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Full length article

Integrated RP-HPLC-FTIR investigation of impurity interconversion and excipient-mediated degradation of venlafaxine tablets

Ivana Mitrevska[a,b,*], Lena Pislevska[c], Monika Stojanovska Pecova[c]

[a] Faculty of Medical Sciences, Goce Delcev University, Krste Misirkov, 10A, 2000 Stip, Republic of North Macedonia
[b] Quality Assurance, ALKALOID AD Skopje, Blvd. A. Makedonski 12, 1000 Skopje, Republic of North Macedonia
[c] Research and Development, ALKALOID AD Skopje, Blvd. A. Makedonski 12, 1000 Skopje, Republic of North Macedonia

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ABSTRACT

Background: Mechanistically informed stability assessment is essential for reliable impurity interpretation and regulatory compliance. Conventional forced degradation studies often overlook analytical-condition-induced interconversion and excipient-mediated effects.

This study aimed to establish an integrated RP-HPLC-FTIR analytical framework for forced degradation profiling and drug-excipient compatibility assessment of venlafaxine hydrochloride tablets.

Methods: Venlafaxine hydrochloride active substance and finished tablets were subjected to hydrolytic, oxidative, thermal, and photolytic stress conditions in accordance with ICH guidelines. Binary mixtures with individual excipients were evaluated under accelerated and exaggerated thermal conditions. Impurity profiling was performed using stability-indicating RP-HPLC with PDA detection, supported by peak purity and mass balance assessment. FTIR spectroscopy was applied to investigate solid-state interactions.

Results: The RP-HPLC method demonstrated adequate selectivity, peak purity, and mass balance values close to 100% for most stress conditions. Venlafaxine hydrochloride showed high stability under photolytic and accelerated storage conditions, while significant degradation occurred under oxidative and severe thermal stress (105°C). Binary mixture studies identified microcrystalline cellulose as the only excipient promoting impurity formation under exaggerated thermal conditions. FTIR results supported the presence of solid-state interactions. The apparent co-elution of pharmacopoeial-specified impurities D and E was shown to result from acid-induced interconversion of impurity E into impurity D during analysis, rather than from insufficient chromatographic resolution.

Conclusion: The integrated RP-HPLC-FTIR approach enables mechanistic differentiation between intrinsic degradation, excipient-mediated effects, and analytical-condition-induced impurity interconversion, providing a robust framework for stability evaluation.

1. Introduction

The development of stability-indicating analytical methodologies is a fundamental requirement in pharmaceutical quality control and formulation development. Such methods must reliably differentiate the active pharmaceutical ingredient (API) from related substances, degradation products, and potential excipient-derived impurities formed under stress conditions, thereby ensuring product safety, efficacy, and regulatory compliance [1–3]. Comprehensive impurity profiling and degradation pathway elucidation are particularly important during early development and throughout the product lifecycle [4]. Venlafaxine hydrochloride (Fig. 1) is a serotonin-norepinephrine reuptake inhibitor widely used in the treatment of depressive disorders and classified as a Biopharmaceutics Classification System (BCS) Class I compound due to its high solubility and permeability [5].

As a basic salt-form molecule, its physicochemical properties including pH-dependent solubility and susceptibility to hydrolytic and oxidative environments necessitate systematic stability evaluation [2,6]. Under stress conditions such as acid/base hydrolysis, oxidation, thermal exposure, and photolysis, venlafaxine hydrochloride may

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* Corresponding author at: Faculty of Medical Sciences, Goce Delcev University, Krste Misirkov, 10A, 2000 Stip, Republic of North Macedonia.
E-mail addresses: ivana.mitrevska@ugd.edu.mk (I. Mitrevska), i.mickoska@Alkaloid.com.mk (M.S. Pecova).

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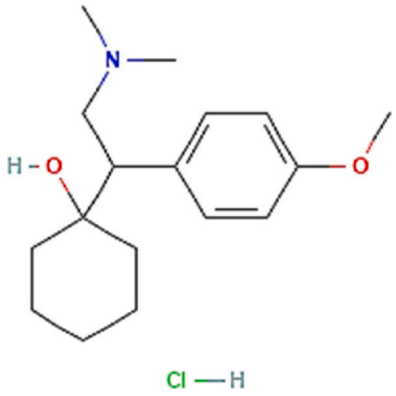

Fig. 1. Chemical structure of venlafaxine hydrochloride.

