Stability-Indicating RP-HPLC Method for the Determination of Vildagliptin in Tablets: Application to Degradation Kinetics

Sharifa Sultana[1], Md. Shahadat Hossain[2*], Uttom Kumar[2], Md. Samiul Islam[2] and Abu Shara Shamsur Rouf[2*]

(Received: April 27, 2025; Accepted: December 19, 2025; Published (web): June 25, 2026)

[1]Department of Pharmacy, Faculty of Health and Life Sciences, Daffodil International University,
Birulia, Savar, Dhaka-1216, Bangladesh
[2]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka,
Dhaka-1000, Bangladesh

ABSTRACT: We developed and validated a stability-indicating high performance liquid chromatography (HPLC) method for the quantitative determination of vildagliptin in tablet formulations, focusing on its degradation products. The method was developed following the ICH guidelines encompassing precision, accuracy, specificity, linearity, and robustness. Vildagliptin exhibited remarkable linearity ($R^2 = 0.999$) and highly sensitive detection and quantification limits determined at 0.05 and 0.5 μg/ml, respectively. Precision, assessed through intra-day (0.48 %) and inter-day (0.975%) analyses, demonstrated % RSD values within 2.0%, while accuracy was confirmed with average recoveries ranging from 99.54% to 101.5%. Robustness testing revealed acceptable % RSD values when varying mobile phase parameters. Then, stress studies revealed vildagliptin's susceptibility to acid, base and oxidative degradation, while maintaining stability under heat and photolytic conditions. Degradation kinetics analysis indicated pseudo-first-order kinetics for degradation in acid, base and oxidative agents. The half-life ($t_{1/2}$), calculated using the Arrhenius plot, of the drug indicates the highest stability ($t_{1/2}$, 990 h) in the heated condition and the lowest stability ($t_{1/2}$, 115.5 h) in the acidic environment. The method's application extends to studying vildagliptin stability, suggesting protective measures during storage, particularly in oxidative, acidic and basic environments, to ensure product stability and efficacy.

Key words. Vildagliptin, stability indicating method, degradation, kinetic study.

INTRODUCTION

Vildagliptin (Figure 1), a potent antidiabetic agent, controls glucose level by blocking dipeptidyl peptidase-4 (DPP-4) and preserving the activity of incretin hormones.[1] These hormones (e.g. glucagon like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP)) trigger beta cells to secret insulin and inhibit alpha cells in reducing release of glucagon within the pancreas.[2,3]

Understanding the degradation profile of vildagliptin is essential for its safe use. A trace amount of degradants can jeopardize the drug's safety, pharmacological efficacy and treatment outcomes in already weak patients. It is important to evaluate the stability to ensure the intrinsic integrity of drug and drug products.[4] Therefore, a stability-indicating analysis method is crucial to analyze both vildagliptin and its degradants. Such a method, a validated quantitative analytical procedure, monitors changes over time in relevant drug properties. These results are instrumental in designing optimized manufacturing process, determining adequate packaging and storage environments, and defining the product's usable lifespan and expiry criteria.[5] The ICH Q1A (R2) guideline underscores the importance of conducting stress testing on one batch of the drug substance under a range of environmental conditions such as temperature, humidity, oxidation, photolysis and hydrolysis at different pH levels [ICH guidelines Q1A (R2), 2005].

Correspondence to: Md. Shahadat Hossain and
Abu Shara Shamsur Rouf
Email address: shahadat@du.ac.bd, rouf321@yahoo.com
Mobile: 01760949818

Dhaka Univ. J. Pharm. Sci. **25**(1): 11-19, 2026 (June)
DOI: https://doi.org/10.3329/dujps.v25i1.91119

Despite the importance of stability studies, the analytical method for vildagliptin is not officially documented in compendia. Existing literature reports limited established analytical techniques, including UV spectrophotometry,[6] HPLC, [2,7-10] and LC-ESI-MS/MS [2,11,12] for estimation of vildagliptin alone or combination with another drug.[13] Most of these methods have high percentage of organic solvent and higher retention time. This study has focused on developing a cost-effective method by reducing the percentage of organic solvent as well as retention time.

