDAGLIPTIN (10 µg/ml)

Densitogram: The solution of Vildagliptin (1000 µg/ml) was prepared. 10 µl (10000 ng/band) of the solution was applied on a pre-activated TLC plate with the help of Hamilton syringe (100 µl), using Linomat 5 sample applicator. The development chamber was saturated with the mobile phase for 15 min. The spotted plate was placed in the saturated chamber and developed up to 80 mm distance. The plate was dried and was scanned over a 90 mm distance at 227 nm. The retention factor was found to be: 0.62 ± 1.92.

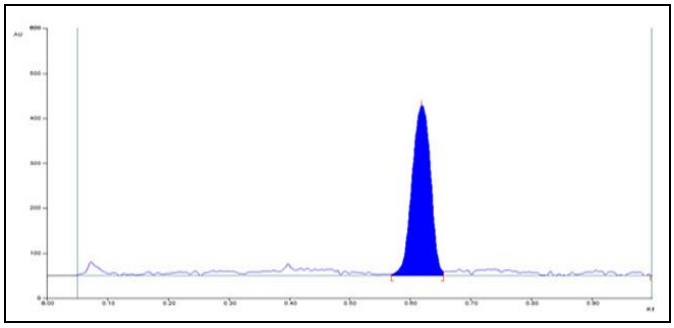

FIG. 2: DENSITOGRAM OF STANDARD SOLUTION OF VILDAGLIPTIN (10000 ng/band)

Summary of Chromatographic Parameters Selected: Chromatographic parameters are summarized in **Table 1**.

TABLE 1: CHROMATOGRAPHIC PARAMETERS

S. no.	Parameter	Conditions used for Analysis
1	Stationary phase	TLC aluminium plate precoated with silica gel 60 F_{254}
2.	Mobile phase	Chloroform: n-Butanol: Methanol (5:2:3 v/v/v)
3.	Detection Wavelength	227 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 study, working standard solution of Vildagliptin subjected to stress condition. Dry heat and photolytic degradation were carried out in solid state.

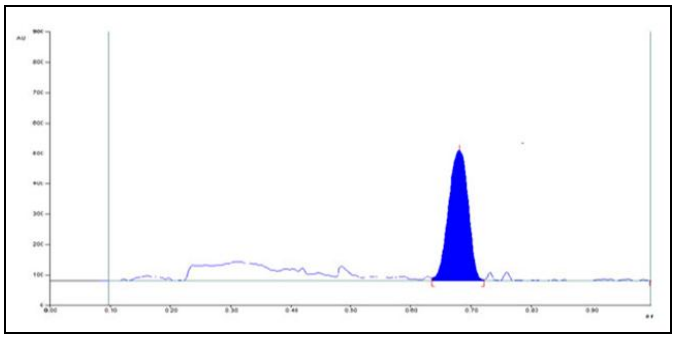

FIG. 3: REPRESENTATIVE DENSITOGRAM OF ACID INDUCED DEGRADATION OF VILDAGLIPTIN (16,000 ng/band)

Degradation Under Acid-Catalyzed Hydrolytic Condition: To 1 ml of stock solution of Vildagliptin (10,000 µg/ml), 1 ml of 1 N HCl was added. The above solution was kept for 4 h at room temperature. The volume was made upto 10 ml with methanol. 16 µl of the resultant solution was then applied at TLC plate and densitogram was developed. 66.34% Vildagliptin was recovered with no peak of degradant. Representative densitogram is shown in **Fig. 3**.

Degradation Under Alkali Catalyzed Hydrolytic Condition: To 1 ml of stock solution of Vildagliptin (10,000 µg/ml), 1 ml of 1 N NaOH was added. The above solution was kept for 4 hours at room temperature. The volume was made up to 10 ml with methanol. 16 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 47.45% of Vildagliptin was recovered with one peak of degradation. Representative densitogram is shown in **Fig. 4**.

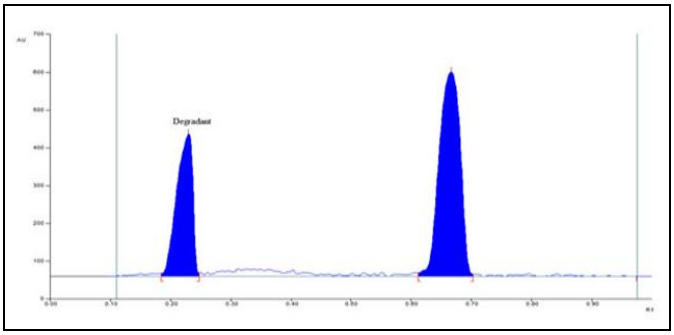

FIG. 4: REPRESENTATIVE DENSITOGRAM OF ALKALI INDUCED DEGRADATION OF VILDAGLIPTIN (16,000 ng/band)

Degradation Under Neutral Hydrolytic Condition: To 1 ml of stock solution of Vildagliptin (10,000 µg/ml), 1 ml of distilled water was added. The above solution was kept for 4 hours at room temperature. The volume was made up to 10 ml with methanol. 16 µl of the resultant solution was then applied at the TLC plate and densitogram was developed. 94.51% of Vildagliptin was recovered with no peak of degradant. Representative densitogram is shown in **Fig. 5**.

