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Stability-indicating RP-UPLC Method for Determination of Vildagliptin in Drug Substance and Its Tablet Dosage Form

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The foremost purpose of this research work is to diminish the analysis time and to establish cost effective method for estimation of Vildagliptin by RP-UPLC.
Study Design: UPLC based Quantification studies.
Place and Duration of Study: Department of Pharmacy, Bhagwant University, Ajmer, Rajasthan, Indiabetween June 2020 and August 2020.
Methodology: A simple, responsive and precised RP-UPLC method with good robustness was developed and validated as per ICH for the analysis of Vildagliptin in drug substance and separation of degradants generated by different forced degradation conditions. Productive separation of Vildagliptin was attained by the use of Luna C18 column (100x2.6mm and 1.6µm) with a mobile phase composition of 0.1% v/v Trifluoroacetic acid and Acetonitrile in 80:20 v/v, which was pumped with 0.5 ml/min flow rate. The eluted substances were examined with PDA detector at 239nm. Stressed degradation studies were performed with proposed method to determine the percentage degradation of Vildagliptin.
Results: The RT of Vildagliptin was observed at 1.56 min. The developed method was validated as per ICHQ2B and proved that the method was precise, sensitive, specific and accurate.The lowest

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concentration of limit of detection (0.05µg/ml) and limit of quantification(0.5µg/ml) of Vildagliptin make obvious about the sensitivity of the method. The correlation coefficient found to be 0.9997 for given range of linear concentrations. The calculated average percentage recoveries of Vildagliptin in spiked solutions were found to be in the range of 99.1-100.5. The calculated % RSD was determined to be less than 2. Determination of degradation of amount of Vildagliptin by forced degradation studies representing the stability indicating nature of the proposed method.

Conclusion: The developed method said to be highly sensitive, accurate, specific and robust, therefore this method has high probability to adopt in pharmaceutical industry for regular analysis of Vildagliptin.

Keywords: Luna C18 column; vildagliptin; stability indicating; validation; specificity; sensitivity.

ABBREVIATIONS

PDA : Photo Diode Array
UPLC:Ultra-Performance Liquid Chromatography
RT : Retention Time
LOD : Limit of Detection
LOQ : Limit of Quantification
ICH :International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
SD : Standard Deviation
RSD : Relative Standard Deviation
API : Active Pharmaceutical Ingredient
FD : Forced Degradation
UV : Ultra Violet
TFA :Trifluoroacetic acid

1. INTRODUCTION

Diabetes or Hyperglycemia is the most of the prevailed diseases across the globe. Elevated blood sugar levels are diagnosed in diabetes. As per WHO study, around 462 million populations were agonized with diabetes [1]. A progressive research is done on diabetes and developing new way of treatments and novel anti-hyperglycemic agents Vildagliptin is one of the novel and efficient oral anti-hyperglycemic agent works by inhibiting dipeptidyl peptidase-4 (DPP-4) results in subdued the actions of glucagon-like peptide-1 (GLP-1) [2-5]. Vildagliptin chemically identified as (2S)-1-[2-[(3-hydroxy-1-adamantyl)amino] acetyl]pyrrolidine-2-carbonitrile. The chemical structure of Vildagliptin was represented in Fig. 1. Till date, few RP-HPLC methods were offered in the literature for the assessment and evaluation of Vildagliptinas as single entity or in blend with other anti-hyperglycemic agents in bulk and formulation forms [6-12]. Along with those few UPLC procedure were existed for vildaglitin alone or in blend with other agents [13].

In the reported method it was observed that complex mobile phase system, longer retention time and high sensitivity for Vildagliptin. Formerly, no RP-UPLC method was described for assessment and evaluation of Vildagliptin as single entity with simple solvent system, lesser retention time (RT) and exploration of degradants produced by stress conditions. The degradants in the drug substance particularly affects the excellence and purity of the drug substance. Hence, a simple UPLC method was developed with simple solvent system, lesser retention time (RT) and exploration of possible degradants produced by stress conditions.

