Stability-Indicating UHPLC Method for the Determination of Desvenlafaxine: Application to Degradation Kinetics

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ABSTRACT: This study was aimed to investigate the degradation behavior and physicochemical stability of desvenlafaxine using reversed-phase ultra-high-performance liquid chromatography (RP-UHPLC) system. The chromatogram was developed on Eclipse XDB-C8 column (150 x 4.6 mm, 5µm). The eluents were monitored through a photo diode array plus (PDA+) detector at 210 nm using an isocratic method with a flow rate of 1.5 ml/min. Mobile phase composition was 30:70 v/v mixture of 0.1 % trifluoroacetic acid (TFA) in water and methanol. Forced degradation studies were performed on drug substance of desvenlafaxine as per International Conference on Harmonization (ICH) prescribed stressed conditions (Q1A(R2) and Q1B) using hydrolytic (acidic, basic, and neutral), oxidative and photolytic methods. The drug substance was found highly labile to acidic (0.5 N hydrochloric acid, 18.65 % degradation in 2 hours at 70°C), basic (1.0 N sodium hydroxide, 11.01 % degradation in 12 hours at 70°C) and oxidative (3 % hydrogen peroxide, 17.05 % degradation in 2 hours at 50°C) stressed conditions, but a great resistance was observed towards dry heat (maximum degradation 0.27 % in 10 days from ambient to higher temperature, 80°C), moist heat (maximum degradation 0.25 % in 2 hours at 80°C and 75 % relative humidity) as well as in photolytic degradation (maximum degradation 0.23 % in 10 days at UV light of 315 - 400 nm). A pseudo-first order kinetic was followed in acidic, basic and peroxide degradation methods which paved a way to calculate the half-life of the drug substance desvenlafaxine under ICH mentioned stressed conditions. The results were also statistically analyzed and the % RSD values were compared with recommended guidelines.

Key words: Desvenlafaxine, RP-UHPLC, ICH, Force degradation, Pseudo-first order kinetic

INTRODUCTION

The ability of a drug substance or drug product to hold its physical, chemical, microbiological, toxicological, protective and informational specifications throughout the retest or expiration dating periods is defined as the stability of the drug substance or the drug product.[1] The stability of chemical molecules in pharmaceutical sector affects the safety and efficacy of drug product which is a matter of great apprehension in this revolutionary era of pharmaceutical development. Polymorphism identification as well as determination of intrinsic stability of drug molecules and drug substances are essential to predict possible reactions and degradation products in the development of a pharmaceutical formulation.[2,3] They are necessary to determine and assure the identity, potency and purity of active ingredients as well as those of the formulated products.[4] Data obtained are applied in developing a suitable manufacturing process, selecting proper packaging, storage conditions, product's shelf life and expiration dates as well as designing a product's life-cycle.[5]

Due to their possible composition, pharmaceuticals are especially sensitive to environmental factors. Precise storage conditions are necessary for the maintenance of quality as well as integrity and product activity. Additionally, some degradants of drug degradation processes are toxic

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and harmful to the patients. Thus, stability study also helps to correlate therapeutic effect with pharmacovigilence.[4]

The US FDA (Food and Drug Administration) and ICH guidance state the requirement of stability testing data to correlate the effects of environmental factors (temperature, humidity and light etc.) and quality of a product with time. So, drug stability is considered to be a secure way to ensure the delivery of therapeutic values to patients.[6]

Desvenlafaxine, the major active metabolite of venlafaxine, is an oral antidepressant of the serotonin-norepinephrine reuptake inhibitor (SNRI) class. Chemical name of desvenlafaxine is 4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl) ethyl] phenol with molecular formula $C_{20}H_{33}NO_7$ and molecular weight of 399.5 g/mol. The clinical effect of desvenlafaxine is thought to occur via inhibition of neurotransmitter reuptake in serotonin, norepine-phrine, and dopamine transporters.[7]

It inhibits serotonin transporters with 10 times the affinity of norepinephrine transporters, and dopamine transporters with the lowest affinity. This drug substance has no inhibitory effect on monoamine oxidase, and almost no affinity for muscarinic, cholinergic, H1-histaminergic, and alpha1-adrenergic receptors in vitro.[7]