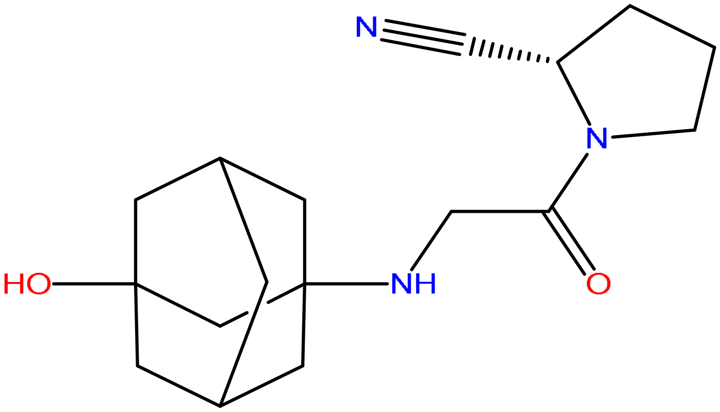

Figure 1. Structure of vildagliptin.

The author developed a precise, robust, specific, and accurate HPLC method using the Quality by Design (QbD) approach with Design of Experiments (DoE).[14] However, among these methods, no author paid attention to the presence of degradants as well as stability indicating assay methods. Thus, we aimed to develop an easy, rapid, precise and robust HPLC stability indicating method to assay vildagliptin avoiding interference from degradants, impurities, as well as excipients used in formulation of its dosage form and bulk. We also applied this method to assess degradation kinetics under ICH recommended stress conditions and to determine half-life at room temperature.

MATERIALS AND METHODS

Materials. Vildagliptin was purchased from Dr. Reddy's Laboratories (India). Potassium dihydrogen phosphate, hydrochloric acid, sodium hydroxide (Daejung Chemicals and Metal Co. Ltd., Korea), acetonitrile (RCI Labscan, Thailand), and nanopure water produced by Evoqua Water Technologies were utilized in the experimental procedures. Galvus® tablets, each containing 50 mg of vildagliptin were used as commercially available samples.

Chromatographic conditions. HPLC analysis was carried out on a Perkin Elmer Flexar HPLC equipped with binary pump, autosampler and photo-diode array plus (PDA+) detector. For the chromatographic separation, we analyzed 20 µl of samples at 239 nm using an X-bridge C_{18} column (150 × 4.6 mm i.d., 5 µm particle size) applying isocratic flow of mobile phase having phosphate buffer (pH 6.8 ± 0.05) and acetonitrile at a ratio of 70:30 at a flow rate of 1 ml/min.

Stock and standard solutions. A stock solution of vildagliptin was formulated at a strength of 1 mg/ml using the mobile phase as the solvent. From this, a 50 µg/ml working standard was obtained. Serial dilutions with the mobile phase were then performed to produce standard solutions within the strengths of 2.5 to 12.5 µg/ml.

Sample preparation. Galvus® tablets were triturated into fine particles, and a quantity equivalent to 100 mg vildagliptin was used to make a 1 mg/ml stock solution. This solution was diluted to 50 µg/ml with mobile phase and filtered via a 0.22 µm Bio disc filter before analysis.

Validation. The limit of detection (LOD) and limit of quantification (LOQ) were determined using signal-to-noise ratios of 3:1 and 10:1, respectively. Precision of the method was evaluated through both intraday and interday assessments. Intraday precision involved triplicate analysis of six test samples—including blanks, standards and vildagliptin formulations—on the same day. Method robustness was verified by applying deliberate, minor changes to key parameters such as flow rate (0.8-1.2 ml/min), mobile phase pH (6.6-7.0) and acetonitrile concentration (25-35%). Accuracy was examined through recovery studies by spiking the samples with vildagliptin at three concentration levels (100% ± 20%) and performing injections in triplicate.

Forced degradation study. Vildagliptin (1 mg/ml) was stressed out under different conditions and analyzed by the developed method. We incubated the drug under acidic and basic hydrolysis, oxidative, thermal and photolytic conditions following ICH guidelines (ICH guidelines Q1A (R1), 2005 or international conference on Harmonization, 2005).

Degradation kinetic study of vildagliptin.