undergo structural transformations leading to the formation of related substances that may impact product quality and shelf-life [2,4]. In addition to intrinsic chemical instability, formulation-related factors, particularly solid-state drug-excipient interactions, may significantly influence degradation kinetics and impurity expression [7]. From a regulatory perspective, impurity control and stability assessment are governed by internationally harmonized guidelines [1,8,9]. The European and British Pharmacopoeias describe specified and unspecified related substances (A-H) associated with venlafaxine hydrochloride, reflecting both process-related and degradation-derived impurities [10,11]. Acceptance criteria require selective and stability-indicating analytical procedures capable of resolving structurally related compounds and stress-induced degradants [3]. Within this context, mass balance evaluation serves as a critical parameter to confirm correspondence between API loss and degradant formation [12,13]. Forced degradation studies are routinely applied to elucidate degradation mechanisms and demonstrate method specificity [2,4]. These studies expose the API or finished dosage form to hydrolytic, oxidative, thermal, and photolytic stress conditions exceeding normal storage environments [8,14]. Controlled degradation levels allow mechanistic interpretation without generating artifactual secondary products [2]. Consequently, the analytical method must ensure adequate chromatographic resolution, peak purity confirmation, and quantitative reliability. Reversed-phase high-performance liquid chromatography (RP-HPLC) with photodiode array detection remains the primary technique for quantitative assay and impurity profiling due to its robustness and selectivity [3,15]. However, chromatographic analysis alone may not sufficiently characterize solid-state drug–excipient interactions contributing to degradation under thermal stress. Fourier Transform Infrared (FTIR) spectroscopy provides complementary molecular-level insight into solid-state interactions through detection of changes in characteristic functional group vibrations [16,17]. Integration of chromatographic and spectroscopic approaches therefore enables a more mechanistically informative stability assessment strategy. Although pharmacopoeial methods describe venlafaxine-related substances [10,11], two important aspects remain insufficiently explored: (i) the potential interconversion of structurally related impurities under acidic chromatographic conditions, and (ii) excipient-mediated degradation behavior under exaggerated thermal stress. The present study therefore aimed to apply an integrated RP-HPLC and FTIR strategy to systematically investigate forced degradation pathways, excipient compatibility, and impurity interconversion phenomena in venlafaxine hydrochloride tablets. Particular emphasis was placed on chromatographic selectivity, peak purity assessment, impurity profiling, binary mixture evaluation, and mechanistic interpretation of stress-induced transformations. While forced degradation studies are routinely applied to investigate stability behavior of pharmaceutical compounds, most reports focus primarily on degradation product identification. In contrast, the present study integrates chromatographic impurity profiling with spectroscopic compatibility analysis to distinguish between intrinsic degradation, excipient-mediated instability, and impurity interconversion occurring under analytical conditions. Such combined mechanistic interpretation of impurity behavior has received limited attention in recent stability studies of pharmaceutical formulations.

2. Materials and methods

2.1. Materials

Venlafaxine hydrochloride reference standard (RS) was provided by Alkaloid AD (Skopje, North Macedonia). Commercial venlafaxine hydrochloride 37.5 mg film-coated tablets and corresponding placebo were obtained from the same manufacturer. Acetonitrile (HPLC grade), triethylamine, ammonium dihydrogen phosphate, orthophosphoric acid (85%), sodium hydroxide, hydrochloric acid (37%), and hydrogen peroxide (30%) were of analytical grade. Purified water was produced using a Milli-Q water purification system. All prepared solutions were filtered through 0.45 μm regenerated cellulose membrane filters prior to chromatographic analysis.

2.2. Forced degradation study

Forced degradation studies were conducted to evaluate the intrinsic stability of venlafaxine hydrochloride in the finished dosage form, and active pharmaceutical ingredient (API). Samples were exposed to photolytic, thermal, acidic, alkaline, and oxidative stress conditions in accordance with ICH stability guidelines and established forced degradation principles [11,14–19]. In addition to the active substance and finished product, placebo samples were subjected to all stress conditions in order to confirm that observed chromatographic peaks originated from the drug substance and not from excipient degradation. Photostability testing was performed following ICH Q1B Option 1 requirements [17]. Thermal stress studies were carried out under both dry heat and humidity-controlled conditions. Samples were exposed to 105 °C for 96 h (dry heat) and to 40 °C/75% relative humidity for 30 days, consistent with accelerated stability testing recommendations [11]. Acidic hydrolysis was performed by incorporating concentrated hydrochloric acid (37% HCl) into the sample preparation procedure. Samples were treated in an ultrasonic bath at 50 °C for approximately 30 min, cooled to room temperature, neutralized to the initial pH, diluted to volume with mobile phase, filtered, and immediately injected. Alkaline hydrolysis was conducted using 1 mol/L sodium hydroxide (NaOH), followed by neutralization to the initial pH, dilution, filtration, and injection. Oxidative degradation was induced using 30% hydrogen peroxide (H_2O_2). After ultrasonic treatment, samples were filtered and injected without neutralization. Unstressed samples were analyzed as controls to enable comparative evaluation and mass balance determination. All stress experiments were performed in duplicate.

2.3. Instrumentation

2.3.1. RP-HPLC system

Quantitative determination of venlafaxine was performed using an in-house reverse-phase HPLC method developed in accordance with general chromatographic and stability-indicating method development principles [14,16]. Analyses were carried out on Thermo Dionex Ultimate 3000 UHPLC and Agilent 1260 Infinity systems equipped with photodiode array (PDA/DAD) detectors. Chromatographic separation for assay determination was achieved on a C18 column (250 × 4.6 mm, 5 μm) maintained at 25 °C. The mobile phase consisted of 30% (v/v) acetonitrile containing 0.1% triethylamine, adjusted to pH 3.5 with orthophosphoric acid. The flow rate was 1.2 mL/min, injection volume 5 μL, and detection wavelength 226 nm. System suitability was evaluated by six replicate injections of the standard solution, with a requirement of RSD ≤ 2.0% for peak areas, in line with validation

