METHOD DEVELOPMENT AND VALIDATION OF VILDAGLIPTIN IN TABLETS AND DOSAGE FORM BY UV SPECTROPHOTOMETER

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ABSTRACT

Current study develops and validates a simple, precise, accurate, specific and highly sensitive method for he determination of Vildagliptin in bulk and pharmaceutical dosage forms. Vildagliptin is an oral anti-hyperglycaemia agent (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs.Vildagliptin inhibits the inactivation of the GLP-1 and GIP by DPP-4, 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. Vildagliptin also shown to reduce hyperglycaemia in type 2 diabetes mellitus. The solvent used is P^H 6.8 Buffer and the λmax or the absorption maxima of the drug was found to be 210nm.The parameters specificity, linearity, accuracy, precision and robustness were evaluated according to international Conference on Harmonization (ICH) Guidelines. A linear response was observed in the range of 10-60μg/ml with a regression coefficient of 0.9901. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.308 and 0.934 mcg/ml respectively

KEYWORDS: Vildagliptin, Hyperglycaemia, UV-Spectroscopy.

1. INTRODUCTION

Vildagliptin is an oral anti-hyperglycaemic agent (anti-diabetic drug) of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Vildagliptin inhibits the inactivation of the

GLP-1 and GIP by DPP-4, 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. Vildagliptin also shown to reduce hyperglycaemia in type 2 diabetes mellitus.

The European Medicines Agency has also approved a combination of vildagliptin and metformin, vildagliptin/metformin (Eucreas by Novartis) as an oral treatment for type-2 diabetes. So, an accurate analytical method is required to estimate Vildagliptin in pharmaceutical formulation.

AIM OF WORK

In the present study, an attempt was made to develop a simple and accurate UV spectrophotometric method for the quantification of Vildagliptin in bulk and pharmaceutical Formulation and to validate the method according to ICH Q2 (R1) guidelines.

2. DRUG PROFILE
MOLECULAR STRUCTURE
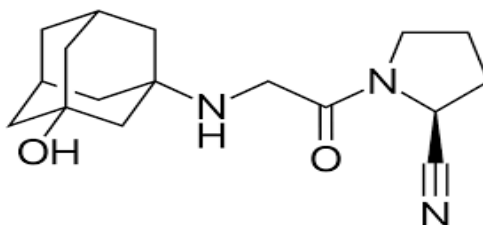
SYNONYM
Vildagliptina
Galvus

IUPAC NAME: (2S)-1-(2-(3-hydroxy-1-adamantyl) amino) acetyl) pyrrolidine-2-carbonitrile

MOLECULAR FORMULA
C17H25N302

MOLECULAR WEIGHT
303.4 g/mol

APPEARANCE

Vildagliptin is white to slightly yellowish or slightly greyish crystalline powder and no polymorphs or solvates have been identified so far.

MELTING POINT: > 145°C

SOLUBILITY

Vildagliptin is soluble in HCL, NaOH, Buffers and organic solvents such as ethanol, Dimethyl sulfoxide (DMSO), and dimethyl formamide (DMF), which should be purged with an inert gas. The solubility of vildagliptin in ethanol and dimethyl sulfoxide is approximately 16 mg/ml and approximately 20mg/ml in dimethyl formamide.

3. MATERIALS AND METHODS

3.1. MATERIALS

In the present study following reagents and materials were employed for Development and Validation of new UV-Visible Spectrophotometric method for estimation of Vildagliptin in Tablet Dosage forms. The solvents utilized in the entire work were MerkPvt.Ltd and all the chemicals were analytical grade.

Chemicals: The Vildagliptin reference standard (assigned purity 98%) was obtained from AUROBINDO, Hyderabad. The commercial pharmaceutical formulations were obtained from local pharmacies. Distilled water was prepared by Aquatron deionizing water system.

