Forced degradation studies of vildagliptin raw material alone and in the presence of excipients using HPLC-UV analysis.

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Received: April 4, 2020*; Accepted:* May 31, 2020

Original Article

ABSTRACT

The chemical stability of a drug formulation can change the potency and safety of the finished medicinal product. A forced degradation study can be a good method to examine the stability of a drug molecule and formulation. An RP-HPLC method was developed with a gradient elution for the optimum separation of vildagliptin and related degradants in the raw material and in the presence of several common excipients under normal and severe conditions. Under acidic and basic conditions, one major degradant at relative retention time (RRT) 1.3 and three major degradants at RRTs 0.4, 0.7 and 1.2 were observed, respectively. By oxidative degradation, five major degradants at RRTs 0.5, 0.6, 0.7, 0.8 and 1.2 were detected. Neutral hydrolysis of vildagliptin produced one major degradant at RRT 0.7 while under thermal and UV conditions no significant degradants were noticeable. The presence of a mixture of excipients together with vildagliptin decreased the percentage of vildagliptin degradation. Individual excipients, including microcrystalline cellulose, magnesium stearate, povidone, sodium starch glycolate, sucrose and lactose when mixed with vildagliptin, decreased the percent of degradation depending on their chemical nature. The presence of excipients decreased the number of degradant products, or affects the formation percentage of these degradants.

KEY WORDS: Degradation products, excipients, RP-HPLC-UV, stressed conditions,vildagliptin

INTRODUCTION

Vildagliptin (Figure 1) (2S)-1-[N-(3-hydroxyadamantan-1-yl)glycyl]-pyrrolidin-2-carbonitril, is a potent and selective dipeptidyl peptidase 4 inhibitor, which improves glycemic control (1). It increases the α- and β-cell's response to glucose thus providing more control over the glucose serum levels (2-3). Vildagliptin can be administered as a single therapy or in combination with metformin (4).

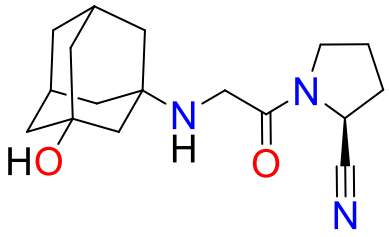

Figure 1 Chemical structure of vildagliptin.

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The chemical behavior of vildagliptin (without metformin) under various stress conditions (acidic, basic, oxidative, thermal, neutral, and photolytic) using HPLC has been published previously. Some of these degradation studies were performed under relatively mild conditions such as a low concentration of reagents, short time, and/or low temperature of reaction (5–12).

Forming stable and homogenous dosage forms requires the addition of excipients, which are pharmaceutically inactive. Some of these excipients (or their impurities or degradation products) may react with the active pharmaceutical ingredient (API) and cause chemical degradation which may result in the formation of toxic degradants and decrease the effectiveness of the drug. For example, dextrose solution added as an excipient to the ampicillin sodium formulation, catalyses the hydrolysis of the ampicillin (13). Similarly, fluoxetine undergoes a Maillard type reaction in the presence of lactose resulting in the formation of a formyl derivative of the drug. (14). Dexamethasone sodium phosphate in the presence of sodium bisulfite (used as an excipient) forms a sulfonated adduct that can affect the efficacy and safety of the finished medicinal product (15). Therefore, the chemical compatibility of the API with the excipients is necessary to assure the stability, efficacy, and chemical identity of the API (16).

Some frequently used excipients in tablet formulations are microcrystalline cellulose (MCC), magnesium stearate, sodium starch glycolate, sucrose, povidone, and lactose. Each excipient has a specific role in the final drug formulation and/or during the manufacturing process (17). The original vildagliptin product (Gulvas®) does not contain either sucrose or povidone. Sucrose was selected for the present study as it can be used in tablet formulations as a binding agent during wet granulation and in the powdered form as a dry binder or as a bulking agent (17). Povidone is used mainly in solid dosage forms as a binder in wet granulation or as a coating agent. Povidone is also used as a solubilizer in oral formulations to enhance the dissolution of poorly soluble drugs (17). Kusama *et. al.*, showed when developing a filler-binder co-propressed excipient that compressibility improved with increasing amounts of povidone. Optimal values of compressibility were obtained in a mixture of 71% MCC (Avicel® PH-200) and 29% povidone (18). Future generic vildagliptin formulations could include these excipients.

Subsuequent to previous research in establishing impurity profiles for pharmaceutical products (19–20), this study aimed at investigating both the chemical stability of vildagliptin under as yet unstudied, stressed conditions and the chemical compatibility of common excipients with the vildagliptin API when combined under normal and stressed conditions. The results could provide degradation data under the worst exaggerated conditions. Under such conditions, new degradants may be formed through the alteration of degradation pathways or mechanisms. Hence, identifying all possible degradants and their forming mechanisms may be accomplished (21). There do not appear to be any previous studies investigating the compatibility of the vildagliptin API formulated with a combination of various excipients at such exaggerated stress conditions.

MATERIALS AND METHODS

Chemicals

Chemicals and reagents used throughout this work were obtained from commercial sources and used as received. Vildagliptin raw material (98.3%) was obtained from Alembic pharmaceutical Co. (Gujarat**,** India). HPLC grade methanol, water, and acetonitrile were purchased from Romil (Cambridge, UK). The following chemicals were purchased from commercial sources and used without further purification: sodium hydroxide (Lab Chem Inc., Zelienople, PA, USA), hydrogen peroxide 30% (JHD Chemicals, India), hydrochloric acid 37% (w/w) (Carlo Erba, France), magnesium stearate (Merck, Darmstadt, Germany), microcrystalline cellulose (Avicel PH112), sodium starch glycolate, sucrose, povidone, lactose, and di-ammonium hydrogen phosphate (Sigma-Aldrich Chemicals, Taufkirchen, Germany). All chemicals were of analytical grade.