principles for stability-indicating methods [14,16]. For assay analysis, twenty tablets were weighed and finely powdered. An amount equivalent to 37.5 mg venlafaxine was accurately transferred to a 100 mL volumetric flask, extracted with mobile phase under sonication for 15 min, diluted to volume, filtered, and injected. The final concentration of the assay solution was 0.4 mg/mL. Standard solutions were prepared from accurately weighed venlafaxine hydrochloride reference standard under identical solvent conditions. Determination of related and degradation products was performed using a method based on the European Pharmacopoeia monograph for venlafaxine hydrochloride with minor in-house modifications [5]. Separation was carried out on a LiChrospher 100 RP-8 column (250 × 4.6 mm, 5 µm) maintained at 25 °C. The mobile phase consisted of 25% (v/v) acetonitrile in ammonium dihydrogen phosphate buffer (11.41 mg/mL), adjusted to pH 4.4 with orthophosphoric acid or ammonia solution. The flow rate was 1.3 mL/min, injection volume 20 µL, and detection wavelength 225 nm. The run time was set to at least ten times the retention time of venlafaxine to ensure elution of late-eluting degradation products. System suitability criteria included resolution ≥ 1.5 between impurity D and venlafaxine and RSD ≤ 5.0% (n = 6), consistent with compendial recommendations [5,8]. According to the European Pharmacopoeia monograph for venlafaxine hydrochloride, system suitability criteria require a minimum resolution of 1.5 between venlafaxine and impurity D. Resolution between impurities D and E is not specified because these impurities exhibit acid-dependent interconversion under the chromatographic conditions, which may result in apparent co-elution during analysis. For related substances analysis, ten tablets were weighed and powdered, and an amount equivalent to 50 mg venlafaxine was transferred to a 50 mL volumetric flask, extracted under sonication, diluted to volume, filtered, and injected. The final test concentration was 1.0 mg/mL. Individual and total impurities were calculated relative to the standard solution, applying a pharmacopoeial disregard limit of 0.05% [5,8]. Specificity under stress conditions was confirmed by PDA-based peak purity assessment. Peak purity was evaluated using PDA/DAD spectral analysis by comparing UV spectra across the chromatographic peak. The detector software calculates a spectral similarity factor (match factor) ranging from 0 to 1000. Values ≥ 990 were considered indicative of spectral homogeneity and absence of co-eluting components. Spectral similarity was evaluated between spectra collected at the upslope, apex, and downslope of the chromatographic peak. Spectral homogeneity across chromatographic peaks was evaluated to exclude co-eluting degradation products and to confirm the stability-indicating capability of the method, as recommended for forced degradation studies [20–24]. The analytical performance of the related substances method was previously evaluated according to internal validation procedures aligned with ICH Q2(R1) recommendations. Key parameters including linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) were verified to confirm suitability of the method for impurity profiling. Furthermore, the in-house RP-HPLC assay method was validated in accordance with ICH Q2(R2) guidelines. The method demonstrated excellent linearity over the concentration range of 0.187–0.751 mg/mL (50–150% of the working concentration), with a correlation coefficient of 1.0000. Accuracy was confirmed with a mean recovery of 100.12% (RSD 0.2%). The method showed high precision, with system precision RSD of 0.1%, method precision RSD of 0.3%, and intermediate precision RSD of 0.3%. Robustness testing demonstrated that small deliberate variations in chromatographic parameters did not significantly affect the results. Standard and sample solutions were stable for at least 172 h at room temperature. A summary of the validation parameters is presented in Table S1.

2.3.2. FTIR spectroscopy

Drug-excipient compatibility was further evaluated using Fourier-transform infrared (FTIR) spectroscopy, a widely accepted technique for solid-state interaction assessment in pharmaceutical systems [25,26]. Binary mixtures of venlafaxine hydrochloride with each individual excipient were prepared in formulation-relevant ratios corresponding to the composition of the investigated tablet formulation. The components were accurately weighed and gently homogenized in a mortar and pestle to obtain a uniform mixture. The use of formulation-relevant ratios allows a more realistic simulation of potential solid-state interactions between the active pharmaceutical ingredient and excipients that may occur in the finished dosage form during manufacturing and storage. FTIR spectra were recorded using a Varian 660 FTIR spectrometer over the spectral range 4000–550 cm^{-1} at a resolution of 4 cm^{-1} , averaging 16 scans per spectrum. Samples were prepared using the potassium bromide (KBr) pellet method. Approximately 1–2 mg of the sample was thoroughly mixed with spectroscopic-grade dry KBr, compressed into a transparent disc under vacuum, and analyzed immediately after preparation to minimize moisture interference. Comparative spectral evaluation focused on characteristic absorption bands of venlafaxine hydrochloride, including regions corresponding to N–H stretching, tertiary amine C–N stretching, aromatic C=C vibrations, and methoxy group absorptions. Spectra of stressed binary mixtures were compared with those of unstressed mixtures in order to identify potential physicochemical interactions, such as hydrogen bonding, protonation effects, or thermally induced structural modifications, consistent with established FTIR-based compatibility assessment approaches in pharmaceutical stability investigations [14,25,26].

The abbreviations used throughout the manuscript include RP-HPLC (reversed-phase high-performance liquid chromatography), FTIR (Fourier transform infrared spectroscopy), API (active pharmaceutical ingredient), and BDL (below disregard limit).

3. Results

3.1. Initial assessment and method specificity

The developed RP-HPLC method was evaluated under unstressed conditions prior to stress application. As summarized in Table 1, both venlafaxine hydrochloride tablets and the isolated API complied with predefined assay and related substances specifications in accordance with regulatory and pharmacopoeial requirements [1,5,8]. Assay values were within 94.0–105.0%, and individual as well as total impurities remained below pharmacopoeial thresholds [5,8].

No unexpected peaks were detected in chromatograms of unstressed samples. PDA-based peak purity analysis confirmed spectral homogeneity of the venlafaxine peak [23], supporting method specificity.

3.2. Photolytic degradation

Results obtained after exposure to photolytic stress are summarized in Table 2. Photostability testing was performed according to ICH Q1B conditions [17]. Representative chromatograms (Fig. S1) demonstrated adequate resolution between venlafaxine and degradation peaks.

Short-term exposure (200 Wh/m^2; 1.2 × 10^6 lx·h) produced no visible changes and no significant assay loss. Total impurities remained ≤ 0.10%, and mass balance values were within 98–102% [22]. After prolonged irradiation (6 × 10^6 lx·h), a slight increase in impurity D and

Table 1
Initial analysis of venlafaxine 37.5 mg tablets and venlafaxine active pharmaceutical ingredient.

