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Stability Indicating HPLC MS Method for Determination of Degradation Products in Vildagliptin

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Abstract

Vildagliptin is an anti-diabetic drug under new class of dipeptidyl peptidase-4(DPP-4) inhibitor. The proposed work is performed to develop simple reverse phase liquid chromatographic method for force degradation behavior of Vildagliptin and its degradation pattern in pharmaceutical dosage forms. The chromatographic separation was obtained on C18 column with mobile phase containing acetonitrile and water (40:60) pH adjusted at 7.0 using triethylamine, with a flow rate of 1 ml/min, UV detection at 220 nm. Retention time was found to be 5.3 min and method was linear in the range of 2-12 ug/ml of R2=0.9999. Limit of detection and limit of quantization were 3.61 and 10.96 ug/ml, respectively.

The method was validated in accordance with International Conference on Harmonization Q2 (R1). Stress studies were carried out using acidic, basic, oxidative, thermal and photolytic conditions and there is no interference of the degradation products was observed with main drug peak.

Keywords: Vildagliptin; Method validation; HPLC MS; Force degradation

Introduction

Vildagliptin (VLD) is an antidiabetic medicine used alone or in combination with other medicines to treat type 2 diabetes in adult patients. It is extensively used as oral anti-hyperglycemic agent of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Pharmacologically, Vildagliptin reduce the inactivation of Glucagon-like peptide-1 (GLP-1) and Gastric Inhibitory Polypeptide (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[1-3]. Several methods have been employed for the estimation of VLD individually and combination with other drugs such as UV and RP-HPLC methods. In literature review various analytical methods such as UV spectrophotometric [4], HPLC methods LCMS [5-8] are reported for vildagliptin alone or in combination with others.

Previous reports on HPLC-MS based analytical quantification of VLD resulted in lesser sensitivity, and high noise. There is need to develop a more efficient, sensitive, simple and rapid stability indicating LC MS method for assessment of degradation behavior of VLD in acidic, basic, oxidative, thermal and photolytic environment.

Materials and Methods

Instrumentation

Jasco HPLC equipped with UV 2075 Plus Detector, UV 2080 Plus Pump connected with auto sampler and analysis performed on Chrom NAV data acquisition software (JASCO Corporation, Japan). The column used was a reversed phase C18 column (250 × 4.6 mm, 5-Hypersil Gold). Liquid chromatography-mass spectrometry (LC–MS) system consisted of a pump. Mass spectrometer used was 6460 triplequadrupole mass spectrometer. The software used was Agilent mass hunter workstation data acqui-sition version 1.18.03.2.2

Chemicals

Vildagliptin were supplied from Merck Laborateries ltd. Andheri, Mumbai. HPLC grade Acetonitrile (ACN) and methanol (Merk, Germany), Analytical grade triethylamine (Sigma Aldrich, Mumbai, India), sodium hydroxide (BDH, Germany), hydrochloric acid and hydrogen peroxide (Merk, Germany) were purchased. High purity water (Millipore) was obtained from college laboratory.

Preparation of stock solution and working standard solution

Stock solutions 1 mg/ml each of Vildagliptin were prepared in methanol.

Preparation of stressed/degradation samples.

The degradation products were prepared as per degradation conditions (hydrolytic, oxidative and thermal) specified in ICH Q2(R1) guideline.

Acid and alkaline hydrolysis

Volumetric flasks (100 ml) containing 10 ml of working standard solution were mixed with 10 ml of 0.1 N HCl. HCl solution for acidic degradation acid or 10 ml of 0.1 N NaOH solutions for alkaline degradation. The solutions were refluxed at 60°C temperature for 30 min. After this period, the acid and alkali degraded solutions were neutralized with volume of 0.1 N NaOH and 0.1 N HCl, respectively.

Thermal and photo degradation

10 ml of working standard solution was transferred to volumetric flask (100 ml) and exposed to 105°C for 30 min in oven (for thermal degradation) or exposed to sun light for 24 hr (for photo degradation). After the specified period of degradation, the resulting solution was diluted with mobile phase.

Oxidative degradation

10 ml of 3% hydrogen peroxide solution was added into a 100 ml volumetric flask containing 10 ml sample solution. After sonication for 1 hr the solutions were filtered and injected.

Stability indicating HPLC method development

HPLC method has been developed by considering separation between the degradation products and the drug, various HPLC parameters like type of column, asymmetry factor, the ratio and pH of mobile phase, flow rate, detection wavelength.