Instrumentation

HPLC analysis was performed using a Dionex Ultimate 3000, equipped with Rapid Separation (RS) UV-Vis wavelength detector (VWD-3400RS), LC pump (LPG-3400SD), auto-sampler column compartment (ACC-3000T), and windows 7-Chromeleon 7.2 software chromatography data system (Thermo Fisher Scientific, Paisley, UK). The LC separation was performed using an Athena C18-column housed in the column oven compartment. pH was measured with an 827 pH lab meter (Metrohm AG, Herisau, Switzerland). An AREX heating magnetic stirrer (VELP Scientifica, Usmate, Italy), with a VTF digital heating controller, was used for solution-state forced degradation studies, while a Gallenkamp oven (Cheshire, England, UK) was used for the solid-state thermal degradation study. A Branson Ultrasonic cleaner (8510E-MTH, Danbury, USA) was used for mobile phase degassing. UV-scans were performed using a Jenway 6700 spectrophotometer (Jenway, Staffordshire, UK).

HPLC analysis

Preparation of buffer solution of 0.02 M di-ammonium hydrogen phosphate

Approximately 2.6 g of di-ammonium hydrogen phosphate was dissolved in 1000 mL water, and the pH of the solution was adjusted to 7.50 ± 0.05 by the addition of phosphoric acid.

Preparation of the mobile phase

Solution (A)

The above buffer solution and methanol were mixed in a ratio of 90:10, respectively, then filtered through a 0.45 µm membrane filter and degassed.

Solution (B)

Methanol, degassed.

Preparation of 0.1 % phosphoric acid solution

1.2 mL phosphoric acid (85 %) was diluted to 1000.0 mL with deionized water.

Preparation of diluent (solvent)

The diluent was prepared by mixing 0.1 % phosphoric acid solution and acetonitrile in a ratio of 90:10.

Preparation of standard solution (1.0 mg/ml)

A solution of vildagliptin was prepared by dissolving 10.0 mg vildagliptin in 10.0 mL diluent.

Selection of maximum wavelength

A UV Scan (190-380) nm for vildagliptin standard solution (1.0 mg/ mL) was performed.

HPLC system and conditions

AC18 - WP, 100A°, (250 mm×4.6 mm), and 5 µm particle size column (selected based on best column performance among several columns used) was used at 40°C in a column oven and all solutions (standard and samples) were stored in the autosampler tray at 20°C. The mobile phase was kept flowing (1 ml/min) using the gradient elution program as described in Table 1; until the baseline became stable at 208 nm. The injection volume was 100 µL.

Table 1 Gradient program of the HPLC method

TIME (MIN)	SOLUTION (A) (%)	SOLUTION (B) (%)
0	80	20
5	80	20
20	50	50
23	80	20
30	80	20

Validation of the HPLC method

System suitability test

A fresh standard solution of vildagliptin in diluent was prepared (1.0 mg/ mL), then injected six times. The relative standard deviation (RSD) % of the peak area,

an average of theoretical plates, and the tailing factor for the vildagliptin peak were calculated.

Linearity test

Five different concentrations of vildagliptin in diluent were prepared to obtain the following concentrations: 0.4, 0.6, 0.8, 1.0, and 1.2 mg/ mL. Each solution was injected in duplicate.

Accuracy test

Three different concentrations of the vildagliptin solutions (0.8, 1.0 1.2 mg/ mL) were prepared in the presence of the same quantity of excipients in the diluent. Two injections were performed from each solution. The assay of each vildagliptin solution was calculated against a fresh standard solution of vildagliptin (1.0 mg/ mL).

Specificity

For degradation studies, a placebo (a mixture of excipients) was dispersed in the diluent, filtered through a 0.45 μm membrane filter and the filtrate injected into the HPLC-UV system. A vildagliptin sample (1 mg/ mL) was injected into the HPLC-UV system for comparison.

Precision

The intra-assay precision (repeatability) was obtained by injecting solutions of 1 mg/mL in triplicate on the same day and at the same experimental conditions, while inter-assay was acquired by injecting the solutions over 4 days. All values of precision were expressed in % RSD.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were determined based on the standard deviation of y-intercept; the slope of the linear calibration equation for vildagliptin. LOD was calculated as 3.3 σ/S and 10 σ/S (σ: standard deviation of the intercept, S: the slope of the calibration curve).

Forced degradation study of vildagliptin raw material

A stock solution of vildagliptin was prepared by dissolving 10.0 mg of vildagliptin in 10.0 mL diluent.

Acidic degradation

Nine milligrams of vildagliptin was dissolved in 2.0 mL of methanol then subjected to acidic degradation by the addition of 3.0 mL of 1 M HCl solution, with the mixture being stored at 80°C for 3 hours. Other experiments were performed by storing the solutions for 5 and 9 hours in an oil bath. Subsequently, the solutions were neutralized to pH 7.0 by the gradual addition of NaOH solution, followed by the addition of diluent to obtain a final concentration of 1.0 mg/ mL.

Basic degradation

Nine milligrams of vildagliptin was dissolved in 2.0 mL of methanol then subjected to basic degradation at different concentration (mmoles) of hydroxide ions, temperature, and time. The solution was neutralized to pH 7.0 by the gradual addition of HCl solution, followed by the addition of diluent to obtain a final concentration of 1.0 mg/mL.

Oxidative degradation

Five milligrams of vildagliptin were dissolved in 2.0 mL of methanol, then exposed to oxidative degradation at different concentrations (mmoles) of peroxide and times followed by the addition of diluent to obtain a final concentration 1.0 mg/mL.

UV photodegradation

Six milligrams of vildagliptin (solid-state) were exposed to UV-light (254 nm) for 8 and 24 hours, followed by the addition of 6.0 mL of diluent. Another experiment was performed by exposing the vildagliptin solution (1.0 mg/ mL) to UV-light (254 nm) for 8 and 24 hours

Neutral hydrolysis degradation

Six milligrams of vildagliptin was dissolved in 2.0

mL of methanol then subjected to neutral hydrolysis by adding 3.0 mL of water and stored at 80°C in a water bath for 1 hour and for 7 hours, followed by the addition of 1.0 mL diluent.