Solubility studies

Solubility studies for Vildagliptin were performed by using various solvents. The solubility of the drug was analyzed in distilled water, phosphate buffer of pH 6.8, sodium hydroxide, hydrochloric acid

Selection of Solvent

Vildagliptin was known to be soluble in solvents like phosphate buffer of pH 6.8, sodium hydroxide, hydrochloric acid. The absorption pattern of resulting solution is measured against respective blank solution in UV range (200-400nm) and λ_{max} was found in each solvent, and it was compared with the UV cutoff of that particular solvent to avoid any interaction between the sample peak and solvent peak. In case of phosphate buffer of pH 6.8, the interactions between solvent peak and sample peak were found to be minimum compared to other solvents and hence phosphate buffer of pH 6.8 was selected as a solvent for estimating the

various parameters of Vildagliptin.

Preparation of Phosphate buffer pH 6.8, Mixed

Dissolve 28.20g of Disodium hydrogen phosphate and 11.45g of Potassium dihydrogen phosphate in sufficient water to produce 1000 ml.

Method Development for Assay of Vildagliptin

Method development for assay of Vildagliptin tablets was initiated based on general method development guidelines and literature.

Preparation of Standard Stock Solution

Standard stock solution is prepared by dissolving 100 mg of Vildagliptin in phosphate buffer of pH 6.8 and the volume was made up to 100ml to obtain 1000 ppm solution and dilutions were made as and when required.

Preparation of Working Standard Solution

Working standard solution is prepared by taking 10 ml of the standard stock solution in the volumetric flask and the volume was made up to 100ml to obtain 100 ppm solution

LINEARITY

Aliquots of working standard solutions of Vildagliptin ranging from 1-6 ml were transferred in to a series of 10 ml volumetric flasks. The volume of each flask was made up to the 10 ml mark with phosphate buffer of pH 6.8 and the absorbances were measured at 210 nm. A graph was drawn by plotting concentration on X axis and absorbance on Y axis and the graph has shown a good linearity with a correlation coefficient of 0.9901.

Table 3.1: Absorbance of Vildagliptin in different concentrations.

S.NO	Concentration (ppm)	Absorbance
1	10	0.321
2	20	0.485
3	30	0.570
4	40	0.671
5	50	0.787
6	60	0.869
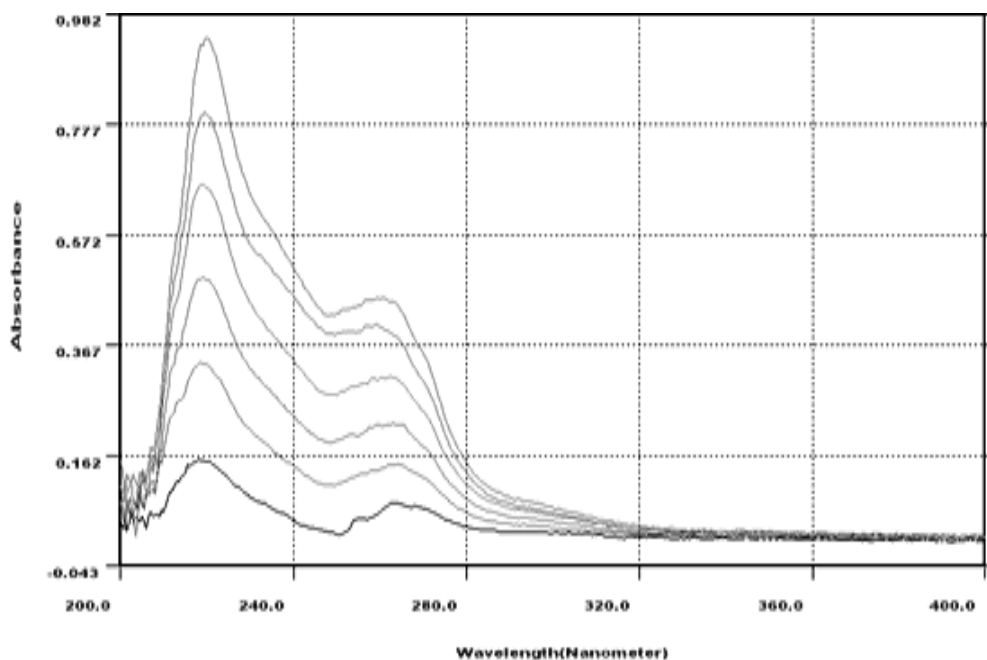
Fig. No. 1: Overlay Spectrum of Vildagliptin.