Method Validation

100 µg/ml stock solution of pure VLD was prepared and used for further analysis in validation parameter [9-11].

Linearity

A stock solution of VLD was diluted up to 1000 µg/ml using mobile phase, which was consequently diluted up to get 2–12 ug/ml serial solutions. The prepared samples were injected in HPLC system. The study was performed in triplicate (n=3). The linearity curve was prepared by plotting the area of peak against the concentration of solution.

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

LOD and LOQ were determined by using standard deviation (SD) of the response and slope of linearity curve. It was calculated using following equations.

LOQ=10 (SD)/Slope

LOD=3.3 (SD)/Slope

Precision (Intra-day and Inter-day precision)

Three samples of three different concentrations of VLD (4, 8, and 12 ug/ml) were examined on the same day for intra-day precision. However, for inter-day precision, three different concentrations of VLD (4, 8, and 12 ug/ml) were analyzed on three consecutive days.

The precision of the method was evaluated by calculating percentage relative standard deviation (RSD) (n=3).

Repeatability

Repeatability of method was performed by spiking 6 ug/ml of the drug from stock solution 3 times on the same day. Peak areas were recorded and the repeatability was evaluated by calculating percentage RSD (n=3).

Accuracy

The accuracy of the HPLC method was determined by applying the drug sample (6 ug/ml) added with the known amount of VLD solution corresponding to get 80%, 100% and 120%. The analysis was performed in triplicate and percentage recovery was calculated.

Quantitative HPLC assay for VLD tablet formulation

Weighed sample of 20 Tablets was powdered. An accurate quantity of powder equivalent to 50 mg of VLD (Tab Galvus 50 mg) was weighed and 50 ml of methanol was added. The solution was sonicated for 30 min and volume was adjusted to 100 ml with methanol. From this solution, an aliquot of 5 ml was diluted up to 50 ml with methanol. A portion of the resultant solution was passed through a 0.45 µm syringe filter and the filtrate was analyzed by HPLC for estimation of percentage purity.

Mass fragmentation study of VLD and its degradation samples

The peak response obtained from the HPLC analysis of each degradation sample was recorded and fractions were collected from the detector system in a systematic way. The collected fractions were further diluted using ACN and injected in LC–MS system. The mass spectra of resulting samples were recorded at a flow rate of 100 µl/min.

Results and Discussion

Optimization of stability-indicating HPLC method

The HPLC method developed in the present study showed the separation of VLD from its degradation peak formed using 0.1 N HCl, 0.1 N NaOH, 3% v/v H_2O_2 and less degradation was observed in photolytic study. This method also consents quantitation of VLD in Tablet formulations. Best results were obtained with a mixture of acetonitrile and water in the ratio of 40:60 (v/v) with pH 7.0 using triethylamine at wavelength 220 nm. The Retention Time (Rt) of pure VLD was found to be 5.3 min Chromatogram shown in Figure 1, however, for degradation products it was within the range of 3.5 to 6.5 min given in Figure 2 and the percent degradation of VLD related to individual degradation process is given in Table 1.

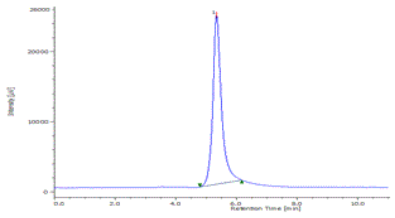

Figure 1: Chromatogram of standard VLD.

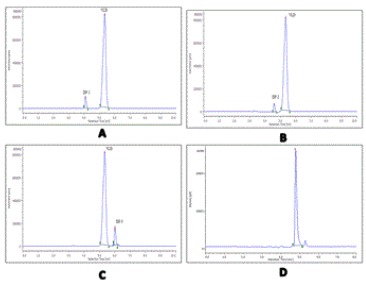

Figure 2: HPLC chromatograms of acid degradation (A) Alkaline Degradation (B) Oxidative Degradation (C) Photolytic Degradation (D).

Sr.no	Degradation type	% Degradation
1	Acidic degradation	13.27%
2	Alkaline degradation	14.76%
3	Oxidative degradation	23.76%
4	Photolytic degradation	1.98%

Table 1: Percent degradation by force degradation studies.

Linearity

The Calibration graph was observed in concentration range of 2–12 ug/ml. The correlation coefficient was observed at 0.9998. Results obtained are shown in Table 2 and Figure 3.