Solid powder degradation

Vildagliptin powder (10.0 mg) was stored at 80°C (120°C and 150°C) in an oven for different times at 1, 3, 5, and 7 hours, then dissolved in 10.0 mL diluent. A summary of the forced degradation conditions is shown in Table 2.

Table 2 Summary of the forced degradation study of vildagliptin raw material.

CONDITION	mmol OF REAGENT
Acidic- 1 M HCl at 80°C for 3 and 5 hours	3.0
Acidic- 1 M HCl at 80°C for 9 hours	3.0
Basic- 3.0 ml of 0.1 M NaOH at 80°C for 1 hour	0.3
Basic- 1.0 ml of 1 M NaOH at 70°C for 1 hour	1.0
Basic- 2.0 ml of 1 M NaOH at 70°C for 1 hour	2.0
Basic- 3.0 ml of 1 M NaOH at RT for 1minute	3.0
Basic- 3.0 ml of 5 M NaOH at RT for 30 minutes	15.0
Basic- 3.0 ml of 1 M NaOH at 80°C for 1 hour	3.0
Basic- 3.0 ml of 0.1 M NaOH at RT for 3 hours	0.3
Oxidative-3 % H_2O_2 at RT for 1 hour	2.2
Oxidative-3 % H_2O_2 at RT for 7 hours	2.2
Oxidative-30 % H_2O_2 at RT for 1 hour	21.6
Oxidative-30% H_2O_2 at RT for 3 hours	21.6
Neutral hydrolysis at 80°C for 1 hour	-
Neutral hydrolysis at 80°C for 7 hours	-
Photolysis- Under UV-light at 254 nm for 8 and 24 hours (solid)	-
Photolysis- Under UV-light at 254 nm for 8 and 24 hours (liquid)	-
Heat of solid state- in oven at 80°C for (1, 3, 5, 7) hours	-
Heat of solid state- in oven at 120°C for (1, 3, 5, 7) hours	-
Heat of solid state- in oven at 150°C for (1, 3, 5, 7) hours	-

Forced degradation studies of vildagliptin API with excipients

Three categories of forced degradation experiments were performed with the excipients as shown below.

Forced degradation of excipients mixture

Preparation of excipients mixture

A mixture of equal masses of microcrystalline cellulose, magnesium stearate, sodium starch glycolate, sucrose, povidone, and lactose was prepared. This mixture was subjected to forced degradation conditions as shown in Table 3 and as described in the procedures of forced degradation study of vildagliptin raw material.

Table 3 Forced degradation conditions applied to the vildagliptin/ excipients mixture

CONDITION	WEIGHT OF EXCIPIENTS OR VILDAGLIPTIN
Acidic- 3 ml of 1 M HCl at 80°C for 9 hours	9.0 mg
Basic- 3ml of 1 M NaOH at 80°C for 1 hour	9.0 mg
Basic- 3 ml of 0.1 M NaOH at RT for 3 hours	9.0 mg
Oxidative-3% H_2O_2 at RT for 7 hours	6.0 mg
Oxidative-30% H_2O_2 at RT for 3 hours	5.0 mg
Neutral hydrolysis at 80°C for 7 hours	6.0 mg
Photolysis under UV light 254 nm for 24 hours	5.0 mg
Heat of solid state- in oven at 150°C for 7 hours	5.0 mg

Forced degradation of excipients mixture with vildagliptin raw material

A vildagliptin powder and mixture of excipients were mixed in a 1:1 (w/w) ratio and treated with 2.0 mL of methanol. The mixture was then subjected to all forced degradation conditions (as shown in Table 3), and the diluent was added at the final step to obtain a concentration of 1.0 mg/ mL vildagliptin.

Forced degradation of individual excipient with vildagliptin raw material

Vildagliptin powder and a specific amount of each excipient in a 1:1 (w/w) ratio, as shown in Table 4, was exposed to all forced degradation conditions as described for vildagliptin standard, as shown in Table 3.

RESULTS AND DISCUSSION

Selection of maximum wavelength (λ_{max})

A UV scan of vildagliptin solution is shown in Figure 2. A λ_{max} of 208 nm was selected for analysis.

Selection of mobile phase

The current HPLC method was developed based on

Table 4 Weight of each excipient component used in the forced degradation study

EXCIPIENT NAME	EXCIPIENT WEIGHT (mg)			
	ACIDIC/BASIC	WATER/3%H_2O_2	30% H_2O_2	UV/THERMAL
Microcrystalline cellulose (Avicel® PH112)	9.0	6.0	5.0	5.0
Magnesium stearate	9.0	6.0	5.0	5.0
Sodiumstarch glycolate	9.0	6.0	5.0	5.0
Sucrose	9.0	6.0	5.0	5.0
Povidone	9.0	6.0	5.0	5.0
Lactose	9.0	6.0	5.0	5.0

preliminary work carried out in a previously published study (10). In brief, a phosphate buffer was used to prepare the mobile phase, with a pH of 1.5 units lesser than the pKa's of vildagliptin 14.71 (acid), 9.03 (basic), which converts the drug into an ionized form (22). The buffer was adjusted to pH 7.50 and then mixed with methanol in a 90:10 ratio to assure adequate solubility, and to prevent the growth of microorganisms in the mobile phase. The mobile phase was mixed with methanol (cut off absorption at 170 nm) in a gradient elution program to provide a lower column head pressure, good peak shape (asymmetry factor = 1.3), and column performance (number of theoretical plates = 13200), as shown in Figure 3.