CALIBRATION CURVE OF VILDAGLIPTIN

y = 0.0107x + 0.2425
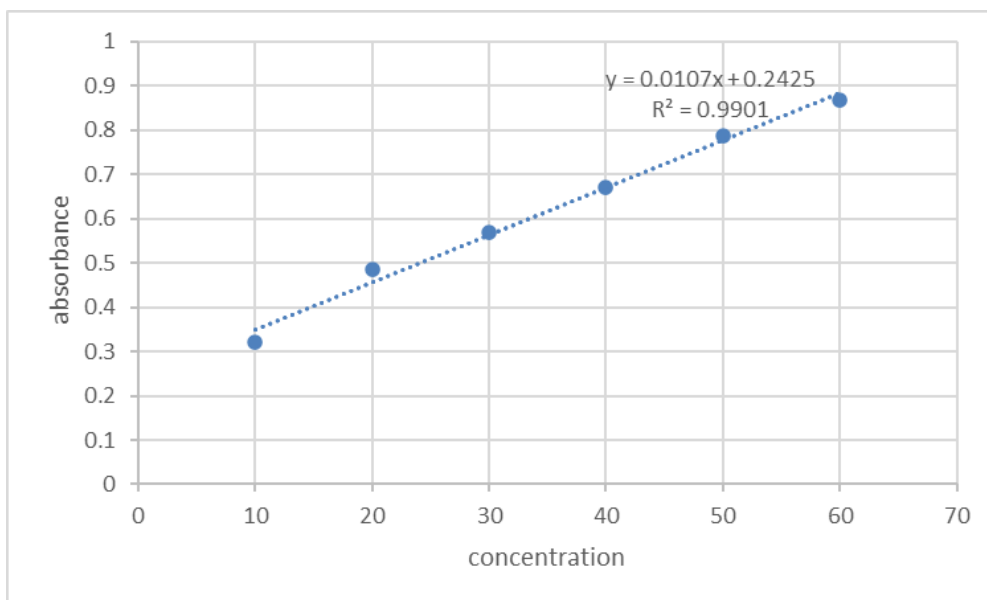R² = 0.9901

Fig. No: 2: Vildagliptin Calibration Curve.

VALIDATION OF SPECTROPHOTOMETRIC METHOD

1. PRECISION

A. Repeatability

The 40ppm concentration of vildagliptin was taken and its absorbance was measured at 210 nm.

B. INTERMEDIATE PRECISION

Analyst to Analyst: Four samples of 40ppm concentration of Vildagliptin were prepared by four different analysts and absorbance was observed under same experimental conditions.

Equipment to Equipment: Three samples of 40ppm concentration of Vildagliptin were prepared and absorbances were observed using different equipment.

Day To Day: Three samples of 40ppm concentration of Vildagliptin were prepared and absorbances were observed. Again, three fresh samples of the same concentration were prepared on the following day and absorbance was measured.

C.REPRODUCIBILITY

The three samples of 40 ppm concentration of Vildagliptin were observed in different labs.

2. ACCURACY (RECOVERY)

Accuracy is performed by diluting the stock solution in three different concentrations at 50%, 100% and 150%. Three samples were prepared from 50% concentration, three samples were prepared from 100% concentration and another three samples from 150% concentration. the absorbance of each sample was noted. Then Percentage recovery and mean percentage recovery are calculated.

3. RUGUDNESS

The absorbance of 40 ppm concentration of Vildagliptin solution was measured at different wavelengths. (210 +/- 5 nm).

4. LINEARITY RANGE

The linearity range of the standard can be found from linearity curve and accuracy data. The results were found to be linear in the concentration range of 10-60 ppm.

5. LIMIT OF DETECTION

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantized as an exact value.
Based on the standard deviation of the responses and the mean of the slopes, the detection limit was found.