There is no official current analytical monograph for powder or dosage forms of desvenlafaxine in the monograph like the British Pharmacopeia or the United States Pharmacopeia. Only few reports were found in the current literature for the quantitative determination of desvenlafaxine through UV spectrophotometry, UHPLC, HPTLC and RP-HPLC techniques.[8-13] But most of these reported methods were less suitable for routine analysis due to poor resolution, complex mobile phase (buffers) preparation, higher retention time which results in extended run time. Furthermore, there is no reported study on the degradation kinetics of this drug molecule. So, the aim of the present work is to develop an accurate, sensitive and rapid RP-UHPLC method using simple mobile phase composition for routine analysis of desvenlafaxine as well as to study its detailed stressed conditions outcomes and degradation kinetics as a part of continued research work on the development, validation and force degradation of other molecules.[14-17]

MATERIALS AND METHODS

Chemicals and reagents. Standard desvenlafaxine was provided as a generous gift from Incepta Pharmaceuticals Limited, Bangladesh. HPLC grade methanol was procured from Merck, USA. Analytical grade TFA and H_2O_2 (30 %) were procured from Panreac Qumica Sau, Spain, Spain and Scharlau, Spain respectively. NaOH pellets were obtained from Merck, India whereas, HCl (37 %) and acetone were purchased from Merck, Germany. Water (HPLC grade) was prepared from Millipore Milli-Q water purification system from Evoqua Water Technologies, USA. Other materials and reagents were of analytical grade.

Chromatographic condition. Chromatographic system used for the investigation was RP-UHPLC Perkin Elmer-flexer series (PerkinElmer Inc., USA) along with FX-15 binary pump, detector and SIL/20A auto-sampler. Chromera software was affiliated with the integrated system for data recovery. Eclipse XDB-C8 column (150 x 4.6 mm i.d., 5 µm particle size) was involved in the chromatographic separation of the eluents in an isocratic mode with a flow rate of 1.5 ml/min at detection length of 210 nm. A 20 µl sample was injected into the column with oven temperature fixed at 25ºC. The mobile phase was a homogenous mixture of 0.1 % TFA in water and methanol in the ratio of 30:70 v/v which was also used as diluting solution in this experiment. It was filtered using vacuum pump and nylon membrane filter of 0.45 µm (Restek, USA) following sonication with sonicator of Human lab Instrument Co., South Korea and degassing. Before injecting into the chromatographic system, all samples were passed through syringe filter of 0.2 µm.

Preparation of standard solutions. Accurately weighed quantity (25.0 mg) of standard desvenlafaxine by electronic balance (Shimadzu,

Japan) was transferred to a 25.0 ml volumetric flask and dissolved in 10 ml of the diluting medium (same as mobile phase composition) and sonicated for 10 minutes. The volume was made up to the mark with mobile phase to get the final concentration of 1 mg/ml. As per requirement, five solutions of concentration 80 µg/ml, 90 µg/mL 100 µg/ml, 110 µg/ml and 120 µg/ml were prepared with proper dilution with the diluting solution.

Validation of the proposed method. The developed method was validated according to current pharmaceutical regulatory guidelines ICH Q2(R1).

System suitability. To validate system suitability, the peak area, theoretical plates, tailing factor and retention time of six replicate injections of working standard of desvenlafaxine of nominal concentration (100 µg/ml) were used. Percentage relative standard deviation (% RSD) values were calculated in each case.

Linearity. For linearity, five different concentrations of standard solution of the drug substance (range 80-120 µg/ml) were analyzed and calibration curve was built. Regression line was calculated as y = mx + c, where, y, m, x and c represent the response (peak area expressed as mAU), the slope of the regression line, the concentration of desvenlafaxine in µg/ml and the intercept of the regression line respectively.

Specificity. Blank along with spiked sample analysis were performed to determine the specificity of the developed method. A blank sample consisting of mobile phase was prepared and treated in the same manner as the investigational spiked samples.

Accuracy (recovery test). Accuracy was evaluated by percent recoveries (mean ± % RSD) from six replicates of standard solution of nominal concentration (100 µg/ml) of desvenlafaxine.

Precision. To study the precision of the developed method, repeatability (intraday precision) and intermediate precision (inter-day precision) were calculated and to do so nominal concentration (100 µg/ml) of desvenlafaxine standard solution was analyzed in six replicates on the same day (intra-day precision) and daily for six times over a period of three days (inter-day precision) and % RSD were compared accordingly.