Acidic hydrolysis. Acidic hydrolysis was conducted by transferring 0.5 ml of the vildagliptin (1 mg/ml) into several volumetric flasks. Each flask received 5 ml of 1M HCl and was incubated at 60, 80, and 105°C for varying durations (up to 8 hrs). After the specific duration, the solutions were neutralized with 1M NaOH and diluted to 50 µg/ml with the mobile phase prior to HPLC analysis.

Basic hydrolysis. Basic hydrolysis was studied by mixing 5 ml of NaOH (1M) to 0.5 ml of vildagliptin (1 mg/ml) in volumetric flasks. These flasks were incubated at 60, 80 and 105°C for predetermined duration (up to 8 hrs). After the treatment, neutralization of the solutions was carried out with 1M HCl. These solutions were then diluted to 50 µg/ml with the mobile phase.

Oxidation with H_2O_2. Oxidation degradation was assayed by adding 5 ml of 10% H_2O_2 to 0.5 ml of vildagliptin (1 mg/ml) into volumetric flasks. In a thermostat water bath with lid (to protect the drug from light), the flasks were heated at different temperatures (30, 40 and 60°C) for 0, 1, 4, 12, 24 and 48 hrs, separately. After specific time interval, the concentration of the solutions was adjusted to 50 µg/ml and injected into HPLC.

Thermal degradation. Thermal degradation was evaluated by incubating 0.5 ml of the drug at 25, 60, 80 and 105°C for 0, 1, 4, 12, 24, and 48 hrs. Later, the solution was adjusted to 50 µg/ml and analyzed by HPLC.

Photo degradation. Two solutions of vildagliptin (1 mg/ml) were assessed for studying light's effect on the drug. The solutions were treated with either day light or UV (254 nm) for 3 days. The strength of the solutions was then adjusted to 50 µg/ml before analyzing by HPLC.

Kinetic investigation. To evaluate the kinetics of degradation of vildagliptin under acidic, basic, oxidative, as well as thermal conditions at various temperatures, the logarithm of the percentage of drug remaining was plotted against time to construct degradation profiles. From these plots, the degradation rate constant (K) and half-life ($t_{1/2}$) were determined. The corresponding kinetic parameters were calculated from the results of triplicate experiments. An Arrhenius plot was used to extrapolate the degradation behavior of vildagliptin at 25°C based on the obtained kinetic data.

RESULTS AND DISCUSSION

Development and validation of the method. A RP-HPLC stability-indicating assay method was established through optimization of various chromatographic conditions to attain effective isolation of vildagliptin from its degradants. To optimize the method, various chromatographic columns (C_8 and C_{18}) with lengths of 150 mm and 250 mm were tested, along with various mobile phase compositions having methanol, acetonitrile, water and phosphate buffer across a pH range of 3 to 7. The flow rates of 0.5 to 2 ml/min were also assessed. Method development and validation were guided by experimental design and data analysis was executed applying Box-Behnken Design (BBD) through the Design-Expert® software (version 10.0.3.1).[14] The optimized chromatographic method employed a C_{18} column (150 mm × 4.6 mm) with a constant mobile phase composition of phosphate buffer (pH 6.8) and acetonitrile (70:30), flowing at a rate of 1 ml/min. Applying these parameters, vildagliptin was eluted at 2.465 min, producing a sharp, symmetrical peak with minimal tailing, and was detected at 239 nm (Figure 2, Table 1). This optimized method demonstrates superiority over previously reported techniques employing acetonitrile-phosphate buffer mobile phases and a photo diode array plus (PDA+) detector.[2,3,16] Additionally, the reduced retention time facilitates rapid analysis of numerous samples with lower mobile phase consumption, enhancing the method's cost-effectiveness.