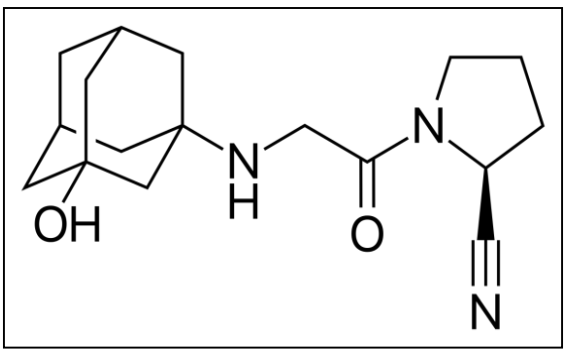


Fig. 1. Chemical structures of Vildagliptin

2. MATERIALS AND METHODS

2.1Reagents and Chemicals

Acetonitrile and Trifluoroacetic acid (TFA) of HPLC grade were procured from local supplier of Merck India. Vildagliptin API was obtained as gift sample from Hetero pharma, Hyderabad.

2.2 Instrumentation

Chromatographic separation was accomplished by using Waters alliance HPLC (2695) with photodiode array detector (PDA) and auto sampler. Data processing and integration was done with the Empower 2 tool.

2.3 Chromatographic Conditions

Productive separation of Vildagliptin was attained by the aid of Luna C18 column (100x2.6mm and 1.6µm) with a solvent composition of 0.1% v/v TFA and acetonitrile in 80:20 v/v, which was pumped with 0.5 ml/min flow rate. The eluted substances are examined in PDA detector at 239nm. Same solvent system was used in the preparation of stock solutions and dilutions.

2.4 Preparation of Standard Solution

Weighed accurately and transfer 50mg of Vildagliptin into 100ml of volumetric flask. Make up the volume with diluent to provide a solution of 0.5g/ml of Vildagliptin. 5ml of the above solution was again diluted to 50ml to get a solution of 50µg/ml of Vildagliptin.

2.5 Preparation of Sample Solution

62mg of Vildagliptin tablet powder was accurately weighed and transferred to 100ml of volumetric flask. Make up the volume with diluent to provide a solution of 0.5g/ml of Vildagliptin. 5ml of the above solution was again diluted to 50ml to get a solution of 50µg/ml of Vildagliptin.

2.6 Method Validation

The adopted method has been validated with respective to Q2 guidelines of ICH.

2.6.1 System suitability

System suitability of the proposed method has been carried out by introducing six homogenous replicate injections of standard solution (50µg/ml). Parameters like theoretical plates, % and tailing factor were evaluated for the gained chromatograms.

2.6.2 Specificity (Selectivity)

The blank, sample solution (50µg/ml), forced degradation solution(50µg/ml and standard solution(50µg/ml) were introduced in to UPLC system one after other. The derived chromatograms were interpreted to assess the occurrence of any interference from the blank, degradants and other substances with Vildagliptin peak.

2.6.3 Linearity

Linear response of the current procedure was established by assessing the correlation coefficient (R2) for the given series of concentrations ranges from 5 to 750 µg/ml Vildagliptin working standard solution.

2.6.4 Sensitivity

Standard deviation method has been adopted to calculate the limit of detection (LOD) and limit of quantification (LOQ). The following formulae were used to determine LOD and LOQ.

$LOD = 3\sigma/S$
$LOQ = 10\sigma/S$

Here, σ is standard deviation of the intercept
S is slope of the calibration curve

2.6.6 Precision

Intra-day precision of the proposed procedure was carried out by injecting prepared standard solution (50µg/ml) for 6 times in a day and inter day or intermediate precision was carried out by injecting prepared standard solution (50µg/ml). for 2 times in a day for 3 days continuously. The %RSD of the attained peak areas of Vildagliptin in both types of precessions was determined. The %RSD should be not more than 2.

2.6.7 Accuracy

To make sure the accuracy of the proposed procedure, percentage recovery procedure was implemented. In which predetermined amount of sample solution was spiked to Vildagliptin standard solution at, 50%, 100%, and 150% specification levels. Each spiked levels were injected in triplicate and average percentage

recovery of sample concentration at different specification levels were assessed.

2.6.8 Robustness

Robustness of the present UPLC method was confirmed by modifies the flow rate and mobile phase composition slightly and deliberately. Standard solution (50µg/ml) was injected in triplicate with each modified parameter. %RSD of the attained peak areas of Vildagliptin in all modified cases were determined. The %RSD should be not more than 2.