Sensitivity. Limits of detection (LOD) and limits of quantitation (LOQ) were calculated based on signal to noise ratio of chromatogram. Pump pressure was monitored and when the fluctuation was negligible, highly diluted standard solutions of desvenlafaxine were injected. LOD and LOQ were the concentrations where the peak area of the chromatogram was about 3.3 times and 10 times higher than the signal to noise ratio.

Ruggedness. Ruggedness of the method was determined by analyzing six replicates of nominal standard concentration of desvenlafaxine (100 µg/ml) by two analysts to check the reproducibility of the test results. For both cases, the percentage recovery and % RSD were calculated.

Robustness. Robustness of the method was investigated by evaluating the effect of flow rate (1.4, 1.5, 1.6 ml/min) and mobile phase composition (0.1 % TFA in water: methanol = 35:65; 30:70; 25:75 v/v). Under these conditions, % RSD were calculated from obtained peak areas of respective chromatograms of three replicates.

Force degradation analysis. 100 ml of drug solution having concentration of 100 µg/ml was subjected to stress degradation under acidic (0.5 N HCl, 5 ml, 70°C, 2 hours), alkaline (1 N NaOH, 5 mL, 70°C, 12 hours), oxidative hydrolysis (3 % H_2O_2, 5 ml, 50°C, 2 hours) and neutral hydrolytic conditions by refluxing at thermal stress (80°C, 75 % RH, 2 hours). Dry heat and photolytic stress were experienced by placing the intact drug substance powder in aluminium foil and kept it in dryer (80°C, 10 days) and photo stability chamber of ACMAS Technologies Pvt. Ltd., India (315-400 nm, 10 days), respectively. The aforementioned forced conditions i.e. strength of stress chemicals and temperature of stress conditions were chosen since, in milder ambience the drug substance illustrated trivial degradation. All force degradation samples were prepared in similar fashion as of method validation sample preparation. Stability of the standard stock

solution of drug substance was tested by storing at room temperature for 24 hours and analyzing the sample on RP-UHPLC in every 2 hours interval.

RESULTS AND DISCUSSION

Validation of analytical method. The method was validated with respect to system suitability (tailing factor 1.149 ± 0.71, theoretical plate 5703.53 ± 0.37 and retention time 2.333 ± 0.15), accuracy with a percentage recovery of 99.256 ± 0.960 % to 100.047 ± 0.036 % and intraday and inter-day precision of 100.013 ± 0.073 and 99.943 ± 0.442, respectively (Tables 1-3). While in table 4 results of robustness study in terms of intentional deviation in flow rate and mobile phase composition showed a percentage recovery of 99.837 ± 0.026 to 100.182 ± 0.025 and 100.01 ± 0.17 to 100.085 ± 0.026, respectively. Moreover, 99.915 ± 0.12 to 99.982 ± 0.068 percentage recoveries were found to back up the ruggedness study results of the developed method. Linearity in terms of correlation coefficient (R^2) was found 0.9999 over the concentration range of 80-120 µg/ml (Fig. 1). In the chromatogram of blank sample good resolution was obtained and no interference of peaks was observed (Fig. 2). The sensitivity of the method was determined through several trials with diluted solution of standard desvenlafaxine and the LOD and LOQ values were obtained at 0.8 µg/ml and 2.4 µg/ml, respectively. The method was proved to be rugged and robust since % recovery as well as % RSD were within ICH Q2(R1) prescribed conditions. The developed validated method was easier and non-cumbersome with regards to mobile phase preparation, while the other published methods used complex mobile phase prepared with potassium dihydrogen phosphate (KH_2PO_4) buffer, methanol and acetonitrile,[12] 2 % triethylamine in 0.05 M ammonium acetate and methanol.[11] It has been proved to be rapid with lower retention time (2.333 minutes) and lesser run time eventually which supports the superiority of this developed process over other methods such as Mallikarjuna's and Huma Rao's published methods in terms of retention time and run time.[12,18] The sensitivity of the method was also superior comparing with Huma Rao's reported method.[18]

Table 1. Outcomes of system suitability parameters (n=6, where n implies the replicate number of sample injection).

