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Forced Degradation Studies for Determination of Vildagliptin in Pharmaceutical Dosage Forms by RP-HPLC

Rohit R. Sawant^{*1}, Kaushal K. Chandrul², Yogesh D. Pawar³

1Research Scholar, Department of Pharmacy, Mewar University, Chittorgarh, Rajasthan

2Research Supervisor, Department of Pharmacy, Mewar University, Chittorgarh, Rajasthan

3Research CoSupervisor, Department of Pharmacy, Mewar University, Chittorgarh, Rajasthan

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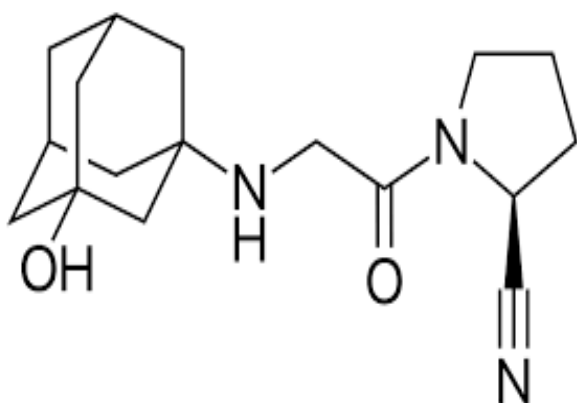
ABSTRACT

Vildagliptin which is DPP-4 inhibitor. The aim of the present study was to develop a simple, precise and stability-indicating HPLC method was developed and validated for the determination of Vildagliptin (VLG) in pharmaceutical dosage forms. In RP-HPLC method, the analytes were resolved using Buffer pH 3.0 and (ACN : Methanol) in the ratio of 80:19:1 as mobile phase at a flow rate of 1.2 ml/min, on HPLC water 2469 system containing of UV/PDA- detector with empower pro software and Waters X-Bridge, C8 column, 150 x 4.6 mm, 5 μ m. The detection was carried out at 210 nm for the drug. The method was successfully validated in accordance to ICH guidelines acceptance criteria for specificity, linearity, accuracy, precision, robustness, and system suitability. Forced Degradation studies revealed that the method was found to be stability-indicating. The results showed that the proposed method is suitable for the precise and accurate determination of Vildagliptin in bulk and its formulation.

Keywords: Vildagliptin, RP-HPLC, Validation, Forced degradation studies.

INTRODUCTION

Vildagliptin (VDG), S-1-[N-(3-hydroxy-1- adamantyl) glycy] pyrrolidine-2- carbonitrile is an oral antihyperglycemic agent (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP4) inhibitor class of drugs^{1,2}. Vildagliptin inhibits the inactivation of GLP1 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 pancreas³.



Vildagliptin has been shown to reduce hyperglycemia in type 2 diabetes mellitus. Literature survey revealed that few analytical methods are used for estimation of Vildagliptin^{1,7-8}.

But there is no analytical method for the determination of Vildagliptin from its pharmaceutical dosage form. Due to lack of published liquid chromatographic methods for VDG, so the aim of the present work was to develop a reversed-phase liquid chromatographic (RP-LC) method that would be suitable for the determination of VDG from its pharmaceutical dosage form^{4,5,6}. The proposed method is simple, accurate, reproducible and suitable for routine determination of Vildagliptin from its pharmaceutical dosage form.

MATERIALS AND METHODS

Drug Vildagliptin: Drug was obtained from Medichem Laboratories Ltd.

Reagents: Water, Acetonitrile, Methanol, Ortho phosphoric acid 88%, Triethylamine, Sodium 1-octonate sulfonate monohydrate all are HPLC grade & GR grade.

Instruments: HPLC model 2693/2998, pH meter, Analytical Balance, Sonicator, Double beam Spectrophotometer UV 1700 Shimadzu, FT-IR, Filter Nylon 0.45µm.

Identification by IR-Spectroscopy

Vildagliptin API: 1mg of Vildagliptin API was mixed properly with 200 mg of dried KBr and then carefully triturated in a mortar pestle. At last this mixture was kept on a die and IR spectrum was taken using the Diffused Attachment reflectance mode.

