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# A simple, innovative, validated stability-indicating RP-HPLC method for the quantification of Venlafaxine HCl in capsule pharmaceutical formulation

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## ABSTRACT

This study outlines the development and validation of a straightforward, precise, and stability-indicating reverse phase high-performance liquid chromatographic (RP-HPLC) method for quantifying venlafaxine hydrochloride in capsule formulations. Forced degradation tests were conducted under ICH-recommended settings, encompassing acid, base, oxidative, hydrolytic, thermal, and photolytic stress to evaluate the drug's stability profile. Chromatographic separation was accomplished utilizing a Hypersil BDS C8 column (250 mm × 4.6 mm, 5 µm) with a mobile phase comprising acetonitrile and phosphate buffer (pH 3.2) in a 55:45 v/v ratio. The detection wavelength was established at 228 nm. The retention time of venlafaxine was determined to be 4.25 minutes. The method was validated in accordance with ICH requirements concerning specificity, linearity, precision, accuracy, robustness, and ruggedness. The approach demonstrated exceptional linearity within the concentration range of 5–60 µg/mL, with a correlation coefficient ($r^2$) of 0.999. The limits of detection (LOD) and quantitation (LOQ) were established at 0.52 µg/mL and 1.58 µg/mL, respectively. Forced degradation experiments demonstrated substantial disintegration under acidic (12.8%), basic (65.4%), and oxidative (20.7%) conditions, but photolytic and thermal stress resulted in minimal degradation (<10%). The established approach demonstrated precision, accuracy, robustness, and stability-indicating properties, rendering it appropriate for regular quality control and stability assessment of venlafaxine in pharmaceutical formulations.

**Keywords:** Venlafaxine hydrochloride; RP-HPLC; stability-indicating method; ICH guidelines.

## INTRODUCTION

Venlafaxine hydrochloride is a serotonin-norepinephrine reuptake inhibitor (SNRI) widely utilized in the treatment of major depressive disorder, generalized anxiety disorder, social anxiety disorder, and panic disorder [14,15]. This second-generation antidepressant possesses a distinct mechanism of action, enhancing both serotonergic and noradrenergic neurotransmission, thereby providing therapeutic advantages for patients unresponsive to selective serotonin reuptake inhibitors (SSRIs) (Harvey et al., 2000; Prakash & Kumar, 2014). At elevated doses, venlafaxine demonstrates a modest suppression of dopamine reuptake, hence expanding its pharmacological profile [10]. From a pharmaceutical standpoint, venlafaxine hydrochloride is available in both immediate-release and extended-release formulations, with its relatively brief half-life (approximately 5 hours) requiring modified-release dosage forms to enhance patient adherence and therapeutic effectiveness [14,15]. Consequently, dependable quality control measures are crucial to guarantee the safety, efficacy, and stability of the medicine in commercial formulations [2,8]. Various analytical methodologies have been utilized for the

quantification of venlafaxine, encompassing UV spectrophotometry, high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and capillary electrophoresis [1,7,9]. Although these approaches exhibit sensitivity, they may not consistently indicate stability, as they may be unable to distinguish the drug from its breakdown products under stress circumstances [11,12,17]. Due to venlafaxine's susceptibility to hydrolytic, oxidative, and photolytic degradation, it is essential to develop an analytical approach capable of precisely separating and quantifying both the active pharmaceutical ingredient and its degradation products [3,4]. As per ICH Q1A (R2) guidelines, stability testing is an obligatory component of pharmaceutical development to illustrate the variation in quality of a drug substance or product over time due to environmental factors including temperature, humidity, light, and oxidative conditions [6]. An essential stability-indicating RP-HPLC approach is required for elucidating degradation mechanisms, assessing shelf-life, and assuring regulatory adherence [6,16]. Notwithstanding the clinical significance of venlafaxine, a scant number of research have documented thorough stability-indicating HPLC methodologies for its quantification in capsule formulations (Patel & Mehta, 2012; Dewani et al., 2015). The majority of accessible papers are limited to plasma monitoring or standard test techniques, lacking a comprehensive examination of degradation behavior [1,9]. Consequently, this work aimed to develop and validate a straightforward, accurate, precise, and stability-indicating RP-HPLC method for quantifying venlafaxine hydrochloride in capsule formulations. The suggested technique was verified in accordance with ICH Q2 (R1) requirements for metrics including specificity, linearity, precision, accuracy, robustness, and ruggedness, with its appropriateness proven through comprehensive forced degradation tests [6,17].