Parameters	Value (Mean ± % RSD)	Acceptable Limit
Peak Area	1834828.31 ± 0.27	% RSD ≤ 1
Tailing Factor	1.149 ± 0.71	≤ 2.0
Theoretical Plate	5703.53 ± 0.37	> 2000
Retention Time	2.333 ± 0.15	% RSD ≤ 0.5

Table 2. Result of accuracy study (n=3, where n implies the replicate number of sample injection).

Amount Added (µg/ml)	% Recovery (Mean ± % RSD)
80	100.004 ± 0.034
90	99.991 ± 0.047
100	99.998 ± 0.018
110	100.047 ± 0.036
120	99.256 ± 0.960

Forced degradation and stability-indicating study of desvenlafaxine. Under different stress conditions, concentration of the drug substance decreased significantly with the formation of different degradation products in case of acid (0.5 N HCl, 18.65 % degradation in 2 hours at 70°C), base (1.0 N NaOH, 11.01 % degradation in 12 hours at 70°C) and oxidative (3 % H_2O_2, 17.05 % degradation in 2 hours at 50°C) hydrolysis (Figures 3-5). However, an eminent resistance was experienced in dry heat (maximum degradation 0.27% in 10 days from ambient to higher temperature, 80°C), moist heat (maximum degradation 0.25 % in 2 hours at 80°C and 75 % relative humidity) as well as in photolytic degradation (maximum degradation 0.23 % in 10 days at UV light of 315-400 nm) (Figure 6). Table 5 shows the percentage degradation of desvenlafaxine under different stress conditions.

Table 3. Findings of precision study (n=6, where n implies the replicate number of sample injection).

Amount added (µg/ml)	Intra-day % recovery (Mean ± % RSD)	Inter-day (% Recovery ± % RSD)			Inter-day % recovery (Mean ± % RSD)
		Day 1	Day 2	Day 3	
100	100.013 ± 0.073	100.019 ± 0.073	99.937 ± 0.054	99.874 ± 0.065	99.943 ± 0.442

Table 4. Result of robustness & ruggedness study (in terms of flow rate, mobile phase composition and analyst variation).

Study Type	Parameters	Variations (ml/min)	Amount added (µg/ml)	Peak area	Retention time (min)	% Recovery (Mean ± % RSD)
Robustness Study(n=3)	Mobile phase flow rate (ml/min)	1.4	100	1831794.7	2.496	99.837 ± 0.026
		1.5	100	1835342	2.330	100.03 ± 0.052
		1.6	100	1838124.7	2.160	100.182 ± 0.025
	Mobile phase composition (% Methanol: % TFA)	68:32	100	1830395	2.434	100.01 ± 0.17
		70:30	100	1835470.3	2.331	100.03 ± 0.056
		72:28	100	1835672	2.276	100.085 ± 0.026
Ruggedness Study (n=6)	Analyst variation	Analyst 1	100	18303951	2.334	99.982 ± 0.068
		Analyst 2	100	18303951	2.335	99.915 ± 0.12