Method validation

Precision, linearity, and accuracy were determined for the developed RP-HPLC method. The aim of method validation was to assure that the method was suitable for its intended purpose according to ICH guidelines (23). The suitability test of the system was performed by injecting six replicates of the vildagliptin standard solution. The peak area, the number of theoretical plates, and the tailing factor of each peak was determined. The RSD of the average peak area

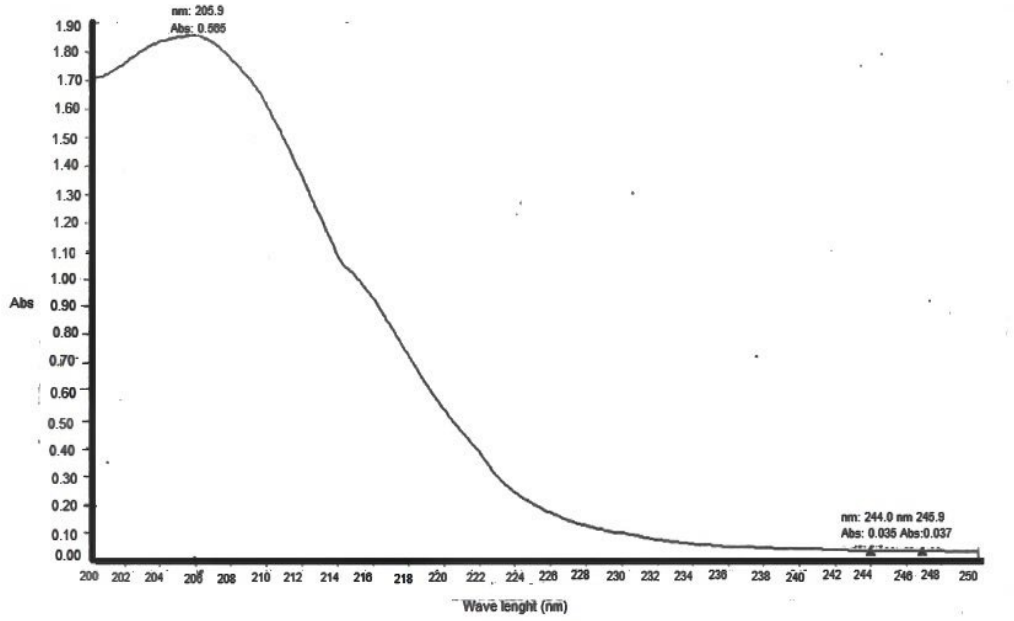

Figure 2 UV scan (190-380) nm spectrum for vildagliptin solution.

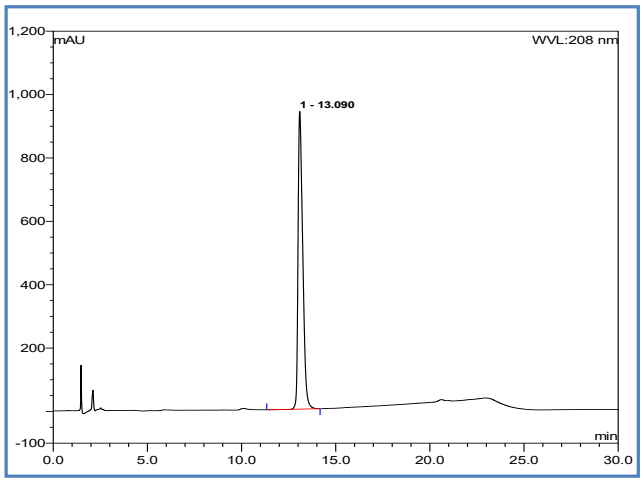

Figure 3 HPLC-UV Chromatogram of vildagliptin solution.

was 0.9%, the average theoretical plate number was > 12000, and the average tailing factor was l.3. The results were within acceptable limits (RSD of the average of peak area <1.5%, theoretical plates >1500, and tailing factor >1.5) (24).

A calibration curve of the linearity test was obtained for five solutions of vildagliptin at 40–120% of the target concentration (1.0 mg/mL) (y = 278.5x + 10.714), where x is the concentration in mg/mL and y is the absolute area under the integrated peak. The regression coefficient was >0.99.

For the accuracy test, the mean values of the assay for each level were 100.9, 99.6, and 98.3% for the concentrations of vildagliptin 0.8, 1.0, and 1.2 mg/mL, respectively. Recovery results were within an acceptable limit (98.0–102.0%) which indicated that none of the excipient components interfered with the vildagliptin peak. Additionally, no peaks were present in the placebo (excipients only) analysis at the RRT of the vildagliptin peak at the specified wavelength. Since a Photo-Diode Array (PDA) detector was not available, this was interpreted as implying a peak purity of 100%. The obtained LOD and LOQ were 0.018 and 0.066 mg/mL, respectively. All obtained values of repeatability were less than 1.0% and precision was greater than 98%.

Forced degradation studies

U.S Food and Drug Administration (FDA) regulations require analysis by HPLC whenever possible (25). After the development of stability-indicating HPLC analytical method for a given API, degradation products can be detected in the drug content after subjecting it to various stressed conditions (26). A wide array of degradation conditions were used in the present study. In some cases, these severe conditions exceeded those recommended by the ICH guidelines for stability. Their purpose was to determine if any additional or alternate degradation pathways or products resulted from exaggerated storage conditions and to verify that the developed HPLC method could resolve these (potentially) new degradants adequately for structural determination.

Forced degradation of the vildagliptin raw material

Table 5 summarizes the degradation results, and HPLC chromatograms are shown in Figures 4 through 7 and additionally in the Supplementary Material.

Forced degradation studies of vildagliptin in the presence of excipients

Vildagliptin was degraded in the presence of individual excipients or the presence of all the excipients and the degradation was followed using the developed HPLC method. A chemical incompatibility between API and excipient(s) is detrimental to drug formulation and storage (27-28).

Forced degradation study of excipients mixture

A mixture of all the excipients (without vildagliptin) was prepared and subjected to forced degradation conditions presented in Table 2. Retention times of the produced degradant peaks did not interfere with the RRT of vildagliptin at the specified measuring wavelength. Also, none of the examined excipients, in this study, eluted at the retention time of vildagliptin. The HPLC chromatograms are shown in the

Table 5 Summary of the major degradants that were obtained from the degradation of vildagliptin raw material under various conditions.

