A Validated Stability Indicating RP-HPLC Method for Milnacipran Hydrochloride, Identification and Characterization of Forced Degradation Products Using LC-MS/MS

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ABSTRACT

A stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for milnacipran hydrochloride. The drug was subjected to forced degradation studies under hydrolytic, oxidative, photolytic and thermal stress conditions according to International Conference on Harmonisation (ICH) guidelines. A single major degradation product (DP-1) was detected under oxidative stress conditions with 30% hydrogen peroxide at room temperature, showing 32% degradation after 4 days. The drug remained stable under acidic, basic, neutral hydrolytic, photolytic and thermal stress conditions. Chromatographic separation was achieved on a Hypersil Gold C18 column (250 mm × 4.6 mm, 5 µm) using gradient elution with 10 mM potassium dihydrogen phosphate buffer (pH 3.5) and acetonitrile as mobile phase at a flow rate of 1.0 mL/min with detection at 220 nm. The degradation product was characterized by LC-MS/MS and identified as milnacipran N-oxide. The method was validated for specificity, linearity, accuracy, precision, robustness and system suitability parameters. The validated method was successfully applied to commercial tablet formulations for stability studies.

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INTRODUCTION

Stability testing of pharmaceutical products is a critical component of drug development and quality assurance programs mandated by regulatory agencies worldwide[1–4]. The primary objective of stability studies is to establish the shelf life of drug substances and products, identify degradation pathways and ensure product quality throughout the intended storage period. Forced degradation studies, also known as stress testing, involve subjecting drug substances to conditions more severe than normal storage to generate potential degradation products[5–7]. These studies provide essential information for developing stability-indicating analytical methods capable of separating and quantifying the active pharmaceutical ingredient (API) in the presence of its degradation products, process-related impurities and excipients[8–10].

Milnacipran hydrochloride, chemically designated as (1RS,2SR)-2-(aminomethyl)-N, N-diethyl-1-phenylcyclopropanecarboxamide hydrochloride, is a serotonin-norepinephrine reuptake inhibitor (SNRI) used in the treatment of major depressive disorder and fibromyalgia[11,12]. The drug exhibits dual inhibition of serotonin and norepinephrine reuptake with relatively balanced potency, distinguishing it from other SNRIs that show preferential selectivity[13]. Milnacipran is marketed as a racemic mixture with a molecular formula of $C_{15}H_{22}N_2O \cdot HCl$ and molecular weight of 282.81

g/mol. The molecule contains a cyclopropane ring, a tertiary amide functional group and a primary amine, structural features that may be susceptible to hydrolytic and oxidative degradation under stress conditions.

Fig. 1: Structure of Milnacipran hydrochloride

Several analytical methods have been reported for the determination of milnacipran in pharmaceutical formulations and biological matrices, including RP-HPLC[14–16], UPLC[17], UHPLC[18], and HPTLC[19,20]. However, comprehensive forced degradation studies with detailed characterization of degradation products and validation of stability-indicating methods are limited in the literature. Pydimarry et al. developed a UPLC method for rapid determination of related substances and degradants in milnacipran, reporting significant degradation under acid, base, water hydrolysis and oxidative conditions[17]. Naresh et al. reported a UHPLC method that resolved milnacipran and five impurities with mass balance near 99.5%[18]. Khatri et al. developed an HPTLC method for stability testing but did not provide detailed structural characterization of degradation products[19]. Saravanan et al. reported an RP-HPLC method but observed minimal degradation under their specific stress conditions[14].

The present study was undertaken to develop a validated stability-indicating RP-HPLC method for milnacipran hydrochloride, conduct comprehensive forced degradation studies under ICH-recommended stress conditions, identify and characterize major degradation products using LC-MS/MS and apply the method to commercial tablet formulations for stability assessment. The method was validated according to ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, robustness, and system suitability parameters[21].

EXPERIMENTAL

Chemicals

Milnacipran hydrochloride reference standard (purity 99.8%) was used for the study. Milnacipran hydrochloride tablets were purchased from a local pharmacy. HPLC-grade acetonitrile and methanol were used. Potassium dihydrogen phosphate, orthophosphoric acid, hydrochloric acid, sodium hydroxide and hydrogen peroxide (30% w/v) were of analytical reagent grade. High-purity water was prepared using a Milli-Q water purification system.

Instrumentation

The HPLC system consisted of a Waters Alliance 2695 separations module equipped with a quaternary pump, autosampler, column oven and Waters 2996 photodiode array (PDA) detector. Data acquisition and processing were performed using Empower 3 chromatography software. Forced degradation studies were conducted using a thermostatically controlled water bath, hot air oven and photostability chamber equipped with UV (254 nm) and visible (400–800 nm) fluorescent lamps providing illumination of 200 W·h/m² and 1.2 million lux·h, respectively. LC-MS/MS analysis was performed on a Waters Acquity system coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer with electrospray ionization (ESI) source operated in positive ion mode.