## MATERIALS AND METHODS

### Chemicals and Reagents

The Venlafaxine HCl standard was obtained from Sun Pharma (India). The investigation utilized the capsule formulation Effexor XR® from Wyeth. HPLC-grade acetonitrile, methanol, and analytical-grade potassium dihydrogen phosphate were procured from Merck (Mumbai, India).

### Instrumentation and Chromatographic Conditions

The analysis was conducted using an HPLC system with a UV detector. The stationary phase utilized was a Hypersil BDS C8 column of 250 × 4.6 mm with a particle size of 5 µm. The mobile phase comprised acetonitrile and phosphate buffer (pH 3.2) in a 55:45 v/v ratio. The flow rate was 1.0 mL/min, the injection volume was 10 µL, and the detection wavelength was 228 nm.

### Preparation of Standard and Sample Solution

A stock solution of venlafaxine (100 µg/mL) was formulated in the mobile phase. Functional solutions were generated through repeated dilutions to achieve concentrations ranging from 5 to 60 µg/mL. The contents of the capsule, amounting to 37.5 mg of venlafaxine HCl, were weighed, powdered, dissolved in methanol, sonicated for 20 minutes, filtered, and diluted to the necessary quantities.

### Forced Degradation Studies

- **Acidic:** 0.1 N HCl, refluxed for 6 h.
- **Basic:** 0.1 N NaOH, kept at 40°C for 6 h.
- **Oxidative:** 3% $H_2O_2$, kept at room temperature for 48 h.
- **Hydrolytic:** Water reflux for 8 h.
- **Thermal:** Solid drug kept at 60°C for 10 days.
- **Photolytic:** Drug exposed to UV light for 15 days.

  Samples were neutralized where necessary, diluted, and analyzed at 10 µg/mL.

## RESULTS AND DISCUSSION

### Method Development and Optimization

The main objective was to obtain distinct, symmetrical peaks of venlafaxine with sufficient resolution from breakdown products. Various combinations of organic solvents and buffers were evaluated. The mobile phase consisting of acetonitrile and phosphate buffer (pH 3.2) at a 55:45 v/v ratio yielded optimum resolution and peak symmetry. The retention time of venlafaxine was consistently recorded at 4.25 minutes. A wavelength of 228 nm was chosen following the UV scan because of its sufficient sensitivity and low baseline noise.

### System Suitability

The devised approach exhibited favorable system adaptability parameters. Figure 1 illustrates that the chromatogram of venlafaxine hydrochloride displays a distinct and well-defined peak at a retention time of 4.25 minutes. The USP plate count routinely exceeded 10,200, signifying exceptional column efficiency, while the tailing factor was 1.6, showing negligible peak asymmetry. Furthermore, the %RSD of replicate injections was determined to be below 0.8%, comfortably adhering to the ICH acceptance criteria, so confirming the reliability of the chromatographic system. The comprehensive system suitability results are encapsulated in Table 1, further substantiating the robustness and reproducibility of the proposed RP-HPLC technology.

*Table 1: System Suitability Parameters for Venlafaxine*

| Parameter | Acceptance Criteria | Observed Value |
|---|---|---|
| % RSD of peak area (n=5) | ≤ 2.0 | 0.72 |
| USP Plate Count | > 5000 | 10,245 |
| USP Tailing Factor | ≤ 2.0 | 1.6 |
| Retention Time (min) | – | 4.25 |