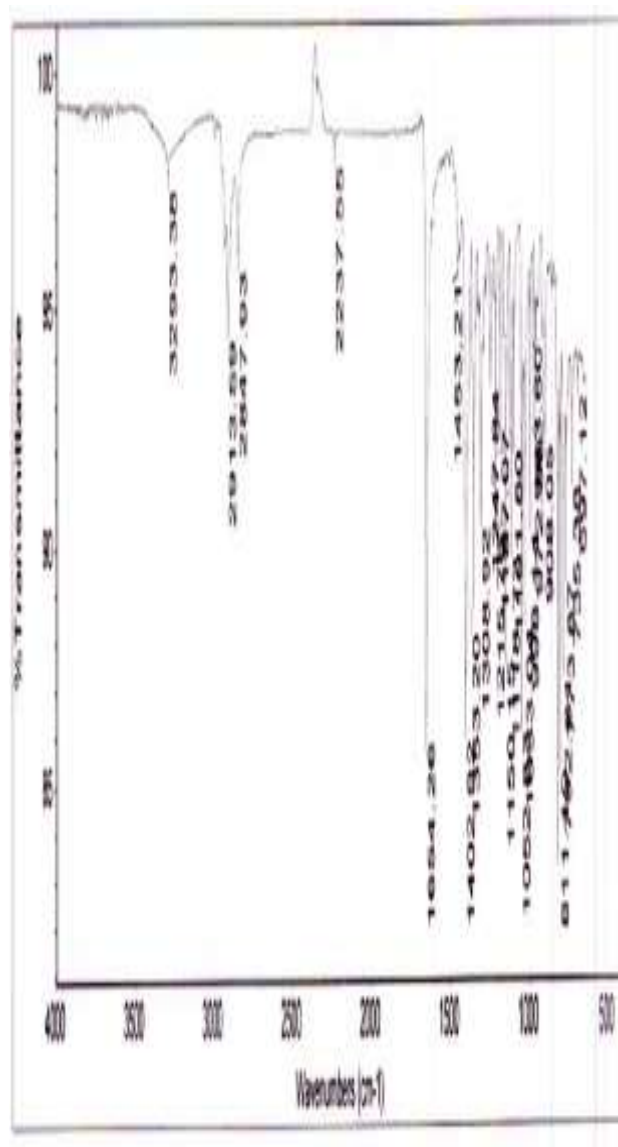


Figure 1: IR spectra of Vildagliptin

Determination of Max wavelength: The standard solutions were scanned separately between 200nm to 400nm. The spectrum, 210nm were recorded for estimation of drug.

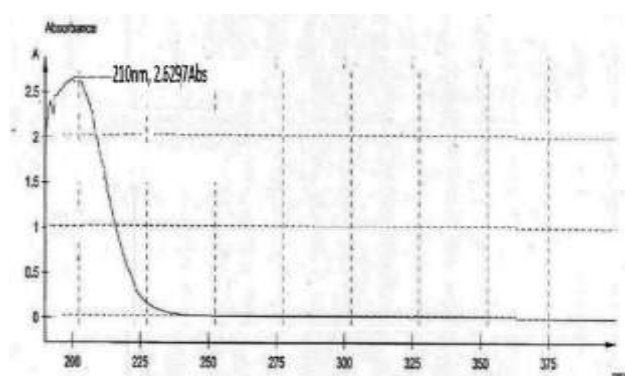


Figure 2: Determination of Wavelength for Vildagliptin

RESULTS AND DISCUSSION

Chromatographic conditions:

Table 1: Chromatographic technique used for Vildagliptin determination

Column	: Waters X-Bridge , C8, 150 x 4.6 mm, 5 μ m
Flow Rate	: 1.2 mL/min
Injection Volume	: 10 μ L
Wavelength	: 210 nm
Column Temp.	: 25°C
Sample Temp.	: 10°C
Run Time	: 10 minutes
Retention Time	: About 6.5 minutes
Needle Wash	: Water : Acetonitrile (10:90)
Seal Wash	: Water : Methanol (90:10)

Preparation of Standard stock solution

A. Vildagliptin: Weighed accurately about 25 mg of Vildagliptin working standard and transferred into 50 mL of volumetric flask. Add about 25 mL of diluent sonicated to dissolved and make up to volume with diluent and mix. (500mcg/mL).