STRESSED CONDITION	DEGRADANT RRT	% AREA OF DEGRADANT	% ASSAY OF VILDAGLIPTIN AFTER DEGRADATION
1 M HCl at 80°C in 3 hours	1.1, 1.3	14.2, 1.4	82.6
1 M HCl in 80°C in 5 hours	1.1, 1.3	15.5, 2.13	84.9
1 M HCl in 80°C in 9 hours	1.3	15.7	86.2
1.0 ml of 1 M NaOH at 70°C in 1 hour	0.4, 0.7	20.5, 79.5	0
2.0 ml of 1 M NaOH at 70°C in 1 hour	0.4, 0.7	41.0, 58.9	0
3.0 ml of 0.1 M NaOH at 80°C in 1 hour	0.4, 0.7	6.2, 85.2	0
3.0 ml of 1 M NaOH at 80°C in 1 hours	0.4, 0.7	79.5, 20.3	0.2
3.0 ml of 5 M NaOH at RT for 30 minute	0.4, 0.7	2.3, 82.7	6.6
5.0 ml of 1 M NaOH at RT for 1 minute	0.7, 1.2	3.3, 65.2	30.5
3.0 ml of 0.1 M NaOH at RT in 3 hours	0.7, 1.2	3.1, 85.9	11.1
30% H_2O_2 at RT in 1 hour	0.7, 1.2	22.5, 73.4	0
3% H_2O_2 at RT in 1 hour	0.5, 0.6, 0.7, 0.8	24.6, 18.5, 21.4, 31.2	0
30% H_2O_2 at RT in 3 hours	0.7, 1.2	9.1, 88.6	0
3% H_2O_2 at RT in 7 hours	0.5	38.9	0
Hydrolysis in 80°C in 1 hour	1.2	11.9	83.9
Hydrolysis in 80C in 7 hours	0.4, 0.7, 1.1, 1.2	7.7, 22.9, 3.0, 7.4	56.9
Thermal stress	ND	NA	100.0
UV stress	ND	NA	100.0

ND = Not detected, NA = Not applicable

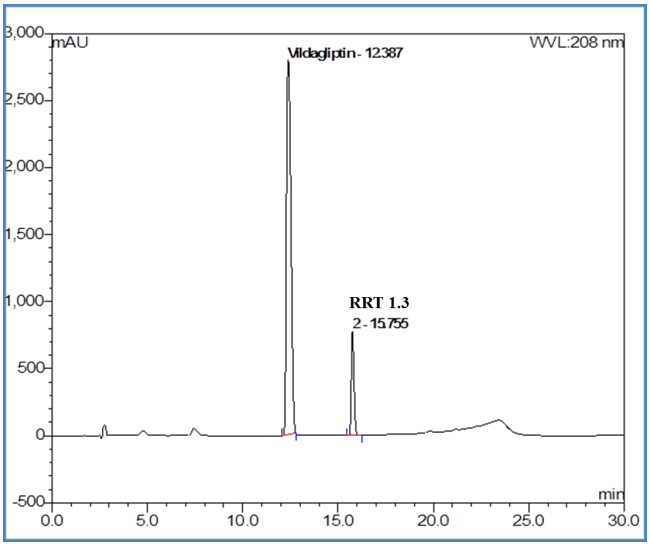

Figure 4 HPLC Chromatogram of acidic degradation of vildagliptin (1 M HCl at 80°C) for 9 hours.

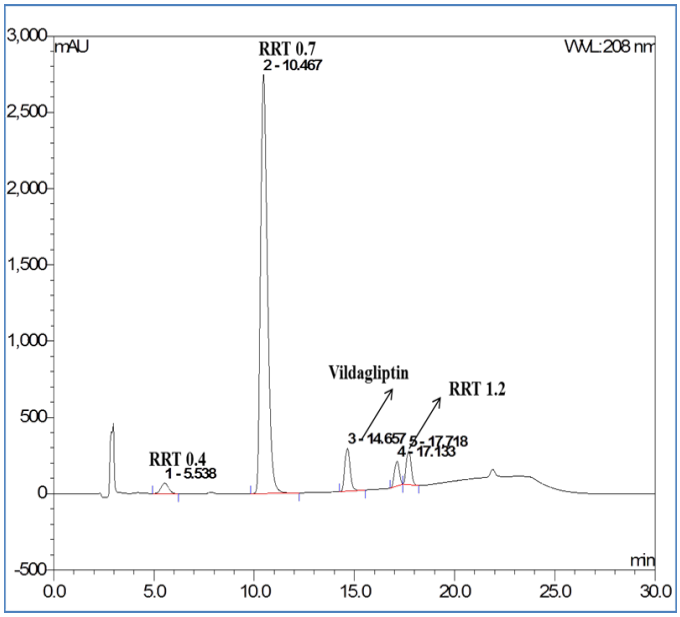

Figure 5 HPLC Chromatogram of basic degradation of vildagliptin, (3.0 ml of 5.0 M NaOH) at RT for 30 minutes.

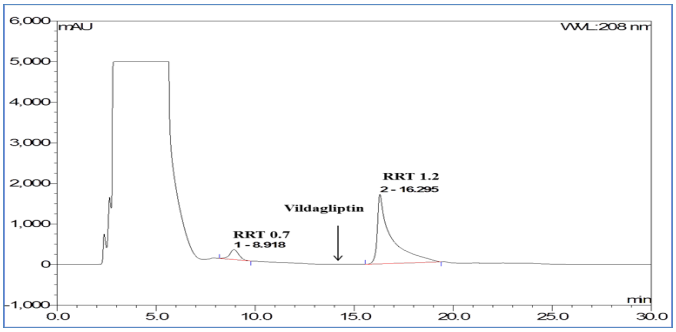

Figure 6 HPLC Chromatogram of oxidative degradation of vildagliptin, (30% H2O2 at room temperature) for 3 hours.

Supplementary Material.

Forced degradation study of vildagliptin with excipients mixture

A solution of vildagliptin and all the excipient components was subjected, separately, to selected forced degradation conditions presented in Table 2. HPLC chromatograms are shown in the Supplementary Material.