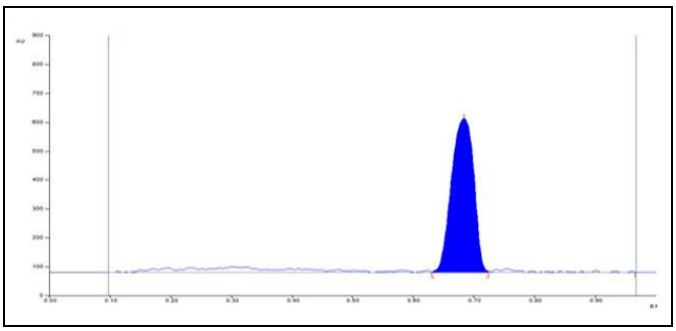

FIG. 5: REPRESENTATIVE DENSITOGRAM OF NEUTRAL DEGRADATION OF VILDAGLIPTIN (16,000 ng/band)

Degradation Under Oxidative Condition: To 1 ml of stock solution of Vildagliptin (10,000 µg/ml), 1 ml of 6% H_2O_2 was added. The above solution was kept for 4 h at room temperature. The volume was made up to 10 ml with methanol. 16 µl of the resultant solution was then applied at the TLC plate and densitogram was developed. Average 86.53% of Vildagliptin was recovered with no peak of degradant. Representative densitogram is shown in **Fig. 6**.

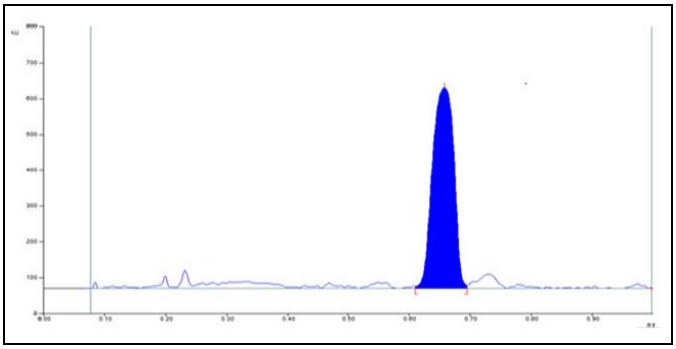

FIG. 6: REPRESENTATIVE DENSITOGRAM OF OXIDATIVE DEGRADATION OF VILDAGLIPTIN (16,000 ng/band)

Degradation Under Dry Heat: Dry heat studies were performed by keeping drug sample in the oven (80 ºC) for a period of 4 h. The sample was withdrawn, dissolved in methanol and diluted to get 1000 µg/ml. 16 µl of the resultant solution was then applied at the TLC plate and densitogram was developed. Average 91.91% Vildagliptin was recovered with no peak of degradant. Representative densitogram is shown in **Fig. 7**.

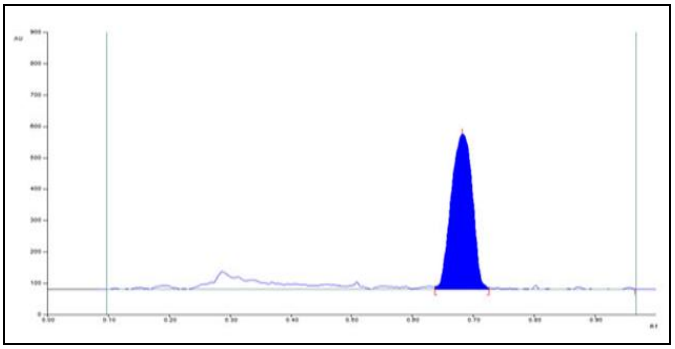

FIG. 7: REPRESENTATIVE DENSITOGRAM OF DRY HEAT DEGRADATION OF VILDAGLIPTIN (16,000 ng/band)

Photo-Degradation Studies: The photodegradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2. After exposure, the sample was withdrawn, dissolved in methanol and diluted to get 1000 µg/ml. 16 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 96.01% of Vildagliptin was recovered with no peak of degradant. Representative densitogram is shown in **Fig. 8**.