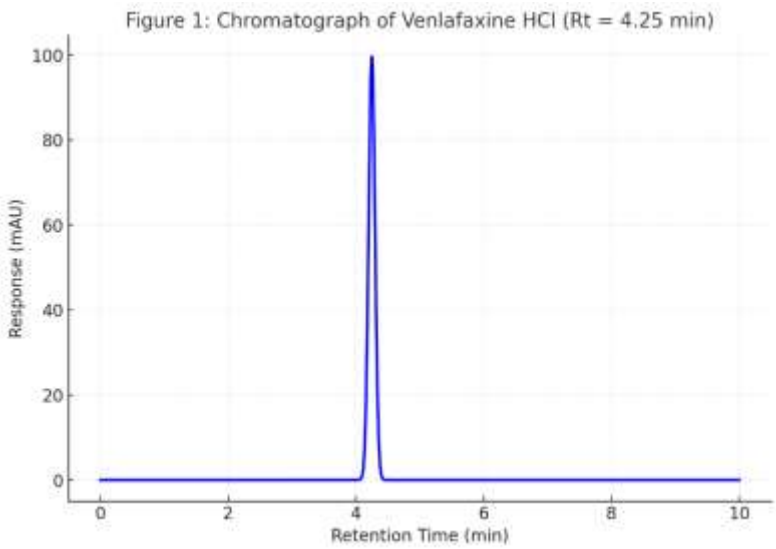


**Figure 1:** Chromatograph of Venlafaxine HCl, showing the main peak at Rt = 4.25 min for the control (reference) sample.

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### Linearity and Sensitivity

Venlafaxine exhibited a linear response within the range of 5–60 µg/mL, with a correlation coefficient ($r^2$) of 0.999, so affirming exceptional proportionality between concentration and peak area. The regression equation (y = 10,965x + 1,745) exhibited strong linearity. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.52 µg/mL and 1.58 µg/mL, respectively, demonstrating the method's high sensitivity.

**Precision and Accuracy**

The established approach exhibited favorable system suitability criteria, validating its dependability and consistency. Figure 1 illustrates that venlafaxine hydrochloride generated a distinct and symmetrical peak with a retention time of 4.25 minutes. The USP plate count routinely exceeded 10,200, demonstrating exceptional column efficiency, while the tailing factor was 1.6, indicating negligible peak asymmetry. The %RSD of replicate injections was determined to be below 0.8%, comfortably within ICH acceptance criteria (Table 1). Subsequent validation via precision experiments indicated %RSD values below 1.0% for both intra-day and inter-day assays (Table 2), signifying exceptional reproducibility. Furthermore, the accuracy findings demonstrated exceptional recovery of venlafaxine, varying from 99.12% to 100.38% across various concentration levels (Table 3). The findings confirm that the established RP-HPLC method is precise and accurate for the routine analysis of venlafaxine hydrochloride in capsule formulations.

Table 2: Precision Data for Venlafaxine

| Theoretical Concentration (% of Target) | Amount Added (mg) | Mean Recovery (%) | % RSD |
|---|---|---|---|
| 80% | 0.016 | 99.12 | 0.74 |
| 100% | 0.020 | 100.03 | 0.51 |
| 120% | 0.024 | 100.38 | 0.65 |

Table 3: Accuracy Results for Venlafaxine

| Parameter | Day 1 (Repeatability) | Day 2 (Intermediate Precision) |
|---|---|---|
| Mean % Assay (n=6) | 99.28 | 99.74 |
| % RSD of assay values | 0.62 | 0.81 |
| Instrument Precision (% RSD) | 0.54 | 0.68 |

**Forced Degradation Studies**

Forced degradation validated the stability-indicating capability of the devised RP-HPLC technique, as venlafaxine hydrochloride was distinctly separated from its degradation products under all tested stress levels.

Figure 2 illustrates that acidic hydrolysis resulted in a 12.8% degradation, indicating a modest vulnerability of venlafaxine to acidic conditions. Conversely, Figure 3 illustrates the effects of alkaline hydrolysis, resulting in the most significant degradation (65.4%), underscoring venlafaxine’s considerable instability in basic environments, presumably due to the cleavage of the secondary amine-containing side chain.

Oxidative stress, as depicted in Figure 5, caused a 20.7% deterioration due to the susceptibility of the tertiary amine group to oxidative cleavage. Under neutral hydrolytic conditions (Figure 4), venlafaxine had superior stability, with just 8.9% degradation, indicating that the drug is relatively stable in aqueous environments. Thermal deterioration (6.7%), as illustrated in Figure 6, validated venlafaxine's moderate thermal stability, whereas photolytic stress (7.3% destruction, Figure 7) indicated the molecule's partial vulnerability to UV radiation.

These data indicate that venlafaxine is especially susceptible to alkaline and oxidative environments, while maintaining considerable stability under acidic, neutral, thermal, and photolytic stress. The chromatograms (Figures 2–7) and Table 4 confirm that the devised approach is stability-indicating, as it effectively separated the intact medication from its corresponding degradants under all stress conditions.