Parameter	Venlafaxine 37.5 mg tablets	Active substance (API)
Appearance	Complies	Complies
Color	Complies	Complies
Assay (%)	98.1	100.1
Total impurities	BDL	BDL

BDL – below disregard limit (0.05%); API – active pharmaceutical ingredient

Table 2
Results of photolytic degradation of venlafaxine 37.5 mg tablets and active pharmaceutical ingredient.

Parameter	200 Wh/m² (2.9 h)	200 Wh/m² Dark Control	1.2 × 10⁶ lx·h (7.1 h)	1.2 × 10⁶ lx·h Dark Control	6 × 10⁶ lx·h (35 h)	6 × 10⁶ lx·h Dark Control	API 6 × 10⁶ lx·h
Appearance	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Color	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Assay (%)	97.68	98.36	97.17	96.85	97.30	97.21	100.42
Peak purity	996–1000 (all cases)						
Impurity A (%)	0.032	0.030	0.032	0.031	0.044	0.028	0.043
RRT 0.69 (%)	0.007	0.007	0.006	0.005	0.005	0.006	–
Impurity C (%)	–	–	–	–	0.002	–	–
RRT 0.85 (%)	0.021	0.012	0.012	0.013	0.017	0.015	–
Impurity D (%)	0.005	0.002	0.006	0.002	0.033	0.004	–
RRT 1.76 (%)	0.003	–	0.002	–	0.020	–	–
RRT 2.16 (%)	0.016	0.016	0.017	0.017	0.019	0.020	0.016
Impurity F (%)	0.019	0.017	0.019	0.023	0.020	0.032	0.012
Total impurities (%)	0.10	0.08	0.09	0.09	0.19	0.10	0.10
Mass balance (%)	99.67	100.35	99.14	98.82	99.38	99.20	100.41

– Not detected or below reporting limit (0.05%); RRT – relative retention time; API – active pharmaceutical ingredient

the peak at RRT 1.76 was observed. However, all impurities remained below specification limits, and total impurities in tablets reached 0.19%. Placebo chromatograms showed no additional peaks.

3.3. Thermal, hydrolytic and oxidative degradation

The results obtained after thermal, hydrolytic, and oxidative stress studies are summarized in Table 3 A and 3B, while representative chromatograms illustrating impurity profiles are presented in Supplementary material Figure S2.

3.3.1. Moderate thermal stress (40 °C/75% RH)

No significant degradation occurred after 30 days. Assay values remained within 94.0–105.0%, impurities were below BDL, and mass balance ranged from 98 to 102% [22]. Minor matrix-related low-level peaks were observed in tablets without stability relevance.

3.3.2. Severe thermal stress (105 °C/96 h)

Marked degradation occurred in the finished product but not in the isolated API. Impurities D, F, A, and the unknown at RRT 1.76 exceeded BDL, and impurity D surpassed its specification limit. This difference indicates formulation-enhanced degradation kinetics at elevated temperature. Thermally induced β-elimination and radical-mediated reactions involving the tertiary amine are plausible mechanisms [14–16]. Increased molecular mobility and oxygen diffusion in the solid matrix may promote oxidative pathways. The absence of macroscopic physical changes despite chromatographic degradation highlights the sensitivity of the analytical method.

Table 3
Results of A) thermal degradation data; B) hydrolytic and oxidative degradation data of venlafaxine 37.5 mg tablets and API.

A)

Parameter	40 °C/75% RH (Tablets)	40 °C/75% RH (API)	105 °C/96 h (Tablets)	105 °C/96 h (API)
Appearance	Complies	Complies	Complies	Complies
Color	Complies	Complies	Complies	Complies
Assay (%)	98.73	100.16	97.42	100.30
Peak purity				
Impurity A (%)	0.028	0.025	0.092	0.032
Impurity C (%)	–	–	0.015	–
Impurity D (%)	0.004	–	0.260	0.005
Impurity F (%)	0.023	0.014	0.062	0.014
Unknown (RRT 1.76) (%)	–	–	0.071	–
Unknown (RRT 2.89) (%)	–	–	–	–
Total impurities (%)	0.10	0.05	0.69	0.07
Mass balance (%)	100.74	100.12	100.01	100.27

B)

Parameter	Alkaline (Tablets)	Alkaline (API)	Acidic (Tablets)	Acidic (API)	Oxidative (Tablets)	Oxidative (API)
Appearance	Complies	Complies	Complies	Complies	Complies	Complies
Color	Complies	Complies	Complies	Complies	Complies	Complies
Assay (%)	99.29	100.84	96.99	99.67	98.47	99.83
Peak purity						
Impurity A (%)	0.025	0.027	0.026	0.030	0.039	0.033
Impurity C (%)	–	–	–	–	0.008	0.005
Impurity D (%)	0.012	0.005	–	–	0.060	0.035
Impurity F (%)	0.021	0.019	0.056	0.077	0.015	–
Unknown (RRT 1.76) (%)	0.076	0.006	–	–	0.494	0.231
Unknown (RRT 2.89) (%)	–	–	–	–	0.061	0.046
Total impurities (%)	0.18	0.07	0.16	0.16	0.79	0.44
Mass balance (%)	101.40	100.82	99.05	99.75	101.18	100.17

– Not detected or below disregard limit (0.05%); RRT – relative retention time; API – active pharmaceutical ingredient

3.3.3. Hydrolytic stress

Alkaline hydrolysis induced a moderate impurity increase in tablets, whereas the API remained largely unaffected. Acidic hydrolysis was performed by addition of 10 mL of 37% hydrochloric acid (HCl) to the sample solution. The prepared samples were treated in an ultrasonic bath for 30 min at 50 °C, cooled to room temperature, neutralized to the initial pH using a suitable base, diluted to volume with the mobile phase, filtered, and immediately analyzed by HPLC. Acidic hydrolysis produced comparable effects in both matrices, with impurity F increasing above BDL but remaining within specifications.