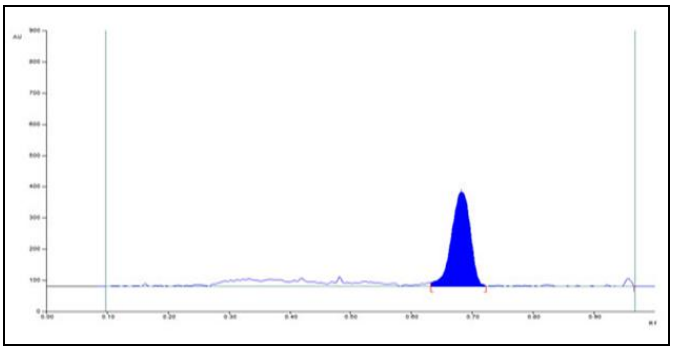

FIG. 8: REPRESENTATIVE DENSITOGRAM OF VILDAGLIPTIN PHOTOLYTIC DEGRADATION (16,000 ng/band)

TABLE 2: SUMMARY OF DEGRADATION

Stress condition / Duration	% Assay of active substance	R_f values of degraded products
Acidic/ 1 N HCl/ at room temperature 4 h	66.34%	-
Alkaline/ 1 N NaOH / at room temperature 4 h	47.45%	0.23
Neutral / H_2O / at room temperature 4 h	94.51%	-
Oxidative/ 6 % H_2O_2 / at room temperature 4 h	86.53%	-
Dry heat / 80ºC/ 4 h	91.91%	-
UV/200 watt hours/square meter / for 24 h	96.01%	-

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 0.998, indicating the non-interference of any other peak of degradation product or impurity.

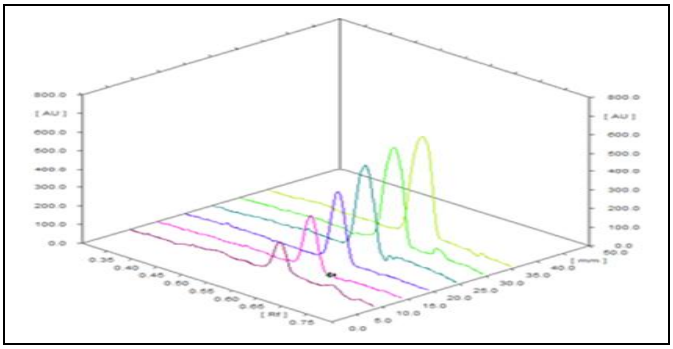

FIG. 9: DENSITOGRAM OF LINEARITY OF VILDAGLIPTIN (2000-20000 ng/band)

Linearity: From the standard stock solution (1000 µg/ml) of Vildagliptin, Six replicates per concentration were spotted. The linearity (the relationship between peak area and concentration) was determined by analyzing six concentrations over the concentration range of 2000-20,000 ng/band for Vildagliptin. The peak areas were plotted against the corresponding concentrations to obtain the calibration curve as shown in **Fig. 10**. The results found to be linear with the regression equation of y=0.8592x+2599 with R^2 = 0.991.

TABLE 3: LINEARITY STUDY OF VILDAGLIPTIN

Replicate	Concentrations of Vildagliptin (ng/band)					
	2000	4000	8000	12000	16000	20000
1	3522	6300.2	9911	13266.7	16927.7	19346.6
2	3421.5	6410.3	9725.3	13376.4	16191.3	19552.8
3	3562.1	6365	9890.3	13571.6	16194.9	19413.2
4	3726.6	6472.3	9886.7	13542.7	16769.5	19102.1
5	3500.2	6253.2	9840.8	13509.9	16547.9	19161.7
6	3542.7	6387.2	9727.6	13437.4	16609.3	19162.7
Average	3545.85	6364.7	9830.28	13484.07	16473.12	19273.18
SD	101.05	78.42	83.63	118.03	193.10	194.61
% RSD	1.84	1.23	0.85	0.87	0.75	0.82

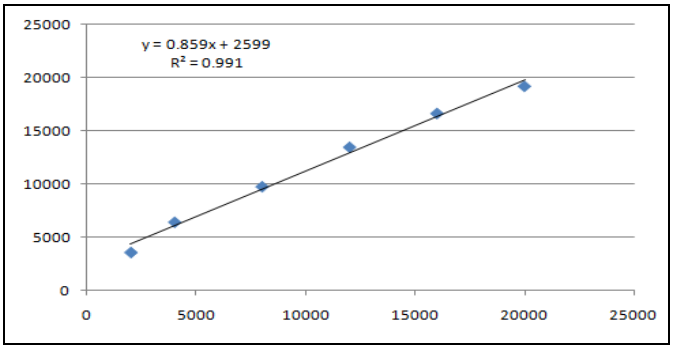

FIG. 10: CALIBRATION CURVE FOR VILDAGLIPTIN

Range: Vildagliptin = 2000-20,000 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 was calculated.

For intraday precision and inter-day precision results obtained are shown in **Table 4**.