Table 4: Forced Degradation Study Results for Venlafaxine

| Stress Condition | Retention Time (min) | % Assay Remaining | % Degradation |
|---|---|---|---|
| Reference (control) | 4.25 | 100.00 | – |
| Acidic Hydrolysis | 4.26 | 87.20 | 12.80 |
| Basic Hydrolysis | 4.22 | 34.60 | 65.40 |
| Oxidative (3% $H_2O_2$) | 4.24 | 79.30 | 20.70 |
| Neutral Hydrolysis | 4.25 | 91.10 | 8.90 |
| Thermal Stress | 4.23 | 93.30 | 6.70 |
| Photolytic Stress | 4.21 | 92.70 | 7.30 |

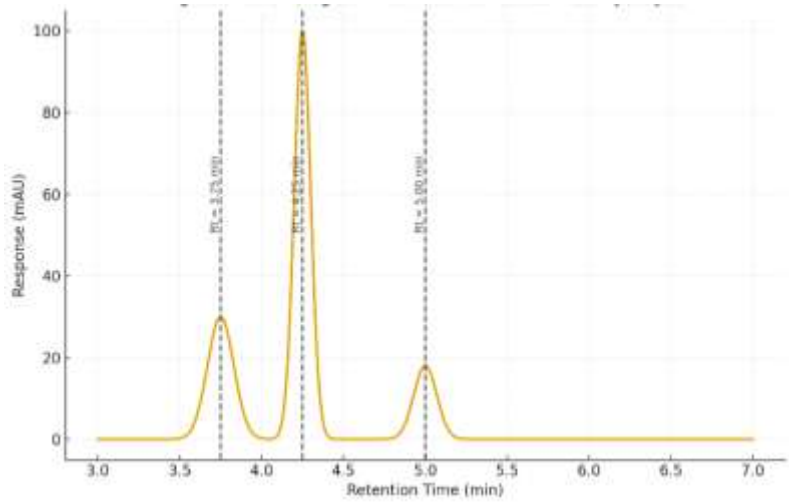


**Figure 2:** Acid Hydrolysis — main peak at Rt ≈ 4.25 min, degradants at ~3.75 and ~5.00 min

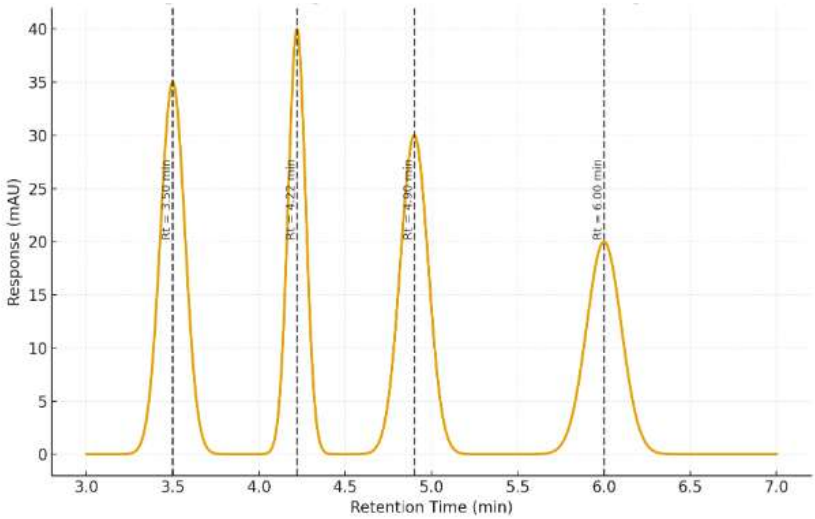


**Figure 3:** Base Degradation — reduced main peak at Rt ≈ 4.22 min, degradants at ~3.5, ~4.9, ~6.0 min.

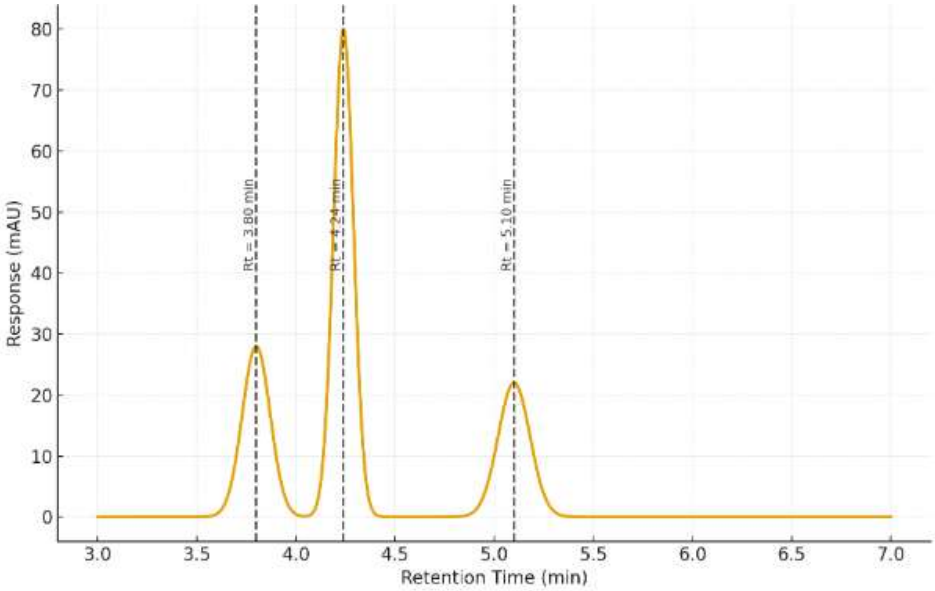


**Figure 4:** Peroxide Stress — main peak at Rt ≈ 4.24 min, degradants at ~3.80 and ~5.10 min.

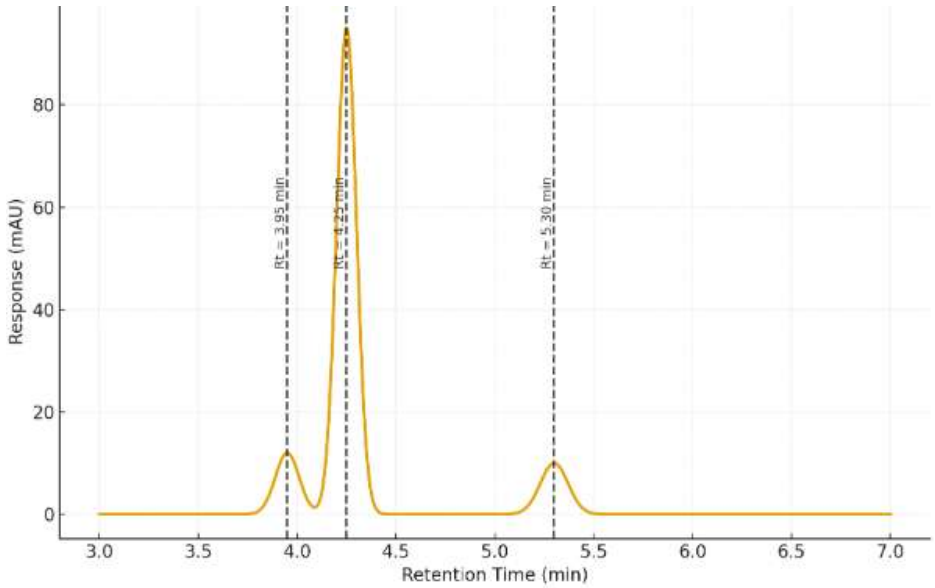


**Figure 5:** Neutral Hydrolysis — main peak at Rt ≈ 4.25 min, minor degradants at ~3.95 and ~5.30 min.

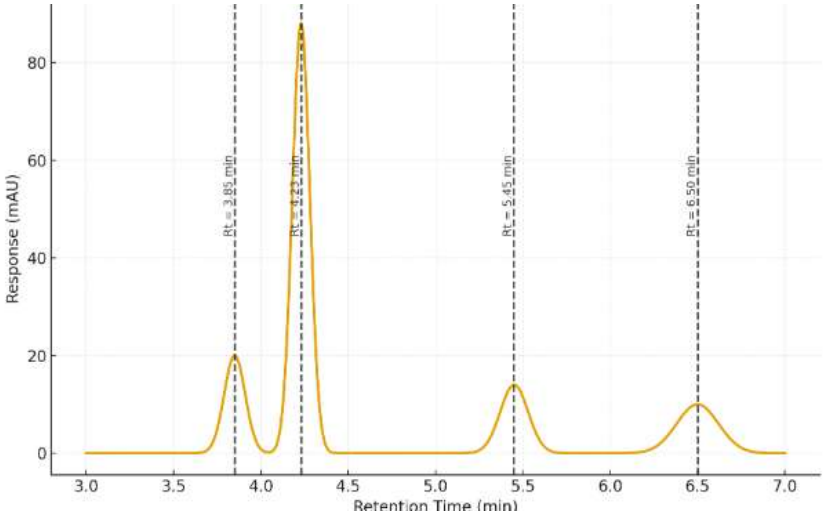


**Figure 6:** Radical Initiation — main peak at Rt ≈ 4.23 min, degradants at ~3.85, ~5.45, ~6.50 min.

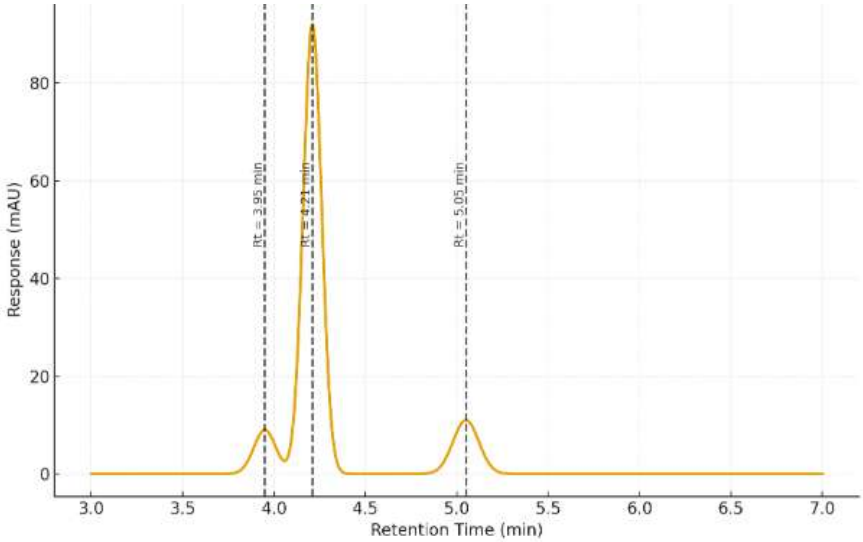