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

6. LIMIT OF QUANTIFICATION

The quantization limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision, accuracy and reliability by the proposed method.

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

ASSAY OF VILDAGLIPTIN FROM ITS DOSAGE FORM

Preparation of Sample Solution

20 tablets of Vildagliptin (VILDAWYN 50) were taken, and all the tablets were crushed to fine powder using a mortar and pestle. Powder to be taken is calculated and 0.206g of Vildagliptin was weighed and transferred in to a 100 ml volumetric flask. The contents were dissolved in phosphate buffer pH 6.8 and sonicated for about 30 min. This solution was filtered through Whatman filter paper and the volume was made up to the mark using phosphate buffer of pH 6.8 and the absorbance of the solution was measured at 210nm.

4. RESULTS AND DISCUSSION

Vildagliptin was analyzed by using proposed UV spectrophotometric method in pharmaceutical formulation. It is soluble in phosphate buffer of pH 6.8 and hence it was selected as a solvent for vildagliptin to obtain UV spectrum in the range of 200-400nm. After the evaluation of the spectrum, vildagliptin showed maximum absorption at 210nm.
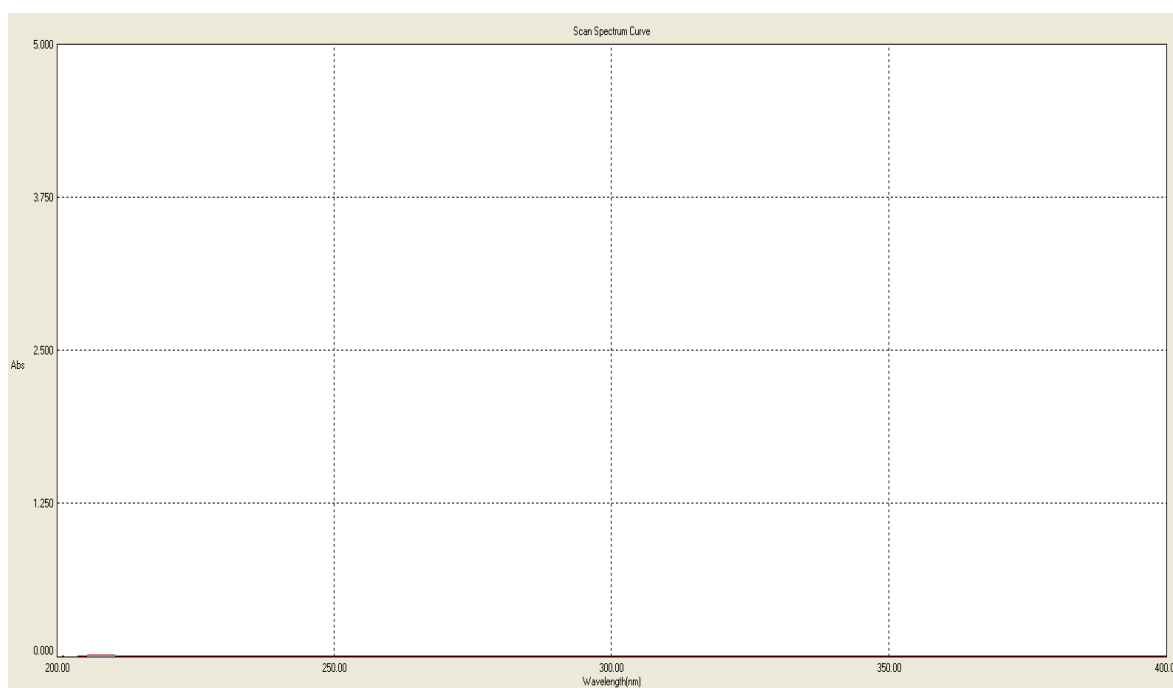
Fig. No: 3: Vildagliptin Blank Spectrum.
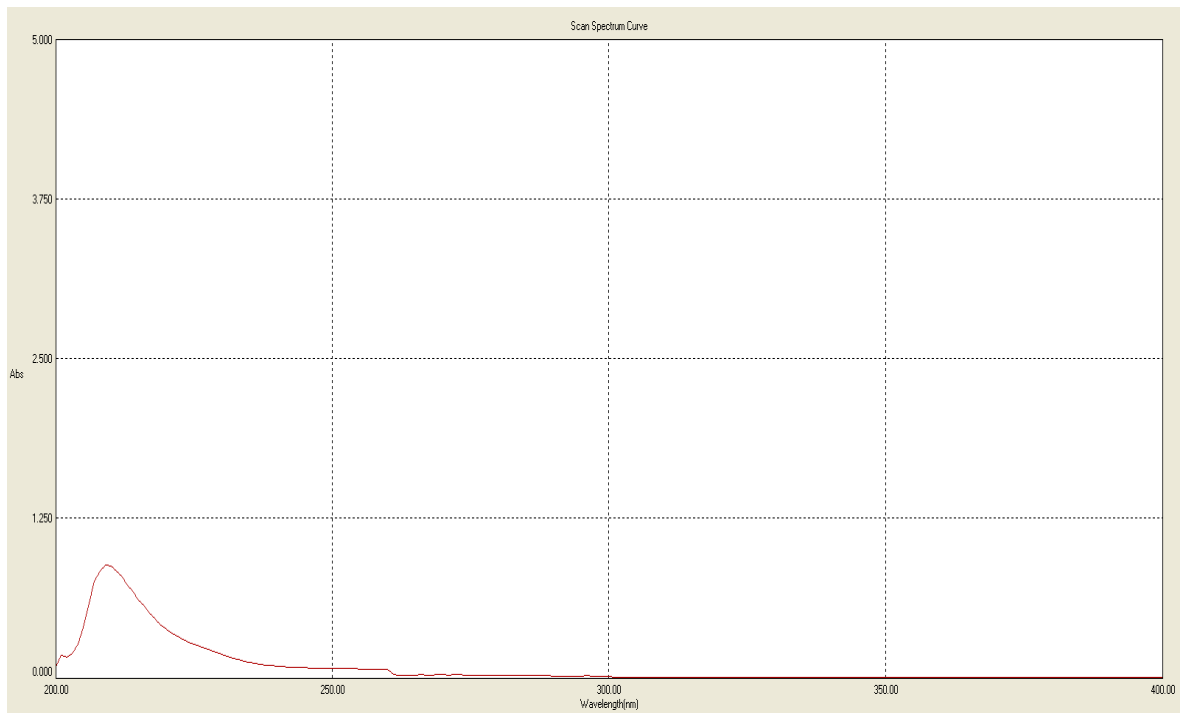
Fig No. 4: Vildagliptin UV Spectrum in Phosphate buffer.