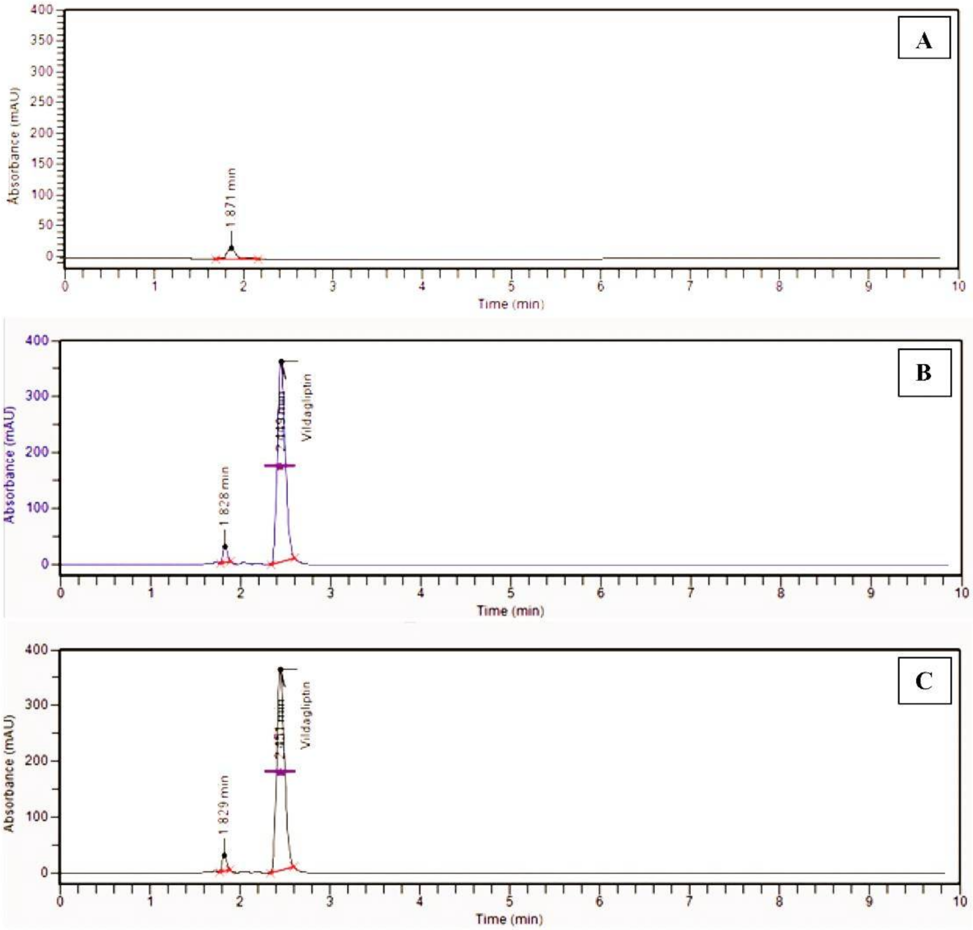

Figure 2. Obtained chromatogram of blank (A), vildagliptin standard (B) and sample (C).

Forced degradation and stability-indicating study of vildagliptin. Vildagliptin exhibited a reduction in concentration under multiple stress conditions, attributed to the formation of degradation products arising from acidic and alkaline hydrolysis, as well as oxidative stress. Figure 3 illustrates chromatograms of stressed samples displaying distinct peaks corresponding to different degradants formed under varied stress conditions. Table 2 provides a detailed breakdown of the percentage of vildagliptin degradation observed under these diverse stress conditions.

During acidic degradation, two additional retention times (R_t) were noted, leading to a 10.79% degradation after 2 hrs. Basic degradation resulted in an 8.86% degradation after 2 hrs, accompanied by three additional degradant peaks. The highest degradation percentage was observed in oxidative conditions, with a 25.58% degradation after two hours and three degradant peaks identified. Notably, the peak resolution between vildagliptin and its degradants remained above 1 in all cases. Vildagliptin exhibited stability under thermal and photolytic stress conditions, with no significant degradation observed under these specific stressors.