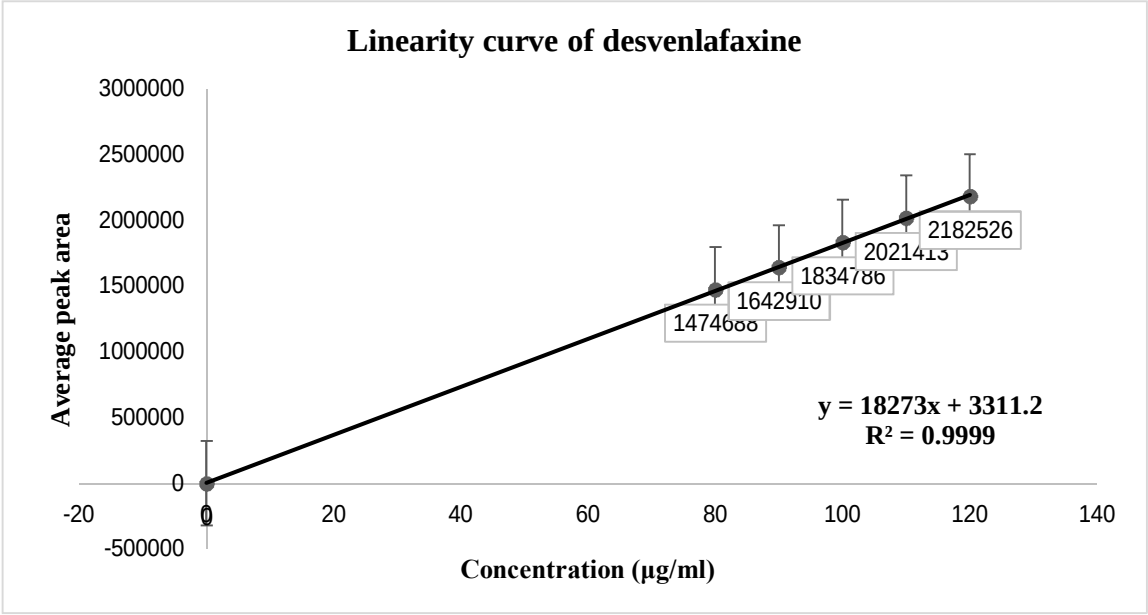

Figure 1. Linearity curve of desvenlafaxine ranging from 80 to 120 µg/ml (Each point represents the mean of 6 determinations).

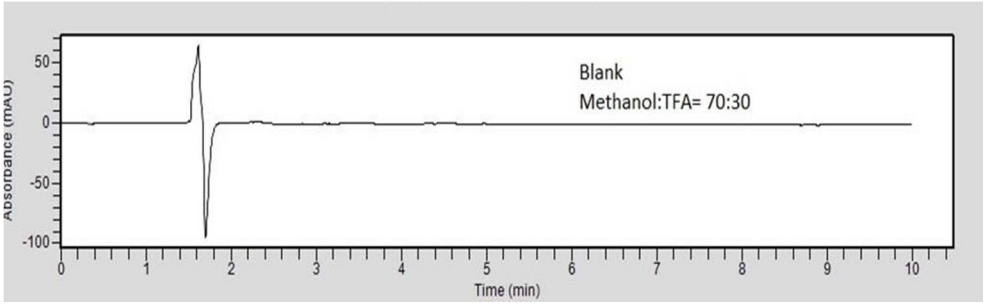

Figure 2. Chromatogram of blank sample.

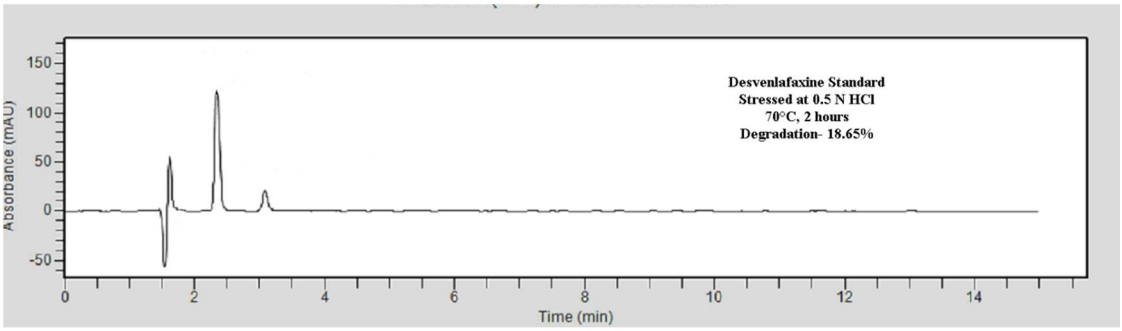

Figure 3. Chromatogram of desvenlafaxine, stressed in 0.5 N HCl at 70°C for 2 hours.