Summary results of the major degradants that were obtained from the degradation study of vildagliptin with excipients mixture under different stress conditions are listed in Table 6**.**

The RRTs of these degradation products were similar to those obtained when the API was subjected to degradation without excipients. This indicated that the presence of these excipients did not interfere with

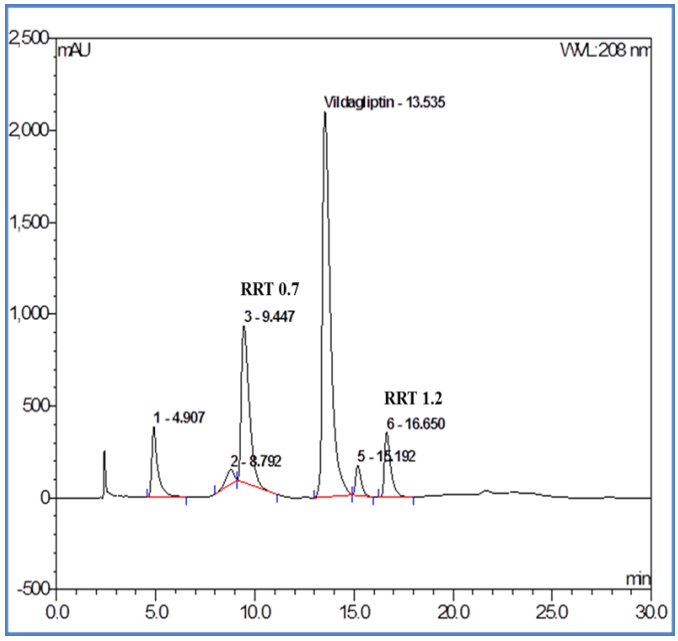

Figure 7 HPLC Chromatogram of neutral hydrolysis degradation of vildagliptin at 80°C for 7 hours

or modify the degradation pathway of the API or introduce new degradation mechanisms. However, the presence of individual excipients in the formulation did decrease the API percent degradation products.

Forced degradation study of vildagliptin with each excipient

A solution of vildagliptin with a specific amount of each excipient was subjected to selected forced degradation conditions presented in Table 2. The solution consisted of a 1:1 (w/w) ratio to increase the possibility of the species to react and to facilitate the detection of degradation products if any (29). Table 7

Table 6 Summary of the results of the major degradants obtained from the degradation of vildagliptin with the excipients mixture.

STRESSED CONDITION	DEGRADANT RRT	% AREA OF DEGRADANT	% ASSAY OF VILDAGLIPTIN
3 ml of 1.0 M HCl in 80°C in 9 hours	1.3	14.1	73.6
3 ml of 1 M NaOH at 80°C in 1 hour	0.4, 0.7	78.9, 20.9	0
3 ml of 0.1 M NaOH at RT in 3 hours	0.4, 1.2	14.6, 63.9	17.6
Thermal at 150°C in 7 hours	ND	NA	100
Hydrolysis in 80°C in 7 hours	1.2	30.4	59.1
3% H_2O_2 at RT in 7 hours	0.5, 0.6, 0.9	32.3, 34.6, 28.0	0

ND = Not detected, NA = Not applicable

shows a summary of the results of the degradation of vildagliptin under different conditions and in the presence of each excipient. Tables showing all the degradation products (Tables S1-S6**)** are listed in the Supplementary Material.

Under acidic degradation conditions, the maximum degradation of vildagliptin was observed with microcrystalline cellulose, even though no significant degradants were observed. Some relevant degradants may have maximum wavelengths that differ from that of the API and hence may not be detectable by the method.

A degradant at RRT 1.3 (vildagliptin with sodium starch glycolate) had an area percent of 4.5% which was lower than what was detected when vildagliptin raw material without the excipient was degraded under the same conditions.

Two different conditions of basic hydrolysis were used and the degradation behavior was almost similar to that of vildagliptin raw material, where two major degradants in each condition were observed at RRTs 0.4, 0.7, and 0.7, 1.2 at 80°C and room temperature, respectively. At 80°C, the maximum area of degradant (RRT 0.4) was 93.9%, and it was formed when vildagliptin was mixed with povidone, while in the presence of other excipients, the maximum area was 77.5% which was slightly lower than what was observed in the degradation of vildagliptin raw material (79.5%). It is worth mentioning that the degradant at RRT 1.2 had a maximum area percent of 86.5% when vildagliptin was mixed with povidone, which was non-significantly greater than the value obtained when vildagliptin alone was degraded (85.9%). The percent formation of a degradant with RRT of 0.7 at 80°C was 21.6% to 57.9% which was greater than what was observed when vildagliptin without excipients was degraded (20.3%). However, this increase in area percent was due to the decrease in area percent of degradant at RRT 0.4 when vildagliptin was mixed with each excipient; except for sodium starch glycolate. An opposite trend was observed at room temperature; there was a decrease in the range of percent formation (9.3-16.9%) of this degradant (RRT 0.7) and an increase in the area percent of formation of degradant at RRT 1.2. These observations are important from the viewpoint of assigning chemical structures to these degradants (out of the scope of this work) since it appears that different degradation pathways are kinetically prominent depending on the temperature.

Lactose, a reducing sugar, provided more protection to the API under the acidic stress conditions than the non-reducing sugar, sucrose. In weakly alkaline medium, however, sucrose provided a larger protective effect. Unlike lactose, sucrose is relatively unreactive under alkaline conditions because it lacks the unsubstituted hemiacetal group present in reducing disaccharides. Hence, it is more effective than lactose in protecting the API under weakly alkaline conditions. There do not seem to exist any plausible mechanisms by which lactose could provide more API protection than

Table 7 Summary of the results of % assay of Vildagliptin under different conditions in the presence of each specific excipient

	1.0 M HCl, 80°C, 9 Hrs	1.0 M NaOH, 80°C, 1 Hr	0.1 M NaOH, RT, 3 Hrs	H_2O_2, 80°C, 7 Hrs	3% ,H_2O_2,, RT, 7 Hrs	30%, H_2O_2,,RT, 3 Hrs
Vildagliptin	73.60	0.20	11.10	56.90	0.00	0.00
Excipient mixture	86.20	0.00	17.60	9.50	0.90	0.00
Microcrystalline Cellulose	85.30	0.00	17.30	62.00	0.60	0.00
Magnesium Stearate	99.28	0.00	16.50	57.90	0.70	0.00
Sodium Starch Glycolate	94.00	0.00	19.60	50.70	0.12	0.00
Sucrose	89.40	0.00	16.60	57.70	0.84	0.00
Povidone	98.58	4.80	10.60	76.40	0.60	0.00
Lactose	96.10	0.00	13.50	57.30	0.10	0.00

sucrose under acidic conditions.