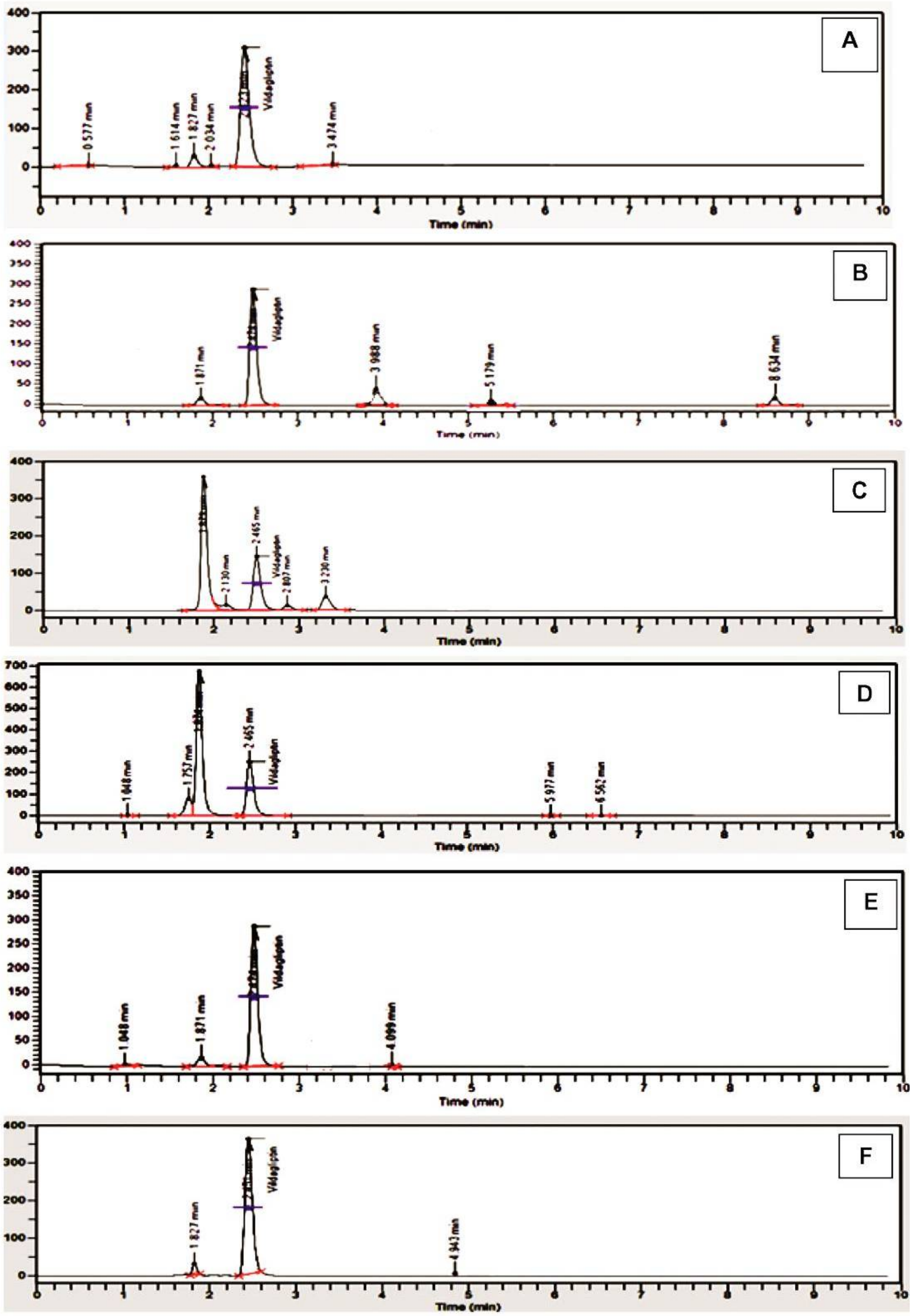

Figure 3. HPLC chromatogram of vildagliptin after acidic hydrolysis (A), basic hydrolysis (B), degradation by oxidation (C), thermal degradation (D), 254 nm UV ray (E) and daylight (F).

Table 2. Vildagliptin stability under various stress conditions.

Stress Parameters	Temp.(°C)	Time (hr)	Rt (min) of Major Degradation Peak	% Degradation (Mean ± % RSD)	α^1	α^2
Acidic hydrolysis (1 M HCl)	80	2	2.034, 3.474	10.79 ± 0.21	1.36	1.21
Basic hydrolysis (1 M NaOH)	80	2	3.988, 5.179, 8.634	8.86 ± 0.16	1.51	1.32
Oxidative (10% H_2O_2)	80	2	2.130, 2.807, 3.230	25.58 ± 0.22	2.03	1.65
Thermal	105	2	5.971, 6.562	5.58 ± 0.35	1.52	1.69
UV radiation (254nm)	RT	72	4.099	2.25 ± 0.19	2.11	1.54
Day light	RT	72	4.943	1.08 ± 0.08	2.25	1.28

$\alpha^1$is the resolution between vildagliptin and the peaks prior to it.
α^2 is the resolution between vildagliptin and the peaks after it.