3.3.4. Oxidative stress

Hydrogen peroxide exposure caused the most pronounced degradation. Impurity D and unknown impurities at RRT 1.75 and 2.89 increased significantly, with the RRT 1.75 impurity exceeding specification limits.

3.4. Binary mixture compatibility studies

Given the enhanced degradation observed in tablets at 105 °C, binary mixtures were prepared to evaluate drug–excipient compatibility in accordance with established solid-state screening strategies [25,26]. Under 40 °C/75% RH, all mixtures remained stable, confirming satisfactory compatibility under accelerated storage conditions. In contrast, exposure to 105 °C for 96 h revealed significant degradation exclusively in the mixture containing microcrystalline cellulose (MCC), characterized by increased levels of impurities D, F, and A and deviation from the mass balance criterion [22]. The summarized results are presented in Table 4, while representative chromatograms are shown in Supplementary Material Figure S3.

FTIR analysis (Fig. 2) supported these findings. Spectra obtained at 40 °C/75% RH were superimposable with untreated controls, whereas after exposure to 105 °C, noticeable shifts were observed in absorption bands corresponding to N–H stretching, C–N vibrations, and aromatic C═C modes.

The enhanced degradation observed in the venlafaxine–MCC system under severe thermal stress may be rationalized by solid-state microenvironmental effects within the cellulose matrix. The FTIR spectrum of microcrystalline cellulose (MCC) is characterized by a broad O–H stretching band around 3340 cm^{-1} , corresponding to hydrogen-bonded hydroxyl groups within the cellulose structure. In the spectrum of venlafaxine hydrochloride, characteristic bands associated with the tertiary amine functionality and aromatic groups are observed in the regions 1250–1350 cm^{-1} (C–N stretching) and 1600 cm^{-1} (aromatic C═C vibrations). In the FTIR spectra of the venlafaxine–MCC binary mixture, a slight broadening and minor shift of the O–H stretching band was observed, suggesting the formation of possible intermolecular hydrogen bonding interactions between the hydroxyl groups of MCC and the tertiary amine functionality of venlafaxine. Additionally, small variations in the C–N stretching region (~ 1260–1300 cm^{-1}) were detected, which further supports the presence of weak solid-state interactions between the drug and the excipient.

3.5. Evaluation of specified impurities and E→D transformation

Using the Ph. Eur.–based method [13], impurities D and E exhibited identical retention times. Mixed standards showed an apparent doubling of the D peak area. FTIR analysis confirmed that impurities D and E are structurally distinct [25,26], excluding identity overlap (Fig. 3). Analysis using both the in-house and British Pharmacopoeia methods [14] reproduced this observation: impurity E solutions consistently generated an additional D peak, whereas impurity D solutions showed no reciprocal formation.

4. Discussion

This study presents a mechanistically informed analytical framework that extends conventional forced degradation approaches by integrating chromatographic impurity profiling with spectroscopic evaluation of solid-state interactions. The key novelty lies in the combined use of RP-HPLC and FTIR techniques to differentiate between intrinsic API degradation, excipient-mediated instability, and analytical-condition-induced impurity interconversion. The absence of additional peaks and the confirmed peak purity under unstressed conditions further support the specificity of the developed method and its suitability for reliable impurity profiling and mass balance assessment. The observed stability of venlafaxine hydrochloride under photolytic conditions is consistent with the known chemical behavior of tertiary amines. Under UV/visible irradiation, such compounds may undergo photooxidative processes, including singlet oxygen-mediated oxidation or radical-driven N-dealkylation reactions, as reported in forced degradation studies [14,16]. The slight increase in impurity D observed after prolonged irradiation suggests limited oxidative transformation; however, the low impurity levels and preserved mass balance indicate that venlafaxine exhibits satisfactory photostability under ICH Q1B conditions. The severe thermal stress applied at 105 °C represents an exaggerated condition used to accelerate degradation and reveal potential degradation pathways and drug–excipient interactions. Such conditions are not intended to directly predict the real-time shelf life of the product. According to ICH stability guidelines, shelf-life determination relies on long-term and accelerated stability studies performed under controlled environmental conditions. Therefore, the 105 °C experiment primarily serves as a diagnostic tool for identifying degradation mechanisms rather than as a predictor of product stability under normal storage conditions. These findings indicate relative hydrolytic stability, consistent with the absence of highly labile ester or amide bonds in the structure. The tertiary amine moiety is highly susceptible to oxidation via N-oxide formation or oxidative N-dealkylation [14,15,23]. Aromatic

Table 4
Results of thermal degradation of binary mixtures of venlafaxine hydrochloride with individual excipients.

Parameter	MCC	Lactose	SSG	Colloidal SiO_2	Mg Stearate	Yellow Fe Oxide	Red Fe Oxide
Assay (%) – 40 °C/75% RH	99.44	94.05	98.26	99.64	100.04	99.75	100.33
Assay (%) – 105 °C/96 h	106.37	94.88	98.66	99.49	100.25	99.60	100.17
Impurity A (%) – 105 °C	0.073	0.028	0.030	0.027	0.026	0.026	0.027
Impurity D (%) – 105 °C	0.154	0.005	0.004	0.006	0.002	0.004	0.003
Impurity F (%) – 105 °C	0.054	0.013	0.012	0.011	0.011	0.012	0.012
Unknown (RRT 1.76) (%) – 105 °C	0.023	–	0.006	–	–	–	–
Total impurities (%) – 40 °C	0.06	0.05	0.05	0.06	0.05	0.06	0.06
Total impurities (%) – 105 °C	0.41	0.06	0.06	0.06	0.06	0.06	0.06
Mass balance (%) – 40 °C	101.71	98.0	100.63	100.72	100.66	101.24	101.28
Mass balance (%) – 105 °C	109.14	98.63	101.05	100.57	100.89	101.10	101.12

Values below 0.05% are considered below disregard limit (BDL).
MCC – microcrystalline cellulose; SSG – sodium starch glycolate.