Sr.no	Concentration (ug/ml)	Area (Au)
1	2	480087
2	4	1066305
3	6	1526285
4	8	2059191
5	10	2582290
6	12	2967540

Table 2: Linearity results for calibration graph.

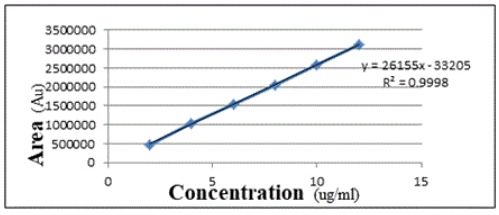

Figure 3: Calibration graph of VLD.

LOD and LOQ

LOD and LOQ were observed as 3.61 ug/ml and 10.96 ug/ml respectively given in Table 3.

Sr.no	Parameter	Result
1	LOD	3.61 ug/ml
2	LOQ	10.96 ug/ml

Table 3: LOD and LOQ for Vildagliptin.

Precision

Intraday and interday precision of VLD comforts the repeatability of test results. The percentage RSD found which was below 2. Results of intraday and interday precision were shown in Table 4.

Sr.no	Precision	Concentration (ug/ml)	SD	%RSD	Mean
1	Interday	4	4546.42	0.45516	
		8	41402.9	2.1044	1.2355
		12	34050.2	1.15064	
2	Intraday	4	3275.58	0.32644	
		8	13599.1	0.68873	0.533
		12	17316.5	0.58405	

Table 4: Precision data for Vildagliptin.

Repeatability

Repeatability of the HPLC method was observed below 2% RSD for 6 ug/ml sample concentration which were within the acceptance criteria as per guidelines shown in Table 5.

Sr.no	Concentration (ug/ml)	Area (Au)	Mean	SD	%RSD
1	6	1530267			
2	6	1528650			
3	6	1522303	1522303	3943.01	0.25902
4	6	1526285			
5	6	1522303			
6	6	1520474			

Table 5: Repeatability study for Vildagliptin.

Accuracy

Accuracy was studied by standard addition method at level 80%, 100% and 120% and percentage recovery found was within accepted Table limit 99%-101%. Results of recovery study are shown in Table 6.

Sr.no	% Level	Initial conc. ug/ml	Amount added	Total amount	Amount recovered	%Recovery	Mean %
1	80	6	4.8	10.8	10.78	99.81	
2	100	6	6	12	12.05	100.4	99.5469
3	120	6	7.2	13.2	12.99	98.4	

Table 6: Recovery studies for Vildagliptin.

Assay

The assay content for VLD Tablet formulations was observed within prescribed limits as per USP monograph given in Table 7 and Figure 4.

Sr.no	Tablet	Lable claim	Observed value	% Purity
1	Galvus	50 mg	49.78 mg	99.50%

Table 7: Assay content of tablet formulation.

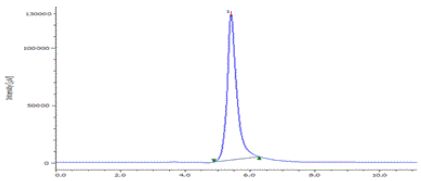

Figure 4: HPLC Chromatogram of tablet formulation.

LC–MS study and predication of possible degradation pathways

Mass spectrum of VLD degradation products were analysed using LCMS instrument on electro spray mode of ionization provided a highly selective method for the determination of the molecular mass of degradation products. Mol mass of degradation products were used to interpret the possible degradation pathways for VLD under various stress conditions given in Figures 5-7 and Table 8.

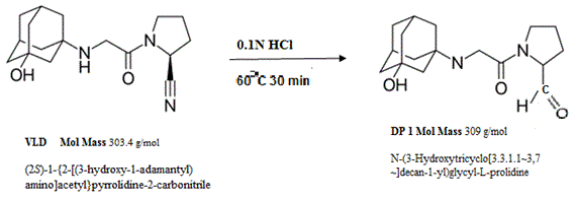

Figure 5: Possible degradation pathway for degradation Product I.

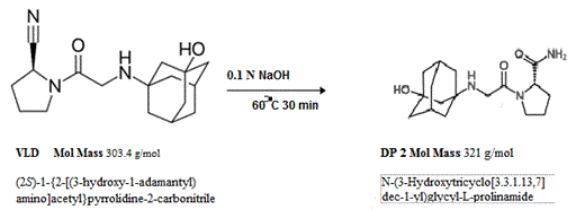

Figure 6: Possible degradation pathway for degradation Product II.