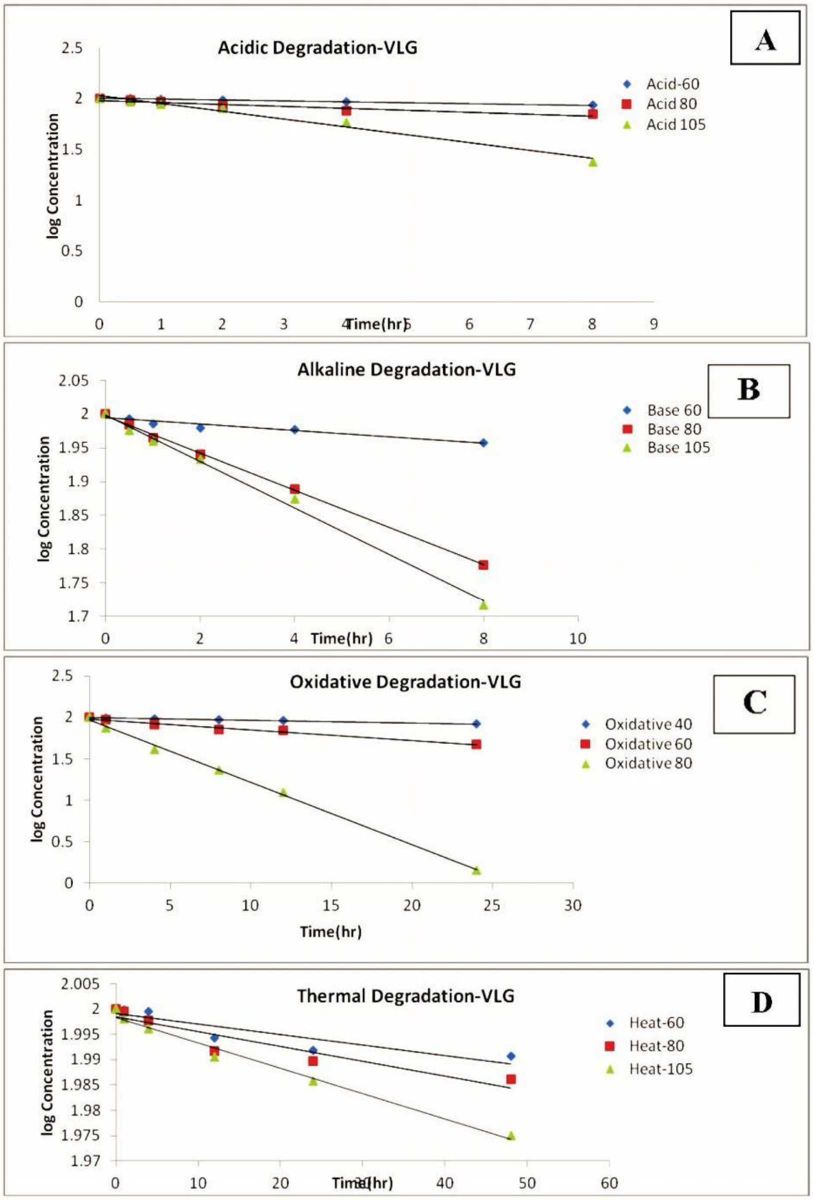

Figure 4. Kinetics studies of degradation at various temperature of acidic (A), alkaline (B), oxidative (C) and thermal (D) stress condition.

Degradation kinetic study of vildagliptin. We conducted examinations on the acidic, basic hydrolysis and thermal degradation kinetics of vildagliptin at 60, 80 and 105°C. Additionally, we studied the oxidation kinetics with H_2O_2 at 40, 60 and 80°C. The degradation kinetics data, depicting distinct decay rates, is illustrated in Figure 4.

The kinetic studies showed that the degradation follows the kinetics of pseudo-first order. Using equation (1), we calculated the rate constants and associated half-lives ($t_{1/2}$) at different temperatures. The calculation was in accordance with previous works.[15,17] A summary of our kinetic treatment is presented in table 2.