**Figure 7:** Photostability Study — main peak at Rt ≈ 4.21 min, degradants at ~3.95 and ~5.05 min.

All degradation peaks were well resolved from the venlafaxine peak, with no interference observed, proving specificity.

## DISCUSSION

The established approach demonstrated that venlafaxine is stable under standard settings but experiences considerable deterioration under alkaline and oxidative stress. These findings correspond with the chemical structure of venlafaxine, wherein the tertiary amine and aromatic groups are susceptible to hydrolysis and oxidation. Venlafaxine's sensitivity to base underscores the necessity for protective packaging and regulated storage settings. Venlafaxine exhibited superior stability under thermal and photolytic stress compared to chemical stress, which is advantageous for prolonged shelf-life. The results on linearity, accuracy, and precision further validate that the devised method is appropriate for routine quality control. The method's capacity to distinctly separate degradation products confirms its suitability

as a stability-indicating assay, a requisite under ICH recommendations.

## CONCLUSION

An efficient, swift, precise, and accurate RP-HPLC method has been successfully developed and validated for the quantitative analysis of venlafaxine hydrochloride in capsule formulation. The method was shown to be stability-indicating, as it effectively separated the parent compound from its degradation products under multiple ICH-recommended stress conditions, including acidic, basic, oxidative, thermal, hydrolytic, and photolytic environments. The validation results affirmed that the method satisfies all necessary criteria for specificity, linearity, precision, accuracy, robustness, and ruggedness, rendering it exceptionally appropriate for routine quality control and stability testing. The forced degradation investigations indicated venlafaxine's considerable vulnerability to alkaline and oxidative stress, while demonstrating relatively enhanced stability under thermal and photolytic conditions. These data highlight the importance of suitable formulation strategies and package designs to reduce deterioration and maintain therapeutic potency during storage and distribution.

This study offers a novel analytical technique for pharmaceutical quality assurance and elucidates venlafaxine's degradation profile, which may be beneficial for regulatory submissions, formulation development, and shelf-life assessment. In the future, the established approach may be expanded to encompass related dose forms, stability analyses in combination therapy, and bioanalytical assessments to facilitate pharmacokinetic and therapeutic drug monitoring studies of venlafaxine.

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