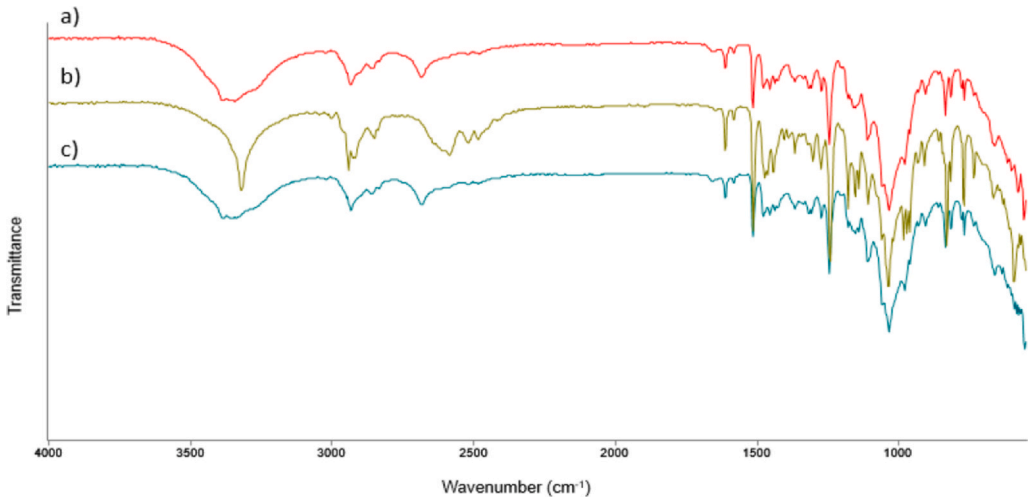

Fig. 2. FTIR spectra of the binary mixture of venlafaxine hydrochloride and microcrystalline cellulose following thermal stress: (a) initial (unstressed) mixture; (b) after exposure to 105 °C for 96 h; (c) after storage at 40 °C/75% RH for 30 days.

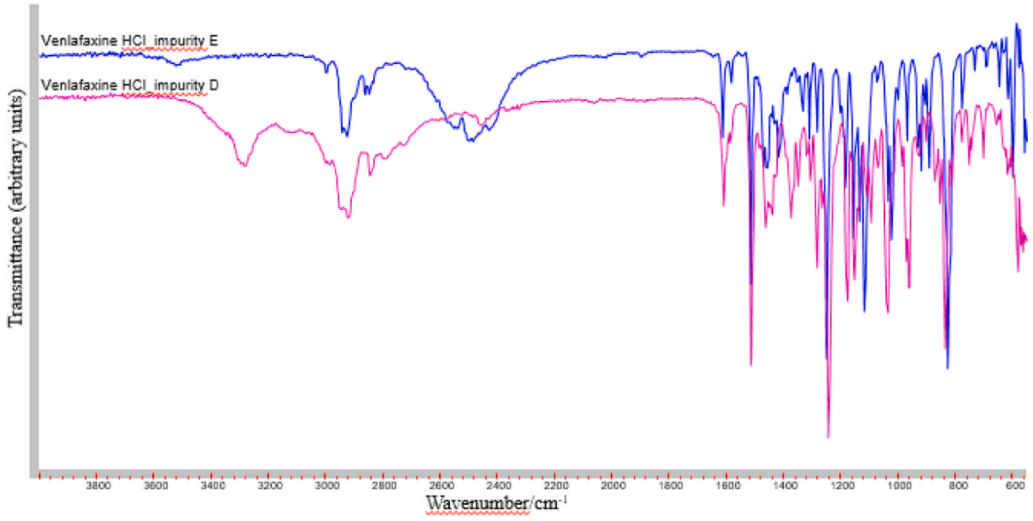

Fig. 3. Overlaid FTIR spectra of impurity D (pink trace) and impurity E (blue trace), showing distinct differences in characteristic absorption bands and confirming that the two compounds are structurally different.

oxidation may also contribute under strong oxidative conditions. Across all stress conditions, mass balance remained within 98–102% [22], and peak purity confirmed method specificity [23].

In the case of the microcrystalline cellulose (MCC) mixture, the mass balance value at 105 °C reached 109.14% (Table 4), driven primarily by assay over-recovery (106.37%) rather than by extensive impurity formation. This positive deviation likely reflects a matrix-related analytical effect under severe thermal stress, such as altered extraction behavior or contribution of co-extracted excipient-derived species to the UV response, rather than true material gain. The observation was specific to the MCC binary mixture and was not seen in the other excipient mixtures or in the stressed API, supporting the interpretation of a matrix-dependent effect associated with the thermally stressed cellulose environment. All experiments were performed in duplicate (n = 2), and the values reported correspond to the mean of two independent determinations, which showed consistent impurity trends. Other excipients showed no comparable chromatographic or spectral changes. Such interactions are consistent with previously reported studies describing hydrogen bonding between cellulose hydroxyl groups and basic nitrogen-containing pharmaceutical compounds, which may influence the microenvironment of the active pharmaceutical ingredient in solid dosage forms. These observations are consistent with altered intermolecular interactions within the excipient matrix rather than intrinsic API degradation alone [25,26]. It should be noted that FTIR provides supportive spectroscopic evidence of interaction, while definitive solid-state characterization would require complementary techniques such as DSC or XRPD. Microcrystalline cellulose contains abundant surface hydroxyl groups capable of forming extensive hydrogen-bond networks and interacting with functional groups present in drug molecules. Such interactions may modify the local microenvironment within the solid matrix and influence degradation kinetics under stress conditions. Previous studies have shown that drug–excipient interactions in solid dosage forms frequently arise from hydrogen bonding, residual moisture, or excipient-derived microenvironmental effects that can promote chemical instability of drug substances [27–29]. In cellulose-based materials, strong hydrogen-bond networks between hydroxyl groups create highly interactive surfaces capable of adsorbing and interacting with small organic molecules, thereby potentially altering reaction pathways at the solid interface. Microcrystalline cellulose is also mildly hygroscopic, and residual adsorbed moisture may contribute to the formation of localized microenvironments within the porous matrix. Although the moisture content of the excipient was not independently quantified in the present study, all binary mixtures were exposed to identical thermal conditions, yet pronounced degradation was observed only in the MCC system, suggesting that moisture alone cannot account for the observed instability.