1. LINEARITY

S.NO	Concentration (PPM)	Absorbance
1	10	0.321
2	20	0.485
3	30	0.570
4	40	0.671
5	50	0.787
6	60	0.869

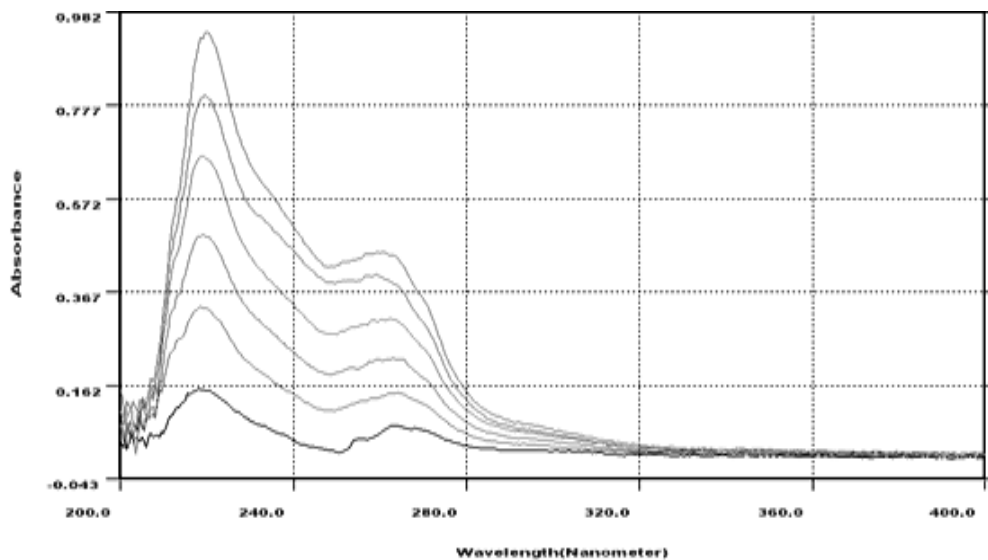**Table No. 4.1: Linearity values of standard vildagliptin.**