2.7 Forced Degradation Studies (Stress Studies)

To find out the stability representing nature of the current method, standard solution (50µg/ml) was stressed by exposing to 1N HCl, 1NaOH, 10% H_2O_2, UV light at 254nm and 80°C/75% RH for 24 hours to produce degradation products. Each kind of stressed solution was injected and assess the percentage degradation of Vildagliptin.

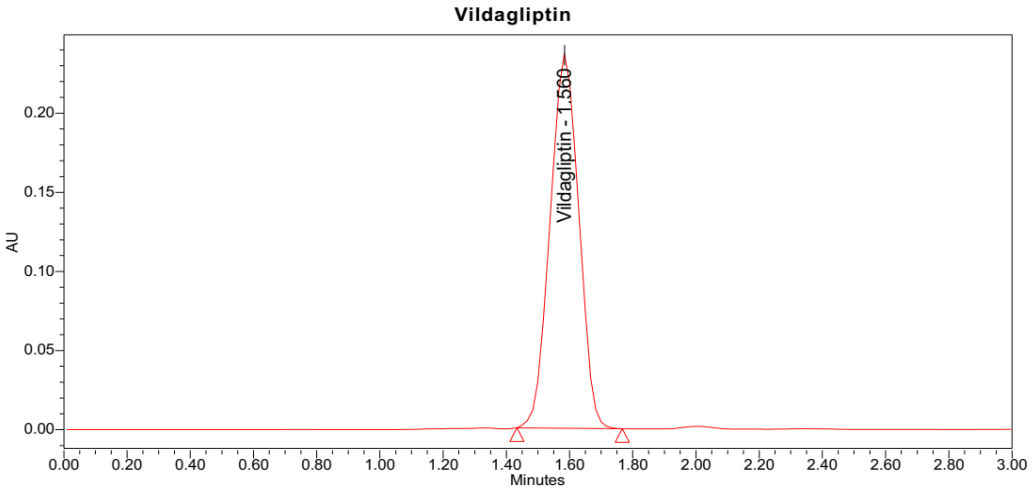
3. RESULTS AND DISCUSSION

3.1 Method Optimization

Productive separation of Vildagliptin was attained by the aid of Luna C18 column (100x2.6mm and 1.6µm) with a solvent composition of 0.1%v/v TFA and acetonitrile in 80:20, which was pumped with 0.5 ml/min flow rate. The eluted substances are examined in PDA detector at 239nm. Same solvent system was used in the preparation of stock solutions and dilutions. The RT of Vildagliptin was observed at 1.56min (Fig. 2). The optimized method condition have been satisfied the all the system suitability parameters (Fig. 2).

3.2 Method Validation

No interference was found at the retention time of Vildagliptin from sample, blank and degradants which extensively illustrates the specificity of the procedure (Fig. 3). The R^2 value calculated to be 0.9997 for Vildagliptin, which indicates good linearity (Fig. 4). The % RSD values of both precisions were etermined to be not more than 2 (Table 1). The obtained results were given in and representative chromatogram in Fig. 5. The percentage recovery of Vildagliptin in spiked sample were in the range of 99.1-100.5 (Table 2), which significantly gives out the accuracy of the concerned method as of ICH limits. The LOD and LOQ values of Vildagliptin were assessed to be 0.05µg/ml and 0.5 µg/ml respectively. Deliberate changes in the flow rate and mobile phase composition could not affect the working properties of the method (Table 3) illustrate the robustness of the method.



Peak Results

	Sample Name	Name	RT	Area	% Area	USP Plate Count	USP Tailing	USP Resolution
1	Sys Prec	Vildagliptin	1.560	2863460	100.00	2236	1.05	