B. Preparation of Standard solution:

Further transferred 20 mL of Vildagliptin Stock Solution into 50 mL volumetric flask and dilute up to the mark with diluent and mix.

C. Preparation of Sample Stock solution: (50/1000 mg):

Weighed and transfer 10 tablets into 1000 mL volumetric flask. Add about 700 mL of diluent, sonicated for 30 minutes with intermittent shaking. Allow it to cool to room temperature and make up to volume with diluent and mix. Let the solution stand for 5 minutes. Filter the sample solution through 0.45 μ Nylon membrane syringe filter. Discard first 3 mL of filtrate.

- **For Vildagliptin:** Dilute 20 mL of stock solution into 50

mL volumetric flask and dilute up to the mark with diluent and mix. (200 mcg/mL). **Similarity Factor:** Two standard solutions were prepared by standard procedure, and result obtained by using HPLC. Calculation:

$$\text{Correlation} = \frac{\text{AS2}}{\text{AS1}} \times \frac{\text{WS1}}{\text{WS2}} \times 100$$

Where,

AS1 : Average area from six replicates of 1st standard solution.

AS2 : Average area from two replicates of 2nd standard solution.

WS1 : Weight of 1st standard solution in mg.

WS2 : Weight of 2nd standard solution in mg.

System suitability

Single injection of Blank (Diluent), six replicate of Standard solution was injected on the system.

Specificity

The analysts should have no interference from other extraneous components and be well resolved from them. Specificity is the procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

Table 2: Result for Vildagliptin

Drug	Parameter	Result
Vildagliptin	Similarity factor	99.5%
	% RSD	0.57
Vildagliptin	Retention time (min)	6.261
	Symmetry factor	1.6
Vildagliptin	Theoretical plate	3887
	Purity angle	0.227
	Purity threshold	0.443
Vildagliptin	Mean recovery	98.8
	%RSD	0.29
Vildagliptin	Acid degradation	91.1
	Base degradation	84.2
	Peroxide degradation	85.4
Vildagliptin	Heat degradation	88.8
	Symmetry factor	1.4
	Theoretical plates	5433

Forced degradation

The study was performed to demonstrate the selectivity

of the method in presence of degradation products. Sample and Placebo (Vildagliptin) were exposed to different stress conditions.

1. Photolytic degradation

20 Tablets were taken (50/1000 mg) in a two separate Petri Plates (One open petri plate and another wrapped with aluminum foil) and exposed it under UV and white light for 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt/square. After exposure, weigh and transfer 10 tablets into 1000 mL volumetric flask. Add about 700 mL of diluent, sonicated for 30 minutes with intermittent shaking. Allow it to cool to room temperature and made up to volume with diluent and mixed. Let the solution to stand for 5 minutes. Filter the sample solution through 0.45 μ Nylon membrane syringe filter. Discarded first 3 mL of filtrate.

a. Placebo preparation: Prepared and Exposed the Placebo solution under UV and white light for 1.2 million lux same as sample solution.

For Vildagliptin: Diluted 20 mL of stock solution into 50 mL volumetric flask with diluent and mixed.

2. Heat degradation

Sample preparation: Weighed and transferred 10 tablets (50/1000 mg) into 1000 mL volumetric flask. Added about 700mL of diluent, sonicated for 30 minutes with intermittent shaking. Heated the solution in the water bath at about 70°C for 24 hrs. Allowed it to cool to room temperature and made up to volume with diluent and mixed. Let the solution to stand for 5 minutes. Filtered the sample solution through 0.45 μ Nylon membrane syringe filter. Discarded first 3 mL of filtrate.