In the hydrolytic stress condition, vildagliptin, in combination with each excipient produced similar degradants at RRTs 0.7 and 1.2. Also, the number of observed degradants were lesser than those found when vildagliptin raw material alone was subjected to degradation. The stabilizing effect was significantly increased when the API was combined with povidone.

Polyvinyl pyrrolidone (PVP) is used as a stabilizer in the formulation of hygroscopic drugs in the solid-state due to its ability to absorb water through osmosis. The decreased availability of reactive water increases the stability of the API - PVP solid dispersions, partly by increasing the activation energy of the degradative process (30). A similar mechanism may be operative in its solution state.

When vildagliptin was combined with all the excipients, a significantly greater degradation was observed than when the API was combined with each excipient, or when the API was subjected to degradation without any added excipient. It can be speculated that the oxidative degradation products of the excipients or their impurities may react with the API or that the physical interaction in the solution of the API with one or more excipients increases the catalytic degradative effect of other excipients, or excipient impurities/degradation products. This effect is significantly lesser in acidic media and in weakly alkaline (0.1 M NaOH) media, because oxidative reactions are thermodynamically less favorable in acidic media. The percent degradation of vildagliptin in the presence of all the excipients followed the order: acidic media < basic media (0.1 M NaOH at RT in 3 hours) < hydrolytic conditions.

The oxidative degradation of mixtures consisting of vildagliptin with each excipient proceeded essentially to completion, with >99% of API degraded at H_2O_2 concentrations 3% and 30%. The number of degradation products observed at 30% H_2O_2 concentration was greater, eluting at RRTs 0.5, 0.7, 0.8, 1.2, and 1.5 minutes with different formation amounts.

The ability to decrease vildagliptin degradation under various stressed conditions is different for each excipient. This may be attributed to the chemical nature of the excipient, or impurities in the excipient, as well as to the chemical nature of the API. DPP-4 inhibitors with a primary or secondary amino group are incompatible with several excipients such as microcrystalline cellulose, sodium starch glycolate, croscarmellose sodium, tartaric acid, citric acid, glucose, fructose, saccharose, lactose, and maltodextrins. The amino group can react with reducing sugars, with reactive carbonyl groups and with carboxylic acid groups formed by the oxidation of microcrystalline cellulose (31). For example, for Sitaglipitin, possessing a primary amino group, magnesium stearate and lactose increase the degradation of the drug in the solid-state (32), whereas, for vildagliptin, there was no significant increase in degradation (relative to API alone) at 80°C for 7 hours in water. Calcium Phosphate has been reported to significantly decrease the stability of vildagliptin (33). Therefore, the stabilizing effect of magnesium stearate in acidic media and weakly basic media at ambient temperature may be due to the stearate anion rather than the Mg^{+2} dication. Lipid monolayers have been reported to slow oxygen diffusion at the air-water interface (34) and this mechanism may be partly responsible for the decrease in oxidation kinetics under these conditions.

CONCLUSIONS

An HPLC method was developed that provided a good resolution between vildagliptin and its degradation products. Using this method, the degradation of the vildagliptin raw material alone and in the presence of excipients was determined within a wide range of stressed conditions. The purpose of subjecting the API and/or the API with excipients to these exaggerated storage conditions was to determine if any additional or alternate degradation pathways or products were formed and to verify that the developed HPLC method could resolve these (potentially) new degradants adequately for structural determination.

No new degradation products were formed (when compared to those formed under the ICH storage stability conditions) under these exaggerated storage

conditions, indicating that no new degradation pathways or mechanisms were operative under these exaggerated conditions. The stability of vildagliptin was increased in the presence of a mixture of selected excipients, especially in acidic media. Also, the results indicated that the chemical nature of the excipients (or their degradation products/impurities) exerted different effects on the degradation behavior and formation percentages of vildagliptin and its degradants, respectively. Further research is needed to elucidate the structure of the degradants and specific degradation pathway mechanisms in the presence of different excipients or excipient degradation products/impurities.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support from the Deanship of Scientific Research at the University of Jordan

CONFLICT OF INTEREST

The Authors hereby declare no conflict of interest.

SUPPLEMENTARY MATERIAL

The supplementary material can be read and downloaded from the manuscript site.

REFERENCES

1 Ito M, Abe M, Okada K, Sasaki H, Maruyama N, Tsuchida M, Higuchi T, Kikuchi F, Soma M. The dipeptidyl peptidase-4 (dpp-4) inhibitor vildagliptin improves glycemic control in type 2 diabetic patients undergoing hemodialysis. Endocr J, 58(11): 979-987, 2011.

2 Shaw RJ, Lamia KA, Vasquez D, Koo S-H, Bardeesy N, Depinho RA, Montminy M, Cantley LC. The kinase lkb1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science, 310(5754): 1642-1646, 2005.

3 Garber AJ, Sharma MD. Update: vildagliptin for the treatment of type 2 diabetes. Expert Opin Inv Drug, 17(1): 105-113, 2008.

4 Abdel-Ghany MF, Abdel-Aziz O, Ayad MF, Tadros MM. Validation of different spectrophotometric methods for determination of vildagliptin and metformin in binary mixture. Spectrochim Acta A Mol Biomol Spectrosc, 125: 175-182, 2014.