Fig. No. 5: Overlay Spectrum of Vildagliptin.
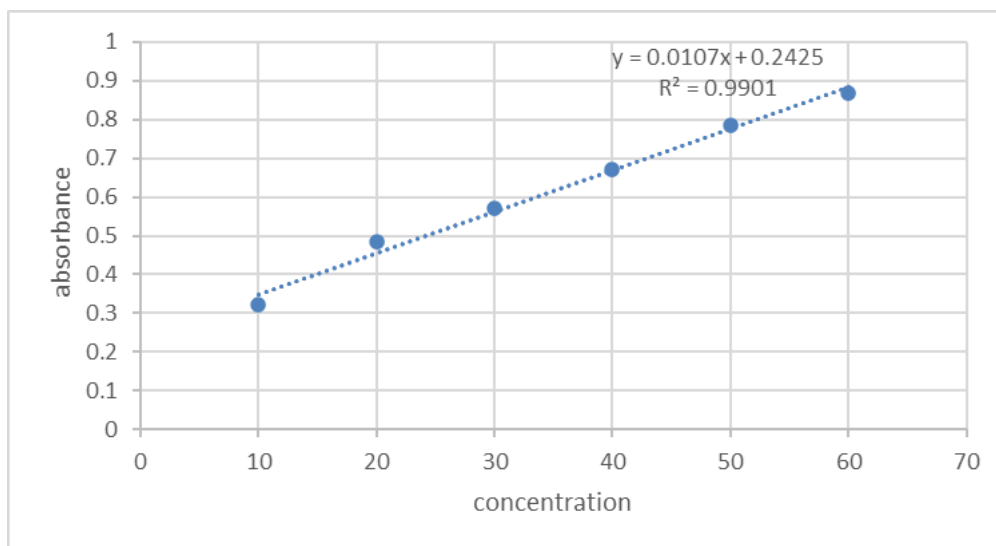
CALIBRATION CURVE OF VILDAGLIPTIN

Fig. No. 6: Calibration Curve of Vildagliptin.

2. REPEATABILITY

Table No: 4.2: Repeatability values of vildagliptin.

S.NO	Concentration(PPM)	Absorbance
1	40	0.628
2	40	0.629
3	40	0.628
4	40	0.630
5	40	0.629
6	40	0.630
Mean = 0.6285		
Standard deviation =0.0010		
% Relative Standard Deviation =0.159		

RSD for absorbance of six measurements was found to be not more than 1.0%

3. INTERMEDIATE PRECISION: ANALYST-ANALYST

Table No: 4.3: Analyst to Analyst (precision) values of vildagliptin.

S.NO	Sample NO	Analyst 1	Analyst 2	Analyst 3	Analyst 4
		ABSORBANCE			
1	Sample-1	0.613	0.607	0.622	0.612
2	Sample-2	0.615	0.612	0.626	0.622
3	Sample-3	0.608	0.615	0.608	0.607
4	Sample-4	0.622	0.617	0.606	0.613
Mean =0.614					
Standard Deviation = 0.0056					
% Relative Standard Deviation =0.91					

B. EQUIPMENT -EQUIPMENT

Table No: 4.4 - Equipment to equipment (precision) values of vildagliptin.