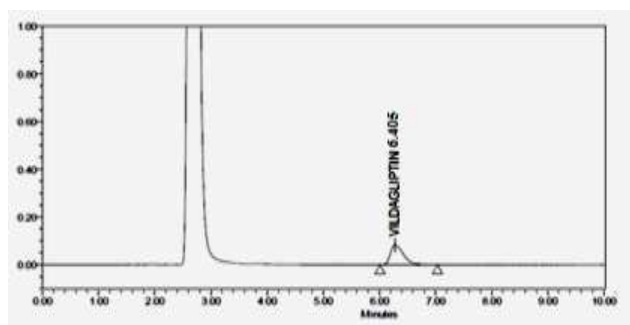


Figure 3: Chromatogram and Purity plot of Heat Treated Sample of Vildagliptin

3. Acid degradation

Sample preparation: 10 tablets (50/1000 mg) was Weighed and transferred into 1000 mL volumetric flask. Added about 700mL of diluent, sonicated for 30 minutes with intermittent shaking. Added 10 mL 1N HCl and kept at RT for 24 hrs. Neutralized this solution before dilution with 10 mL 1N NaOH. Allowed it to cool to room temperature and made up to volume with diluent and mixed. Let the solution to stand for 5 minutes. Filtered the sample

solution through 0.45 μ Nylon membrane syringe filter. Discarded first 3 mL of filtrate.

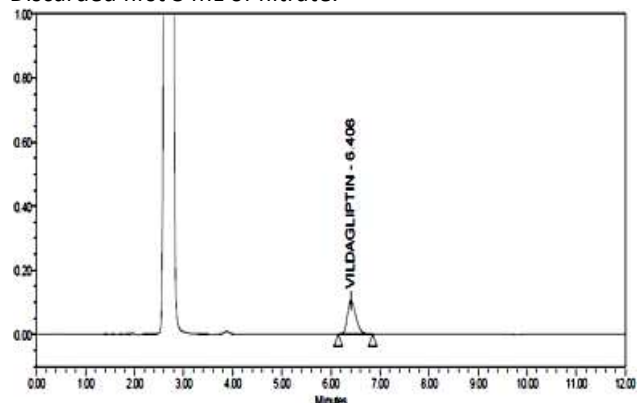


Figure 4: Chromatogram and Purity plot of Acid sample of Vildagliptin

4. Base degradation

Sample preparation: Weighed and transferred 10 tablets (50/1000 mg) into 1000 mL volumetric flask. Added about 700mL of diluent, sonicated for 30 minutes with intermittent shaking. Added 10 mL 1N NaOH and kept at RT for 24 hrs. Neutralized this solution before dilution with 10 mL 1N HCl. allowed it to cool to room temperature and made up to volume with diluent and mixed. Let the solution to stand for 5 minutes. Filtered the sample solution through 0.45 μ Nylon membrane syringe filter. Discarded first 3 mL of filtrate.

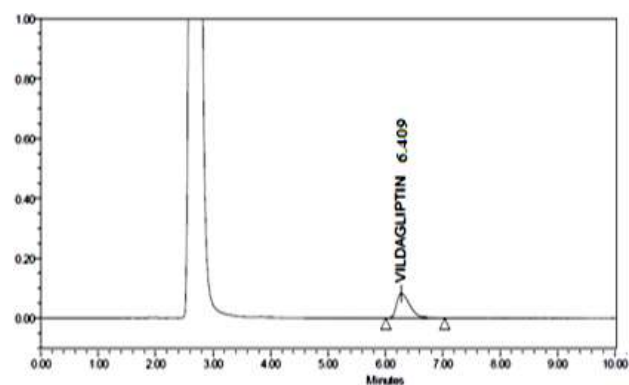


Figure 5: Chromatogram and Purity plot of base sample of Vildagliptin

5. Peroxide degradation

Sample preparation: Weighed and transferred 10 tablets (50/1000 mg) into 1000 mL volumetric flask. Added about 700mL of diluent, sonicated for 30 minutes with intermittent shaking. Added 10 mL 30% Hydrogen peroxide solution and kept at RT for 24 hrs. Allowed it to cool to room temperature and made up to volume with diluent and mixed. Let the solution to stand for 5 minutes. Filtered the sample solution through 0.45 μ Nylon membrane syringe filter. Discarded first 3 mL of filtrate.