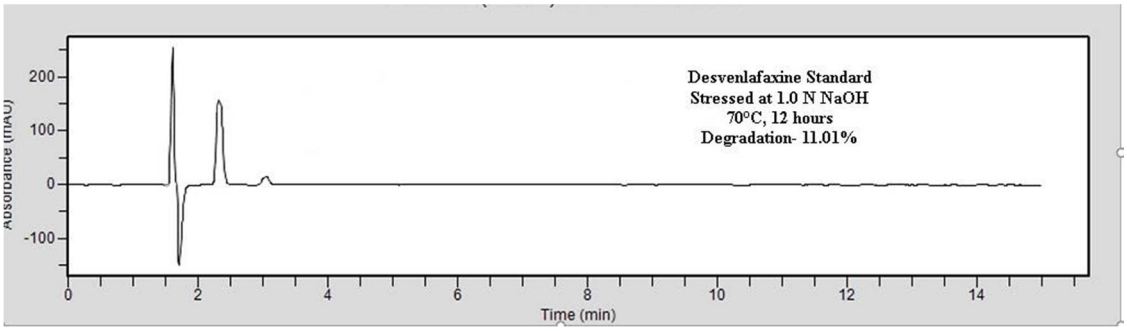

Figure 4. Chromatogram of desvenlafaxine, stressed in 1.0 N NaOH at 70°C for 12 hours.

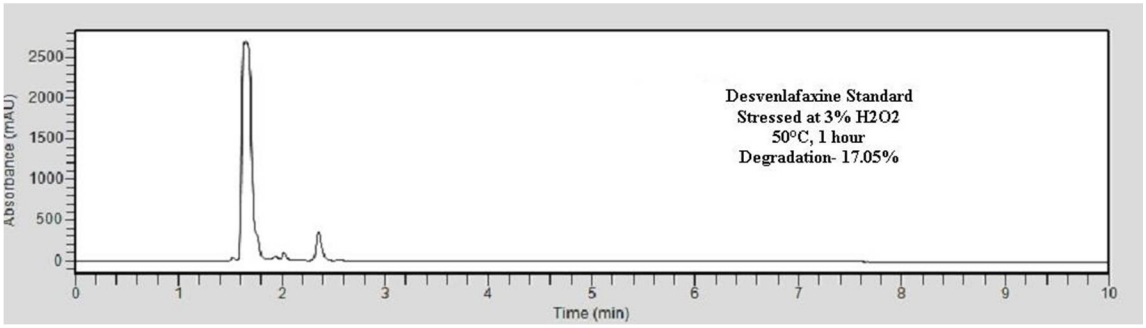

Figure 5. Chromatogram of desvenlafaxine, stressed in 3 % H_2O_2 at 50°C for 2 hours.

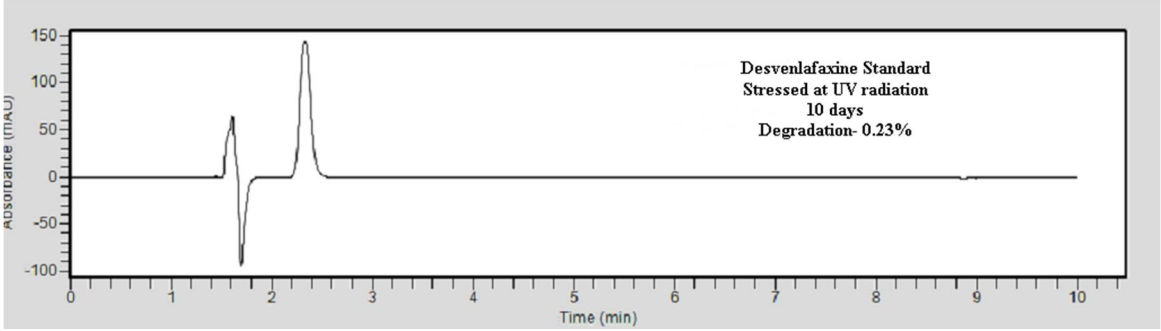

Figure 6. Chromatogram of desvenlafaxine, stressed in UV light for 10 days.

Table 5. Results of stress degradation studies of desvenlafaxine (n=6, where n implies the replicate number of sample injection).

Sl. No	Stress conditions	Time	% Recovery (Mean ± % RSD)	% Degradation (Mean ± % RSD)
1	Acid Degradation	2 hours	81.55 ± 0.33	18.45 ± 0.33
2	Base Degradation	12 hours	88.13 ± 0.21	11.87 ± 0.21
3	Peroxide Degradation	2 hours	86.11 ± 0.34	13.89 ± 0.43
4	UV Degradation	10 days	99.77 ± 0.22	0.23 ± 0.22
5	Thermal Degradation (Dry Heat)	10 days	99.73 ± 0.43	0.27 ± 0.43
6	Thermal Degradation (Moist Heat)	2 hours	99.75 ± 0.17	0.25 ± 0.17