TABLE 4: INTRADAY AND INTERDAY VARIATION STUDIES DATA FOR VILDAGLIPTIN

Concentration (µg/ml)	Intra-day Precision			Inter-day Precision		
	Average area	% Recovery	% R.S.D	Average area	% Recovery	% R.S.D
4000	6065	100.85		6051.2	100.06	
	6087.2	101.50	1.15	6077.5	100.83	0.45
	6010.3	99.26		6050.2	100.03	
8000	9490.3	100.26		9440.8	99.309	
	9486.7	100.21	0.40	9465.6	99.670	0.40
	9440.8	99.54		9411	98.876	
12,000	13076.4	101.62		13009.9	100.80	
	12931.3	100.21	0.70	13035.4	101.05	0.71
	13022.7	101.10		12896.7	99.70	

Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ are calculated from the formula: -

$$LOD = 3.3\ \sigma / S$$

$$LOQ = 10\ \sigma / S$$

Where, σ = standard deviation of Y-intercept
S = slope of the calibration curve.

LOD of Vildagliptin = 357.31 ng/ band

LOQ of Vildagliptin = 1082.765 ng/band.

Assay: Galvus 50 mg tablet formulation analysis was carried out as mentioned under the preparation of sample solution**.** The procedure was repeated six times. The sample solution was injected and the area was recorded. Concentration and % recovery were determined from linear equation **Table 5**.

TABLE 5: ASSAY OF MARKETED FORMULATION

Drug	Peak Area	Amount Recovered (µg/ml)	% Recovery	± %RSD
Vildagliptin	5988.7	3945.18	98.62	1.40
	5956.2	3907.35	97.68	
	6050.5	4017.10	100.42	
	6038.3	4002.91	100.07	
	6051.5	4018.27	100.45	
	6089.5	4062.52	101.56	

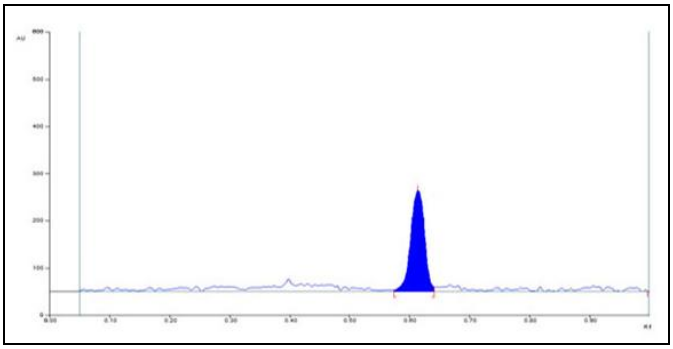

FIG. 11: DENSITOGRAM OF SAMPLE SOLUTION OF VILDAGLIPTIN (4000 ng/band)

Accuracy: To check the accuracy of the method, recovery studies were carried by spiking the standard drug to the blend, at three different levels 50, 100 and 150%. The basic concentration of the sample chosen was 4000 ng/band. The % recovery was determined from the linearity equation. The results obtained are shown in **Table 6**.

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 the method is robust. The results obtained are shown in **Table 7**.

Summary of Validation Study: The summary of validation parameters are summarized in **Table 8**.

TABLE 8: SUMMARY OF VALIDATION PARAMETERS

S. no.	Parameter	Vildagliptin
1	Linearity	Y = 0.859 x + 2599
2	Range	2,000 – 20,000 ng / band
3	Precision	% RSD
	Intraday	0.40 – 1.15
	Interday	0.40 – 0.71
4	Assay	99.8 %
5	Accuracy	% Recovery (Mean)
	50%	98.380
	100%	99.066
	150%	100.017
6	LOD	357.31 ng / band
7	LOQ	1082.76 ng / band
8	Specificity	Specific
9	Robustness	Robust

TABLE 6: RECOVERY STUDIES OF VILDAGLIPTIN

Level	Amount of sample taken (ng/band)	Amount standard spiked (ng/band)	Area	% Recovery	±% RSD
50%	4000	2000	7687.3	98.70	0.60
			7635.5	97.69	
			7689.3	98.74	
100%	4000	4000	9427.4	99.34	1.79
			9277.6	97.16	
			9520.3	100.69	
150%	4000	6000	11130.7	99.29	0.92
			11164.1	99.68	
			11282.6	101.06	

TABLE 7: ROBUSTNESS STUDY

S. no.	Parameters	Variation	Concentration (ng/band)	%RSD
1	Time from application to development	(0, 30, 60, 90 min.)	2000	0.48
			8000	0.76
			16000	0.82
2	Time from development to scanning	(0, 30, 60, 90 min.)	2000	0.42
			8000	0.51
			16000	0.39
3	Scanning wavelength	227 ± 1 nm	2000	0.33
			8000	0.43

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

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CONFLICTS OF INTEREST: Nil

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