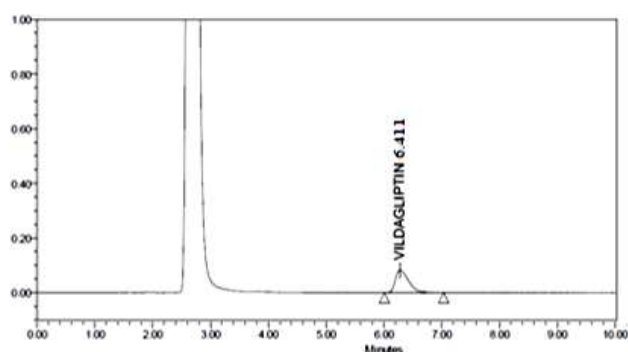


Figure 6: Chromatogram and Purity plot of peroxide sample of Vildagliptin

6. Humidity degradation:

Sample preparation: 10 tablets were taken (50/1000 mg) and transferred in a Petri Plate and exposed it at 25°C and 90% RH chamber for 2 days. After exposure weighed and transferred 10 tablets into 1000 mL volumetric flask. Added about 700 mL of diluent, sonicated for 30 minutes with intermittent shaking. Allowed it to cool to room temperature and made up to volume with diluent and mix. Let the solution to stand for 5 minutes. Filtered the sample solution through 0.45µm Nylon membrane syringe filter. Discarded first 3 mL of filtrate.

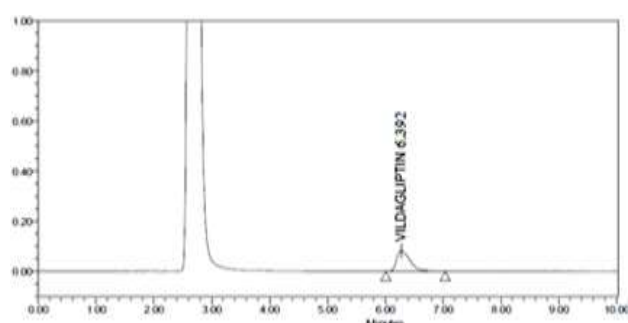


Figure 7: Chromatogram and Purity plot of Humidity sample of Vildagliptin

Linearity and Range

Evaluated linearity in the range of 50% to 150% of the working concentration level of Vildagliptin. As the working concentration level of Vildagliptin is 200 ppm the range proposed is 50% to 150% of 200 ppm for Vildagliptin.

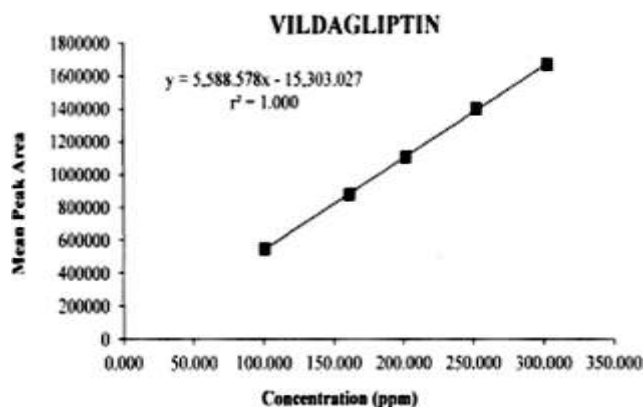


Figure 8: Linearity Plot for Vildagliptin

Accuracy (Recovery)

Evaluated accuracy at three levels 50%, 100% and 150% of the working concentration level for Vildagliptin. As the working concentration level of Vildagliptin is about 200 mcg/mL each level prepared in triplicates.

Precision

System Precision: Single injection of Blank (Diluent) and six replicate injections of standard solution were injected on the system.

Method Precision: Six independent sample preparations were prepared and injected in duplicate on the HPLC.

Intermediate Precision (Ruggedness): Same procedure of method precision is followed by another Analyst by using same lot of Vildagliptin Tablets on different instrument, different column and on different day.

CONCLUSION

The method shows good reproducibility; moreover the RP-HPLC method is accurate, precise, specific, and reproducible. The analysis of dose formulation of Vildagliptin can also be successfully performed by the RP-HPLC method. No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies. The results from table clearly indicate that the RP-HPLC technique can be successfully applied for the estimation of above-mentioned drugs in their solid dosage formulation.

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