Fig. 2. Chromatogram of Vildagliptin with optimized conditions

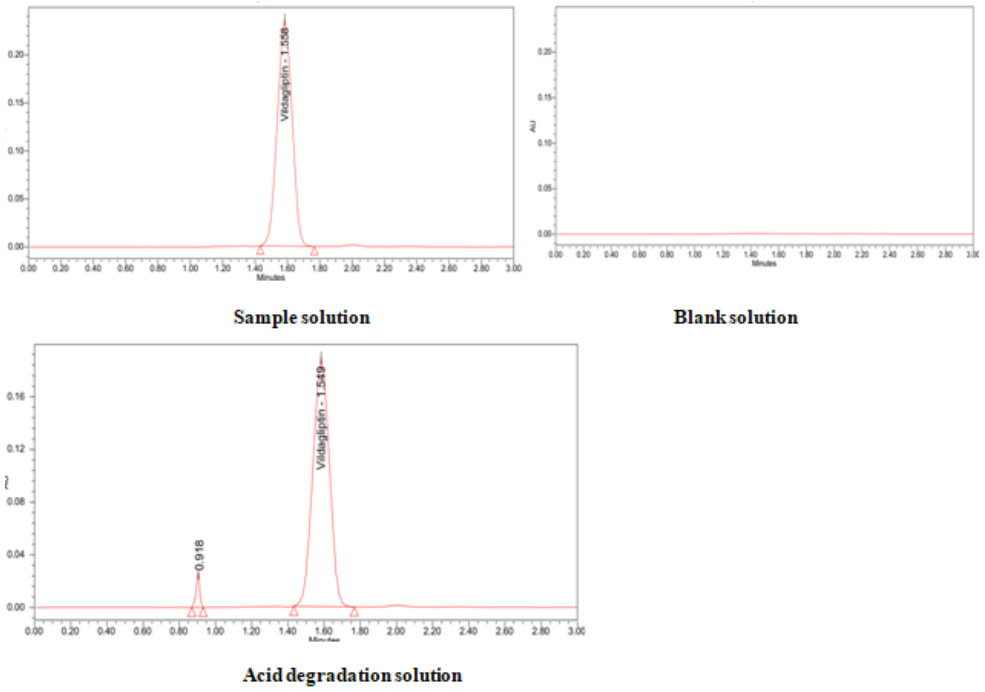


Fig. 3. Chromatograms representing the specificity of UPLC method

S.No.	Concentration µg/ml	Peak Area
1	5.00	284880
2	12.50	759113
3	25.00	1496209
4	37.50	2106347
5	50.00	2834505
6	62.50	3578517
7	75.00	4255617
Correlation coefficient -		0.9997

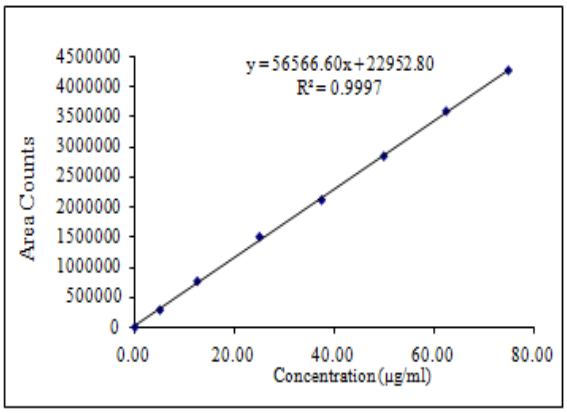


Fig. 4. Results representing the linearity of UPLC method

Table 1. Results of intra-day and inter day precision of Vildagliptin

Injection	Peak area	
	Intra-day precision	Inter day precision
1	2891923	2870847
2	2875508	2897995
3	2887820	2913999
4	2891040	2907151
5	2858217	2883482
6	2863460	2896710
Mean (n=6)	2877995	2895031
SD	14624.04	15725.96
% RSD	0.5	0.54

Table 2. Results of accuracy of Vildagliptin by % recovery method

% level	Amount added (µg/ml)	Standard solution peak area	Spiked peak area	Amount recovered (µg/ml)	% Recovery	Mean % Recovery
50	25	1435660	1435660	24.94	99.8	100.5
50	25	1446679	1446679	25.13	100.5	
50	25	1458142	1458142	25.33	101.3	
100	50	2908150	2908150	50.52	101.0	100.3
100	50	2890631	2890631	50.22	100.4	
100	50	2860249	2860249	49.69	99.4	
150	75	4256287	4256287	73.95	98.6	99.0
150	75	4293520	4293520	74.59	99.5	
150	75	4274616	4274616	74.26	99.0	

Table 3. Results of robustness by deliberate changes in flow rate and mobile phase

Peak area	Flow rate		Mobile phase (Organic phase)	
	Plus	Minus	Plus	Minus
Mean	2552409	3275929	2729862	3029897
SD	4848.78	5048.07	3134.68	9003.14
% RSD	0.19	0.154	0.115	0.297