Acidic and basic hydrolytic degradation of the molecule were investigated at room temperature, 50°C and 70°C; but at room temperature and 50°C, the rate of hydrolysis was so slow that no notable degradation was achieved. In acidic degradation, strength of HCl was increased up to 0.5 N where negligible degradation occurs at lower concentrations. Whereas, the strength of NaOH was increased up to 1.0 N to get significant amount of degradation. Thus, 0.5 N HCl was used for acid

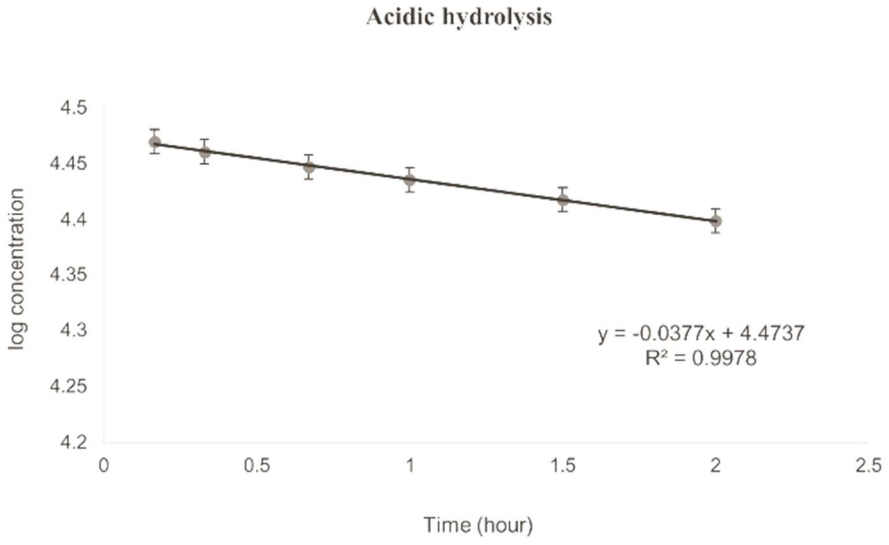

Figure 7. Desvenlafaxine degradation kinetics at 70°C under 0.5 N HCl stressed conditions.

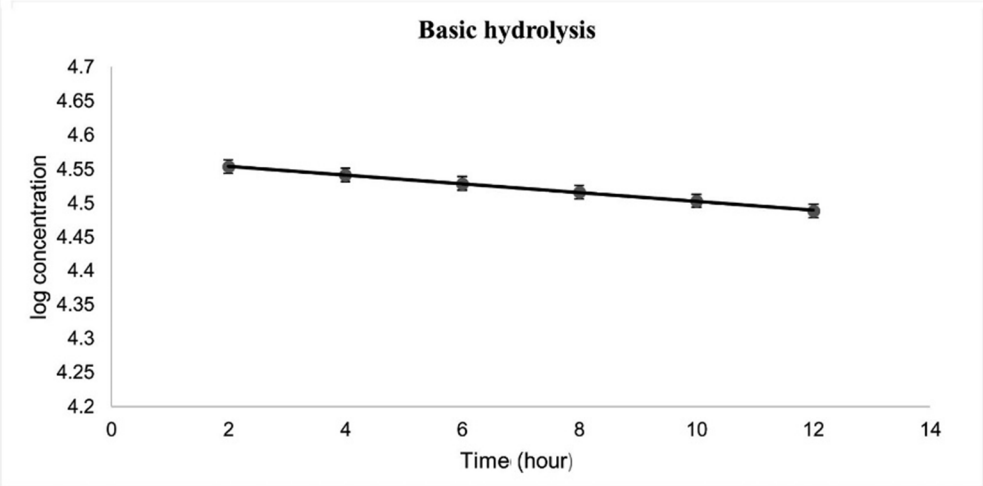

Figure 8. Desvenlafaxine degradation kinetics at 70°C under 1.0 N NaOH stressed conditions.

Table 6. Results of degradation kinetics study for acidic, basic, peroxide hydrolysis (n=6, where n implies the replicate number of sample injection).