$\log C = \log C_0 - Kt/2.303$ ....(1)

The lines' slope in our calculations is represented by $-K/2.303$, where C and C_0 denote the concentrations of the drug at time 't' and initially, respectively. Here, 'K' represents the rate constant of degradation. The half-life ($t_{1/2}$) of vildagliptin at 25°C was estimated by deriving the Arrhenius plots. These plots involved plotting the logarithm of the calculated K values obtained from equation (1) versus the inverted absolute temperature (1/T).

This stability-indicating HPLC method proves to be a straightforward and accurate assay, effectively isolating and quantifying vildagliptin amidst its degradants in solid dosage form. Additionally, it allows for estimating degradation rates and the half-lives at ambient temperature. PDA plus detector played a pivotal role in ensuring peak purity and identifying vildagliptin under various degradation conditions.

Compared to previous reports, our method boasts a significantly reduced run time of 2.474 min, compared to the previously reported 10 min, thus offering a more time-efficient approach.[2,7,16] Vildagliptin displayed notable degradation during hydrolysis and oxidative processes but remained stable under heat and photolytic degradation conditions.

System suitability parameters were thoroughly evaluated to confirm alignment with USP and FDA guidelines. The resolution between vildagliptin and its degradation products exceeded 1.21 min, demonstrating efficient separation. This study also presented degradation kinetics of vildagliptin in tablet dosage form under acidic, alkaline, oxidative, and thermally forced environment. Vildagliptin exhibited a pseudo-first-order degradation kinetics behavior under acidic, alkaline, oxidative and thermal conditions, with respective half-lives ($t_{1/2}$) at 25°C of 115.5 h, 346.5 h, 385 and 990 h (Table 3). Notably, no significant degradants were observed under thermal and photolytic stress conditions.

Table 3. Temperature effect on rate constant of degradation and the half-lives at various degradation environment.

Stress	Temp(°C)	R^2	$K(h^{-1})$	$t_{1/2}(h)$
Acidic	105	0.980	0.076	9.12
	80	0.929	0.018	36.28
	60	0.996	0.008	86.625
	25*		0.006	115.5
Alkaline	105	0.994	0.034	20.38
	80	0.998	0.027	25.67
	60	0.924	0.004	173
	25*		0.002	346.5
Oxidative	80	0.998	0.074	9.37
	60	0.973	0.013	53.31
	40	0.997	0.003	231
	25*		0.0018	385
Heat	105	0.984	0.009	77
	80	0.975	0.004	173.25
	60	0.942	0.002	346.5
	25*		0.0007	990

*: Room temperature

Overall, the method provides valuable insights into the degradation pattern of vildagliptin under diverse forced environment, highlighting the impact of temperature, oxidation, acid, base and light exposure on its stability. The results indicate that vildagliptin degradation is most pronounced in presence of oxidative stress, followed by acidic and then basic hydrolysis at room temperature.

CONCLUSION

The optimized RP-HPLC stability-indicating method for vildagliptin assay has been established as simple, reproducible, accurate, robust and specific, even in presence of its degradants at various stress conditions. Validation was conducted according to ICH guidelines. The drug displayed degradation under acidic, alkaline and oxidative environment following a degradation kinetics of pseudo-first-order. The current study reported on the oxidative and hydrolytic lability of the drug, so protection against these two conditions is recommended during storage and handling. However, it remained stable under thermal and photolytic stress. The method's low retention time and detection limit underscores its high sensitivity, straightforward application and strong selectivity, making it well-suited for routine quality control of both bulk substances as well as solid dosage forms in the pharmaceutical industry. Moreover, it serves as a valuable tool in stability-indicating methods and during in vivo studies for quantifying drug substances in biological fluids.

ACKNOWLEDGEMENT

We are grateful to "Higher Education Quality Enhancement Project (HEQEP), AIF, Round-III, Window-2, CP-3245, Award No. 26, University Grants Commission (UGC), Bangladesh".

DECLARATION

There is no conflict of interest.