5 Malakar A, Bokshi B, Nasrin D. Development and validation of rp-hplc method for estimation of vildagliptin from tablet dosage form. Int J Pharm Life Sci, 1(1): 1-8, 2012.

6 Rao KH, Rao AL, Sekhar K. Development and validation of hplc method for the estimation of vildagliptin in pharmaceutical dosage form. Int J Pharm Chem Biol Sci, 4: 361-366, 2014.

7 Butle S, Deshpande P. Validated stability-indicating hptlc method development for determination of vildagliptin as bulk and tablet dosage form. Eur J Pharm Med Res, 2: 234-237, 2015.

8 ?ao Z, Deng Y, Chen Y, Wang A, Hu X. Synthesis of main impurity of vildagliptin. Asian J Chem, 26: 3489–3492, 2014.

9 Barden AT, Salamon B, Schapoval EE, Steppe M. Stability-indicating rp-lc method for the determination of vildagliptin and mass spectrometry detection for a main degradation product. J Chromatogr Sci, 50(5): 426-432, 2012.

10 Kumar N, Devineni SR, Singh G, Kadirappa A, Dubey SK, Kumar P. Identification, isolation and characterization of potential process-related impurity and its degradation product in vildagliptin. J Pharm Biomed Anal, 119: 114–121, 2016.

11 Boovizhikannan T, Palanirajan VK. RP-HPLC determination of vildagliptin in pure and in tablet formulation. J Pharm Res, 7(1): 1113-116, 2013.

12 Khatun R, Mirazunnabi M. A validated reversed-phase HPLC method for the determination of vildagliptin from tablet dosage form. Int J Pharm Life Sci 2(3): 90-98, 2013.

13 Ivashkiv, E., Ampicillin. Analytical Profiles of Drug Substances. Vol. 2, Elsevier, pp 1-61, 1973.

14 Wirth DD, Baerrtschi SW, Johnson RA, Maple SR, Miller MS, Hallenbeck DK, Gregg SM. Millard reaction of lactose and fluoxetine hydrochloride, a secondary amine. J Pharam SCi, 87(1): 31-39, 1988.

15 Cao X, Jia F, Taq QF. Characterization of impurity-I in dexamethasone sodium phosphate injection. Chin J Pharm Anal, 33(6): 1016-1020, 2013.

16 Wu Y, Levons J, Narang AS, Raghavan K, Rao VM. Reactive impurities in excipients: profiling, identification and mitigation of drug–excipient incompatibility. AAPS PharmSciTech, 12(4): 1248-1263, 2011.

17 Rowe, RC; Sheskey, PJ; Owen, SC., Handbook of Pharmaceutical Excipients. 5th Ed. Pharmaceutical press London, 2006.

18 Kusuma AP, Fudholi A, Nugroho AK. Optimization direct comprssion's co-pressed excipient microcrystalline cellulose pH 102 and povidone® K30. ISOR J Pharm Biol Sci, 9(2): 65-69, 2014

19 Arar S, Sweidan K, Qasem S. Identification and characterization of the products of prasugrel hydrochloride tablets using lc-ms technique. J Liq Chromatogr R T, 41(1): 14-23, 2018.

20 Sweidan K, Elayan M, Sabbah D, Idrees G, Arafat T. Study

of forced degradation behavior of amisulpride by lc-ms and nmr and development of a stability-indicating method. Curr Pharm Anal, 14(2): 157-165, 2018.

21 Blessy M, Patel RD, Prajapati PN, Agrawal Y. Development of forced degradation and stability indicating studies of drugs—a review. J pharm anal, 4(3): 159-165, 2014.

22 Yehia AM, Helmy AH, Fayek YN. Functionalized β-cyclodextrin-based potentiometric membrane for the selective determination of vildagliptin in presence of its in-process impurity. Anal Bioanal Electrochem, 10: 1414-1425, 2018.

23 ICH (2005). ICH Q2 (R1), International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Methods Text and Methodology, 2005.

24 Aksoy B, Küçükgüzel I, Rollas S. Development and validation of a stability-indicating hplc method for determination of ciprofloxacin hydrochloride and its related compounds in film-coated tablets. Chromatographia, 66(1): 57-63, 2007.

25 Prathap B, Dey A, Johnson P, Arthanariswaran P. A Review-importance of rp-hplc in analytical method development. Int J Novel Trends Pharm Sci, 3(1): 15-23, 2013.

26 Venkatesan P, Valliappan K. Impurity profiling: theory and practice. J Pharm Sci & Res, 6(7): 254-259, 2014.

27 Fathima N, Mamatha T, Qureshi HK, Anitha N, Rao JV. Drug-excipient interaction and its importance in dosage form development. J Applied Pharma Sci, 1(06): 66-71, 2011.

28 Chadha R, Bhandari S. Drug–excipient compatibility screening—role of thermoanalytical and spectroscopic techniques. J Pharmaceut Biomed Anal, 87: 82-97, 2014.

29 Ahlneck C, Zografi G. The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid state. Int J Pharm, 62(2-3): 87-95, 1990.

30 Osman YB, Liavitskaya T, Vyazovkin S, Polyvinylpyrrolidone affects thermal stability of drugs in solid dispersions, Int. J. Pharm., 551(1-2): 111-120, 2018.

31 US Patent 9415016B2, "DPP-IV inhibitor combined with a further antidiabetic agent, tablets comprising such formulations, their use and process for their preparation", assigned to Boehringer Ingelheim International GmbH.

32 Gumieniczek A, Berecka A, Mroczek T, et. al., Determination of chemical stability of sitagliptin by LC-UV, LC-MS and FT-IR methods., J. Pharm. Biomed. Analysis, 164: 789-807, 2019.

33 Patent WO 2008/144730 A2 "Stable pharmaceutical formulation for a DPP-IV inhibitor" assigned to Phenomix Corp., San Diego, CA, USA

34 Guskova RA, Fedorov GE, Belevich NP, Akhobadze VV, Ivanov II, Effect of lipid monolayers on diffusion of oxygen through the air/water interface, Biofizika, 45(4): 654-659, 2000.