Conditions	Time (h=hour)	Concentration (log C), µg/ml	% Degradation	Rate constant (k), h^{-1}
0.5 N HCl	0.167	87.31	12.69	0.0868
	0.33	86.52	13.48	
	0.67	85.37	14.63	
	1	84.4	15.60	
	1.5	82.89	17.11	
	2	81.35	18.65	
1.0 N NaOH	2	94.98	5.02	0.01497
	4	93.83	6.17	
	6	92.65	7.35	
	8	91.48	8.52	
	10	90.32	9.68	
	12	88.99	11.01	
3 % H_2O_2	0.137	88.37	11.63	0.0783
	0.33	87.72	12.28	
	0.67	86.61	13.39	
	1	85.52	14.48	
	1.5	84.23	15.77	
	2	82.95	17.05	

hydrolysis, whereas 1.0 N NaOH was used for the basic hydrolysis degradation kinetic study. In case of oxidation with H_2O_2, the degradation kinetics was studied at 50°C with 3 % concentration (Figure 5) showing notable decomposition rate. In all tested kinetic studies, the degradation followed pseudo-first order kinetics as log concentration of percent remaining drug substance vs time depicted a straight line (Figures 7-9).

The degradation rate constants were calculated (Table 6) from the slopes of the straight lines according to log C = log C_0 –Kt/2.303; where, the slope of the line is −K/2.303 (here, K is the degradation rate constant) and C and C_0 are the drug concentrations measured at a given time t and at zero time (in hour), respectively.[19,20] The found rate constants can be used to determine the half-lives of the drug in stressed conditions as there is no reported data found in this field.

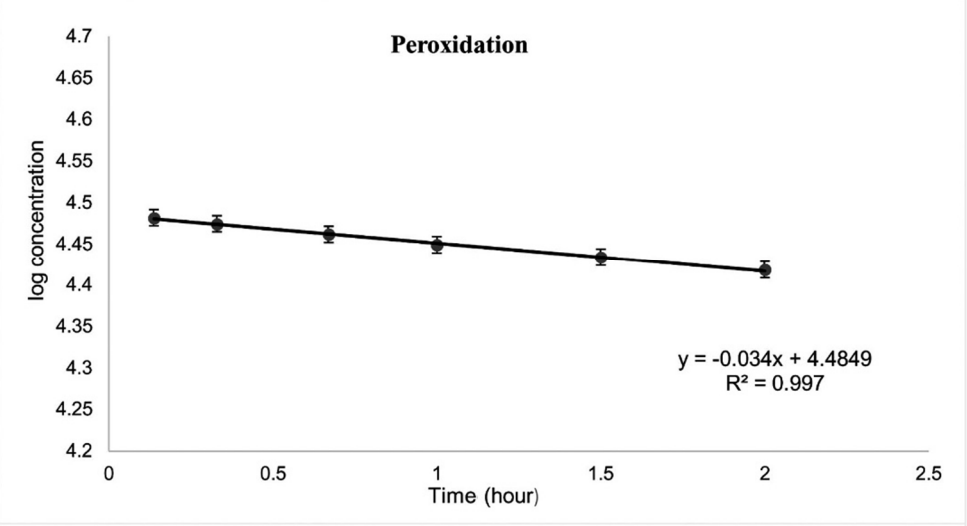

Figure 9. Desvenlafaxine degradation kinetics at 50°C under 3 % H_2O_2 stressed conditions.

CONCLUSION

The newly developed method has been proven to be simple, sensitive, rapid and versatile for the determination of desvenlafaxine in the presence of its degradation products, to calculate its degradation kinetics rates and half-lives at different stressed conditions according to ICH guideline. In this study the drug exhibited degradation under acidic (0.5 N HCl), alkaline (1.0 N NaOH) and oxidative (3 % H_2O_2) stressed conditions, following a pseudo-first-order degradation kinetics behavior. But, it was found to be stable under thermal and photolytic accelerated stressed conditions. The mobile phase composition was simple with commonly available and inexpensive reagents which render the method suitable for routine analysis as well as further research on desvenlafaxine.

AUTHORS' CONTRIBUTIONS

The idea was generated and supervised by ASSR. The laboratory work was performed by HA with active assistance of MMA. The statistical analysis was performed by MRR. HA, MMA and MRR drafted the manuscript and was finally approved by ASSR.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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