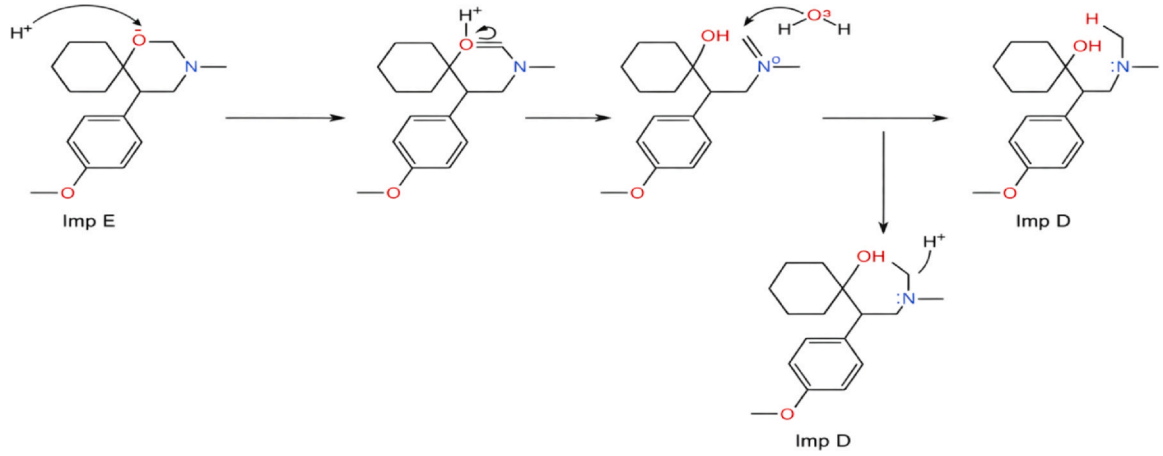

Fig. 4. Proposed acid-catalyzed interconversion of impurity E to impurity D.

At elevated temperature, increased molecular mobility within the porous matrix may create localized microdomains of altered polarity and oxygen diffusion. Furthermore, residual moisture associated with MCC may generate microenvironments that facilitate thermally accelerated oxidative or β-elimination pathways involving the tertiary amine functionality of venlafaxine [14–16]. Hydrogen-bonding interactions between the API and cellulose hydroxyl groups may also modify the electronic environment of the tertiary amine, potentially lowering activation barriers for degradation reactions. In addition, degradation within the venlafaxine–MCC system may proceed via heterogeneous solid-state kinetics rather than homogeneous bulk processes [23]. In such systems, reaction rates are governed by interfacial phenomena, including surface adsorption, localized diffusion constraints, and restricted molecular mobility within the excipient matrix. The high surface area of MCC may therefore increase the effective contact interface between API and excipient, promoting surface-mediated reactions that would be kinetically unfavorable in the isolated crystalline API. Under exaggerated thermal stress, such heterogeneous pathways may dominate degradation behavior, leading to matrix-dependent impurity formation patterns distinct from those observed for the pure drug substance. The absence of comparable degradation in other binary mixtures indicates that this behavior is matrix-specific rather than a manifestation of general thermal instability. Collectively, these results demonstrate that excipient surface chemistry and solid-state microenvironment can significantly modulate degradation kinetics under exaggerated thermal conditions.

Although crystallite size can influence surface interactions in solid-state systems, all materials used in this study originated from the same production batches and were handled under identical preparation conditions, minimizing variability associated with particle or crystallite size. Particle size distribution of the API and excipients was not independently determined in this study; however, all materials were pharmaceutical-grade substances from the same production batches, which reduces variability related to particle size effects on surface-mediated degradation. The integrated RP-HPLC-FTIR approach enabled clear differentiation between intrinsic API instability and excipient-mediated degradation effects.

The observed peak behavior of pharmacopoeial impurities D and E indicates that the apparent co-elution is not due to insufficient chromatographic resolution, but rather to an analytical-condition-induced transformation. The formation of an additional D peak in impurity E solutions, without reciprocal conversion, suggests an acid-catalyzed interconversion mechanism occurring under chromatographic conditions. This finding has direct regulatory relevance, as impurity levels observed during routine quality control may not necessarily reflect true degradation pathways but may arise from transformations occurring during chromatographic analysis. Therefore, caution is required when interpreting impurity limits for such systems, and method conditions may need to be critically evaluated to avoid misinterpretation of impurity profiles. The observed conversion of impurity E to impurity D is most plausibly explained by an acid-catalyzed structural rearrangement occurring during solution preparation or chromatographic analysis (Fig. 4). All solutions were analyzed immediately after preparation in order to minimize possible time-dependent transformations in the sample vial. The consistent formation of impurity D during chromatographic analysis using acidic mobile phases suggests that the observed E→D conversion occurs predominantly under analytical conditions rather than as a progressive transformation in the vial prior to injection. All applied chromatographic methods employed acidic mobile phases, creating conditions under which protonation of nitrogen-containing heterocycles is favored. The observed behavior was reproducible using the European Pharmacopoeia, British Pharmacopoeia, and the in-house chromatographic methods, all employing acidic mobile phases, further supporting the hypothesis of acid-induced impurity interconversion occurring in solution.