S.NO	Equipment	Absorbance-1	Absorbance-2	Absorbance-3
1	Equipment-1	0.615	0.612	0.607
2	Equipment-2	0.622	0.613	0.610
Mean =0.613				
Standard Deviation =0.0050				
% Relative Standard Deviation =0.81				

DAY-DAY

Table No: 4.5: Day to Day (precision) values of vildagliptin.

S.NO	Day	Concentration	Absorbance– 1	Absorbance- 2	Absorbance- 3
1	Day 1	40	0.682	0.676	0.674
2	Day 2	40	0.672	0.674	0.672
Mean = 0.675					
Standard Deviation =0.0037					
% Relative Standard Deviation =0.54					

4. REPRODUCIBILITY

Table No: 4.6: Reproducibility values of vildagliptin.

S.NO	LAB	Absorbance-1	Absorbance - 2	Absorbance - 3
1	Lab - 1	0.632	0.632	0.624
2	Lab - 2	0.626	0.636	0.632
3	Lab - 3	0.629	0.644	0.626
Mean = 0.631				
Standard Deviation = 0.0042				
% Relative Standard Deviation =0.66				

RSD for measured absorbance was found to be not more than 1.0% in all the cases indicating

that the method has good precision.

5. ACCURACY (RECOVERY)

Table No. 4.7: Accuracy values of vildagliptin.

S.NO	Accur-acy Level	Wt. Of Sample	Sample Absorbance	Amt Added	AMT Found	% Recovery	Mean % Recovery	STD DEV	% RSD
1	50	193.43	0.299	25.15	25.11	100.1			
2	50	193.43	0.295	25.16	25.45	98.8			
3	50	193.43	0.297	25.15	25.58	98.3	99%	0.932	0.939
4	100	386.86	0.671	50.34	50.01	100.6			
5	100	386.86	0.675	50.34	49.80	101.8			
6	100	386.86	0.674	50.34	50.14	100.3	100.6%	0.35	0.34
7	150	773.72	0.891	75.45	74.90	99.3			
8	150	773.72	0.875	75.45	75.86	98.1			
9	150	773.72	0.864	75.45	76.68	98.3	98.5%	0.648	0.657

Mean recovery was found to be between 98%-102% indicating that the test method has an acceptable level of accuracy.

6. Ruggedness

Table No: 4.8 - Ruggedness values of vildagliptin.

S.NO	Concentration(PPM)	Wavelength (nm)	Absorbance
1	40	205	0.686
2	40	206	0.679
3	40	207	0.676
4	40	208	0.682
5	40	209	0.679
6	40	210	0.686
7	40	211	0.683
8	40	212	0.688
9	40	213	0.694
10	40	214	0.683
11	40	215	0.686
Mean = 0.683			
Standard Deviation = 0.0050			
% Relative Standard Deviation =0.73			

7. LIMIT OF DETECTION

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

$=3.3 \times 0.001\ /\ 0.0107$

$=0.308$ ppm

8. LIMIT OF QUANTIFICATION

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

$=10 \times 0.001\ /\ 0.0107$

$=0.934$ ppm

CALCULATION FOR VILDAGLIPTIN

$$Assay = \frac{SPL\ abs}{STD\ abs} \times \frac{STD\ wt.}{STD\ dilln} \times \frac{SPL\ Dilln}{SPL\ wt.} \times \frac{100}{LC} \times Avg\ wt. \times \frac{\%\ potency}{100}$$

$$= \frac{0.682}{0.671} \times \frac{100}{100} \times \frac{10}{0.386} \times \frac{100}{50} \times 1.94 \times \frac{98}{100}$$

$= 99.4\%$

ANALYTICAL PERFORMANCE PARAMETERS OF VILDAGLIPTIN

Table No. 5.9: Analytical performance parameters of vildagliptin.

S.No	Parameters	Values
1	Linearity range	10-60ppm
2	Slope (M)	0.0107
3	Intercept (c)	0.2425
4	Correlation coefficient	0.99
5	Standard deviation	0.001
6	LOD	0.308 ppm
7	LOQ	0.934 ppm
8	Accuracy	98-102%
9	Assay	99.4%

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