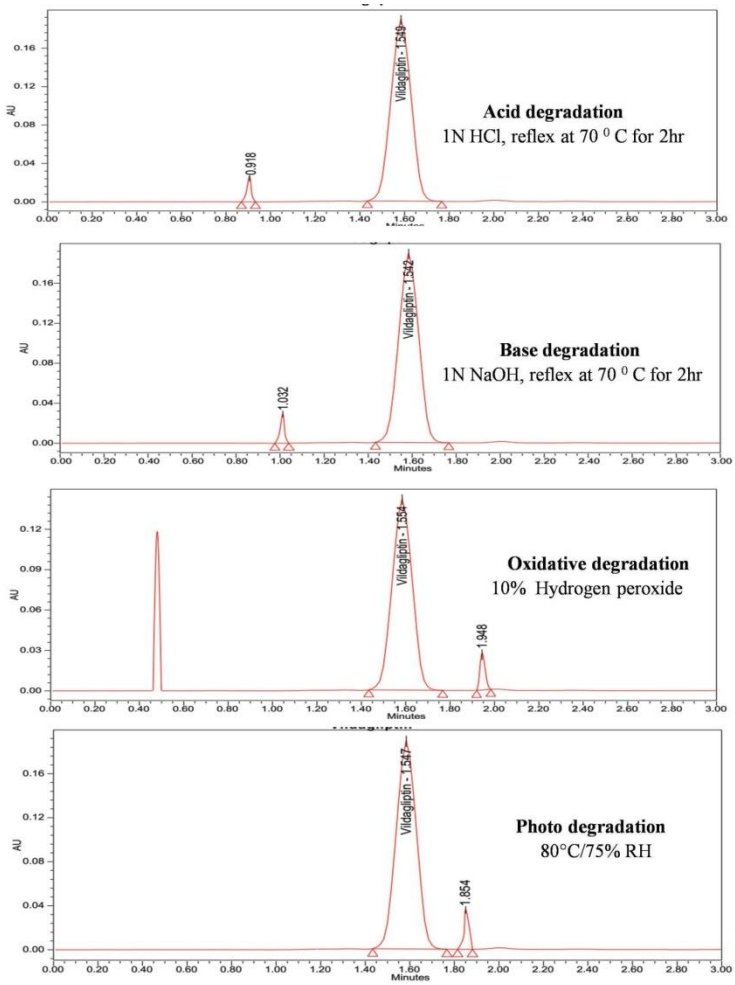


Fig. 5. Chromatograms representing the degradation of Vildagliptin at different FD conditions

Table 4. Percentage degradation of Vildagliptin at different FD conditions

Type of FD	Conditions	Time	% Degradation
Acid degradation	1N HCl, reflex at 70 0 C for 2hr	24 hrs	17.4
Base degradation	1N NaOH, reflex at 70 0 C for 2hr	24 hrs	15.9
Oxidative degradation	10% Hydrogen peroxide	24 hrs	16.6
Photo degradation	80°C/75% RH	24 hrs	19.7
Thermal degradtaion	UV cabin -254nm	24 hrs	19.1
Neutral degradation	Water (pH 7)	24 hrs	18.8

The stability indicating property of the proposed method was confirmed through the degradation of Vildagliptin and well resolution of generated degradant peaks. The forced degradation conditions with percentage degradation in each stressed conditions were mentioned in Table 4 and Fig. 5. As comparison with available LC methods, the proposed method has less retention time of 1.55min, simple mobile phase composition of 0.1% TFA and Acetonitrile with good sensitivity in terms of LOD and LOQ.

In most cases stability indicating RP-HPLC method has noteworthy role in the analysis of the drugs. Until recently, a single stability indicating RP-HPLC method with high sensitivity and lower RT was not reported for Vildagliptin. In available methods sensitivity, RT and linear concentration range was not good. Hence attempt was made to develop an efficient, responsive stability indicating RP-HPLC method. The RT in the present developed method was 1.5 min for Vildagliptin outlines the method with lower RT, can be said as economical method. The statistical outcomes of the validation parameters of the current method were in the acceptance range ICH guidelines.

4. CONCLUSIONS

Based on established experimental results the proposed RP-UPLC method was reliable, highly sensitive, and cost effective for evaluation of Vildagliptin in drug substance and tablet form. The optimized FD conditions were effectively satisfying the stability indicating property of the established UPLC method. Hence it can be implemented in the regular analysis of Vildagliptin in API and tablet form in the pharmaceutical manufacturing units.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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