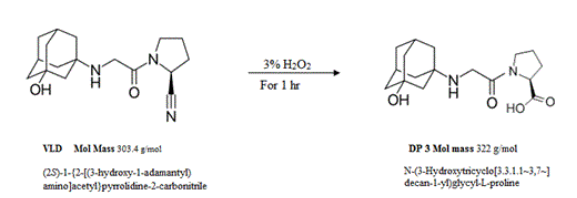

Figure 7: Possible degradation pathway for degradation Product III.

Sr.no	Degradation product	IUPAC Name
1	DP I	N-(3-hydroxytricyclo [3.3.1.1~3,7~]decan-1-yl)glycyl-L-prolidine
2	DP II	N-(3-hydroxytricyclo [3.3.1.13,7]dec-1-yl)glycyl-L-prolinamide
3	DP III	N-(3 hydroxytricyclo[3.3.1.1~3,7~]decan-1-yl)glycyl-L-proline

Table 8: Degradation products with IUPAC name.

Mass spectrum of degradation Product-I (m/z: 309 g/mol)

The degradation peak observed during acidic degradation of VLD using 0.1 N HCl was further studied for LC MS analysis. In LC–MS, the peak was obtained shown in Figure 8 at mass to charge ratio of '309' which is unknown degradation product of VLD. The IUPAC name of possible structure might be N-(3-hydroxytricyclo [3.3.1.1~ 3,7 ~]decan-1-yl)glycyl-L-prolidine.

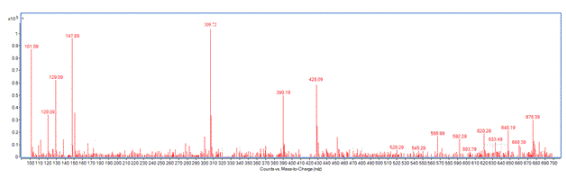

Figure 8: Mass spectrum of Degradation Product I.

Mass spectrum of degradation Product-II (m/z: 321 g/mol)

The degradation peak observed during acidic degradation of VLD using 0.1 N NaOH was further studied for LC MS analysis. In LC–MS, the peak was obtained shown in Figure 9 at mass to charge ratio of

'321' which is unknown degradation product of VLD. The IUPAC name of possible structure might be N-(3-hydroxytricyclo [3.3.1.13,7]dec-1-yl)glycyl-L-prolinamide.

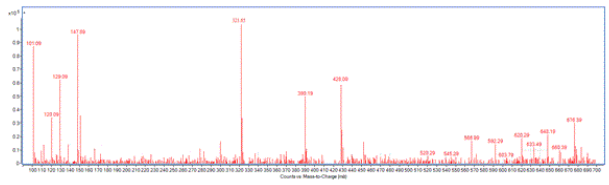

Figure 9: Mass spectrum of Degradation Product II.

Mass spectrum of degradation Product-III (m/z: 322 g/mol)

The degradation peak observed during acidic degradation of VLD using 3% v/v H_2O_2 was further studied for LC MS analysis. In LC–MS, the peak was obtained shown in Figure 10 at mass to charge ratio of '322' which is known major amide impurity of VLD reported in USP. The IUPAC name of possible structure might be N-(3 hydroxytricyclo[3.3.1.1~3,7~]decan-1-yl)glycyl-L-proline.

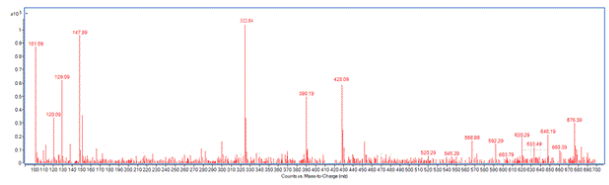

Figure 10: Mass spectrum of degradation Product III.

Conclusion

In the current study, the HPLC method was developed and validated as per ICH guidelines. The developed method is efficient to separate VLD and its degradation products using acetonitrile and water (40:60) pH 7.0. The HPLC method developed was found to be linear (R2=0.999) within range of 2-12 ug/ml for VLD. Recovery study performed at 80, 100 and 120% of VLD concentration showed mean percentage recovery as 99.54%. Moreover, the forced degraded samples of VLD were examined by a developed method which exhibited degradation of VLD to a reasonable extent in acidic, alkaline, and oxidative stress conditions.

Acknowledgement

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