REFERENCES

1. El-Bagary, R.I., Elkady, E.F. and Ayoub, B.M. 2011. Liquid chromatographic methods for the determination of vildagliptin in the presence of its synthetic intermediate and the simultaneous determination of pioglitazone hydrochloride and metformin hydrochloride. *Int. J. Biomed. Sci.* **7**, 201-208.
2. Barden, A.T., Salamon, B., Schapoval, E.E.S. and Steppe, M. 2012. Stability-indicating RP-LC method for the determination of vildagliptin and mass spectrometry detection for a main degradation product. *J. Chromatogr. Sci.* **50**, 426-432.
3. Latha, K. and Ramachandran, D. 2013. Method development and validation for the simultaneous estimation of vildagliptin and metformin in tablet dosage form by RP-HPLC. *Int. J. Pharm. Pharm. Sci.* **5**, 459-463.
4. Jamrógiewicz, M. and Pieńkowska, K. 2019. Recent breakthroughs in the stability testing of pharmaceutical compounds. *TrAC, Trends Anal. Chem.* **111**, 118-127.
5. Moreno, A.H. and Salgado, H.R.N. 2012. Stability study and degradation kinetics of ceftazidime in pharmaceutical preparations. *Adv. Anal. Chem.* **2**, 1-5.
6. Naveed, S., Rehman, H., Qamar, F. and Zainab, S. 2014. Method development and validation of Vildagliptin using UV spectrophotometer. *Int. J. Pharm. Sci. Res.* **5**, 714-717.
7. Chaphekar, M. M. and Hamrapurkar, P. D. 2016. Development and validation of RP-HPLC assay method for vildagliptin using QbD approach and its application to forced degradation studies. *Int. J. Pharm. Sci. Drug Res.* **8**, 157-165.
8. Khatun, R. and Mirazzunnabi, M. 2013. A validated reversed-phase HPLC method for the determination of vildagliptin from tablet dosage form. *Int. J. Pharm. Life Sci.* **2**, 90-98.
9. Sultana, R., Bachar, S.C. and Rahman, F. 2013. Development and validation of stability indicating assay method of vildagliptin in bulk and tablet dosage form by RP-HPLC. *Int. J. Pharm. Life Sci.* **4**, 2530-2534.
10. Ramachandra, P., Vasudevan, M. and Chandan, R. 2014. Development and validation of RP-HPLC method for the estimation of vildagliptin in pharmaceutical dosage forms. *World J. Pharm. Pharm. Res.* **3**, 2125-2132.
11. He, Y., Yamaguchi, M., Ito, H., Terao, S. and Sekiguchi, K. 2010. Pharmacokinetics and pharmacodynamics of vildagliptin in Japanese patients with type 2 diabetes. *Int. J. Clin. Pharmacol. Ther.* **48**, 582-595.
12. Pharne, A., Santhakumari, B., Ghemud, A., Jain, H. and Kulkarni, M. 2012. Bioanalytical method development and validation of vildagliptin a novel dipeptidyl peptidase IV inhibitor by RP-HPLC method. *Int. J. Pharm. Pharm. Sci.* **4**, 119-123.
13. Shakoor, A., Ahmed, M., Ikram, R., Hussain, S., Tahir, A. and Jan, B.M. 2020. Stability-indicating RP-HPLC method for simultaneous determination of metformin hydrochloride and vildagliptin in tablet and biological samples. *Acta Chromatogr.* **32**, 39-43.
14. Sultana, S., Kumar, U., Hossain, M.S., Lira, D.N. and Rouf, A.S.S. 2017. QbD approach for the development and validation of RP-UHPLC method for quantitation of vildagliptin. *Dhaka Univ. J. Pharm. Sci.* **16**, 107-117.

15. Mourad, S.S., El-Kimary, E.I., Hamdy, D.A. and Barary, M.A. 2016. Stability-indicating HPLC-DAD method for the determination of linagliptin in tablet dosage form: application to degradation kinetics. *J. Chromatogr. Sci.* **54**, 1560-1566.
16. ElBagary, R.I., Azzazy, H.M., ElKady, E.F. and Farouk, F. 2016. Simultaneous determination of metformin, vildagliptin, and 3-amino-1-adamantanol in human plasma: Application to pharmacokinetic studies. *J. Liq. Chromatogr. Relat. Technol.* **39**, 195-202.
17. Gawad, D.A. and Belal, T.S. 2017. HPLC-DAD stability indicating determination of pentoxyverine citrate: Application to degradation kinetics and assay of syrup dosage form. *Arabian J. Chem.* **10,** S2908-S2918.