Impurity E contains a spirocyclic azaspiroundecane framework. Under acidic conditions, protonation of the tertiary amine increases the electrophilicity of adjacent carbon centers within the spiro system. This activation facilitates cleavage of the C–N bond through a protonation-induced ring-opening mechanism, yielding an open-chain amino alcohol consistent with the structure of impurity D [2,9,15]. Acid-promoted ring opening of spirocyclic amines is a recognized transformation in heterocyclic chemistry and has been reported in pharmaceutical degradation and stress-testing studies under low pH conditions [11,15]. Protonation lowers the activation barrier for C–N bond cleavage and shifts the equilibrium toward thermodynamically favored open-chain derivatives.

Quantitative data demonstrated consistent mass redistribution (E + formed D ≈ D alone), strongly supporting chemical interconversion rather than chromatographic co-elution. A quantitative summary of the peak area redistribution observed in the mixed standard experiments is provided in Supplementary Table S2. The reproducibility of the E→D transformation across two independent chromatographic systems confirms a solution-phase chemical process rather than insufficient chromatographic selectivity. Collectively, the chromatographic behavior, quantitative mass redistribution, and mechanistic consistency support acid-catalyzed spiro ring opening as the most plausible origin of the apparent co-elution phenomenon.

Validation studies demonstrated that both standard and sample solutions remained stable for extended periods at room temperature, with recoveries within the acceptable range (90–110%) for at least 90 h under analytical conditions. These findings suggest that the observed E→D transformation is unlikely to result from general solution instability in the vial, but is more consistent with a specific structural interconversion occurring under the acidic chromatographic conditions used in the method.

From a regulatory perspective, the observed E→D interconversion highlights the importance of considering potential analyte instability under analytical conditions. Because the transformation appears to occur during chromatographic analysis in acidic media, the detected level of impurity D may partly reflect conversion from impurity E rather than independent formation of impurity D during product degradation. Consequently, quality control laboratories should interpret elevated impurity D levels with caution when impurity E may also be present. While the pharmacopoeial methods remain suitable for routine impurity monitoring, awareness of potential impurity interconversion is important for accurate interpretation of impurity profiles obtained under acidic analytical conditions.

Despite these findings, several limitations should be acknowledged. Solid-state interactions were primarily evaluated using FTIR spectroscopy, which provides supportive but not definitive structural evidence. Additional characterization using complementary techniques such as differential scanning calorimetry (DSC) or X-ray powder diffraction (XRPD) would provide more comprehensive insight into the physicochemical nature of drug–excipient interactions. Moreover, structural identification of minor degradation products was not confirmed by LC–MS analysis, which could further support degradation pathway elucidation.

Future studies integrating chromatographic, spectroscopic, and mass spectrometric techniques, as well as computational or in silico approaches, may enable more comprehensive degradation pathway mapping and improved understanding of impurity behavior in pharmaceutical systems.

5. Conclusion

This study presents an integrated RP-HPLC–FTIR analytical strategy for mechanistically driven stability profiling of venlafaxine hydrochloride tablets. The developed chromatographic method demonstrated clear stability-indicating capability, supported by adequate selectivity, peak purity, and mass balance values close to 100% for most applied stress conditions. Venlafaxine hydrochloride exhibited substantial stability under photolytic (ICH Q1B) and accelerated storage conditions, as well as limited sensitivity to hydrolytic stress. In contrast, exaggerated thermal exposure (105 °C) and oxidative conditions were identified as the primary drivers of degradation, defining the dominant instability pathways of the molecule.

Binary mixture investigations revealed excipient-dependent degradation behavior, with microcrystalline cellulose emerging as the only formulation component promoting impurity formation under severe thermal stress. The high surface area and abundance of hydroxyl functionalities within the cellulose matrix may create a reactive solid-state microenvironment capable of facilitating hydrogen-bond rearrangements, localized oxidative processes, and enhanced molecular mobility at elevated temperature. FTIR spectral alterations support microenvironment-driven interactions, indicating that excipient-mediated effects rather than intrinsic API instability contribute to the observed degradation under extreme conditions. Although FTIR provided supportive spectroscopic evidence of solid-state interactions, further confirmation of these effects would benefit from complementary techniques such as DSC or XRPD. Importantly, the apparent chromatographic co-elution of pharmacopoeial impurities (D and E) was demonstrated to result from acid-induced interconversion of impurity E into impurity D during analysis rather than insufficient chromatographic resolution. This finding underscores that impurity behavior observed under compendial analytical conditions may reflect analytical-environment-induced chemical transformation rather than intrinsic manufacturing- or storage-related degradation.

Collectively, the integration of forced degradation profiling, excipient compatibility screening, and impurity interconversion analysis establishes a unified and mechanistically informed framework for stability evaluation. The proposed approach enhances interpretative reliability in regulatory impurity assessment and provides a robust platform applicable to tertiary and spirocyclic amine-containing pharmaceutical systems beyond venlafaxine formulations.

Clinical Trial No

Not Applicable.

CRediT authorship contribution statement

Ivana Mitrevska: Writing – original draft, Methodology, Conceptualization. **Lena Pislevska:** Formal analysis, Data curation. **Monika Stojanovska Pecova:** Writing – review & editing.

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not applicable.

Consent to participate

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Data availability

The manuscript includes all necessary data; related data may be provided upon request from the corresponding author.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cpan.2026.04.002.

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