Chromatographic Conditions

Chromatographic separation was achieved on a Hypersil Gold C18 column (250 mm × 4.6 mm, 5 µm particle) maintained at 30°C. The mobile phase consisted of (A) 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 with orthophosphoric acid and (B) acetonitrile. Gradient elution was performed as follows: 0 - 5 min, 10% B; 5 - 15 min, 10 - 30% B; 15 - 25 min, 30 - 50% B; 25 - 30 min, 50 - 70% B; 30 - 35 min, 70% B; 35 - 36 min, 70 - 10% B; 36 - 45 min, 10% B (equilibration). The flow rate was 1.0 mL/min, injection volume was 20 µL and detection was performed at 220 nm. The total run time was 45 minutes.

Preparation of Solutions

Standard stock solution: Milnacipran hydrochloride reference standard (25 mg) was accurately weighed and transferred to a 25 mL volumetric flask. The flask was filled to volume with mobile phase (50:50 v/v buffer: acetonitrile) to obtain a stock solution of 1000 µg/mL. Working standard solutions were prepared by appropriate dilution of the stock solution with mobile phase.

Sample solution: Twenty tablets were weighed and finely powdered. An accurately weighed portion of powder equivalent to 25 mg of milnacipran hydrochloride was transferred to a 25 mL volumetric flask, dissolved in mobile phase with sonication for 15 minutes and diluted to volume. The solution was filtered through a 0.45 µm nylon membrane filter before injection.

Stress Decomposition Studies

Forced degradation studies were performed on milnacipran hydrochloride bulk drug and tablet formulation according to ICH Q1A(R2) and Q1B guidelines[1,2]. The optimized stress conditions are summarized in Table 1.

Table 1. Optimized Stress Conditions for Milnacipran Hydrochloride

Stress Condition	Stressor	Temperature	Duration	% Degradation	Degradation Products
Acidic hydrolysis	2 N HCl	80°C	4 days	2.1	None detected
Acidic hydrolysis	2 N HCl	80°C	7 days	3.8	None detected
Acidic hydrolysis	2 N HCl	80°C	13 days	5.2	Minor products (<1%)
Basic hydrolysis	2 N NaOH	80°C	4 days	3.5	Minor products (<1%)
Basic hydrolysis	2 N NaOH	80°C	7 days	5.8	Minor products (<1%)
Basic hydrolysis	2 N NaOH	80°C	13 days	7.4	Minor products (<1%)
Neutral hydrolysis	Water	80°C	13 days	1.8	None detected
Oxidative	30% H_2O_2	25°C	1 day	12.5	DP-1 (10.8%)
Oxidative	30% H_2O_2	25°C	2 days	24.3	DP-1 (21.5%)
Oxidative	30% H_2O_2	25°C	4 days	32.1	DP-1 (28.2%)
Photolytic (solid)	UV + Visible	40°C/75% RH	2 days	0.8	None detected
Photolytic (solid)	UV + Visible	40°C/75% RH	7 days	1.5	None detected

Photolytic (solid)	UV + Visible	40°C/75% RH	13 days	2.3	None detected
Photolytic (solution)	UV + Visible	40°C/75% RH	2 days	1.2	None detected
Photolytic (solution)	UV + Visible	40°C/75% RH	7 days	2.1	None detected
Photolytic (solution)	UV + Visible	40°C/75% RH	13 days	3.5	Minor products (<1%)
Thermal	Dry heat	50°C	7 days	1.1	None detected
Thermal	Dry heat	50°C	14 days	1.8	None detected
Thermal	Dry heat	50°C	21 days	2.5	None detected

Hydrolytic degradation: For acidic hydrolysis, 10 mg of drug was dissolved in 10 mL of 2 N hydrochloric acid and heated at 80°C in a water bath for 4, 7 and 13 days. For basic hydrolysis, 10 mg of drug was dissolved in 10 mL of 2 N sodium hydroxide and heated at 80°C for 4, 7 and 13 days. For neutral hydrolysis, 10 mg of drug was dissolved in 10 mL of water and heated at 80°C for 13 days. After the specified time intervals, aliquots were withdrawn, neutralized with equivalent concentration of acid or base, diluted to 100 µg/mL with mobile phase, filtered through 0.45 µm membrane filters and analyzed by HPLC.

Oxidative degradation: For oxidative stress, 10 mg of drug was dissolved in 10 mL of 30% hydrogen peroxide and kept at room temperature (25 ± 2°C) in the dark for 4 days. Aliquots were withdrawn at 1, 2 and 4 days, diluted to 100 µg/mL with mobile phase, filtered and analyzed.

Photolytic degradation: For photolytic stress, solid drug (spread as a thin layer in a petri dish) and drug solution (10 mg in 10 mL water in a transparent glass vial) were exposed to UV light (254 nm) and fluorescent light (visible range) in a photostability chamber at 40°C and 75% relative humidity for 2, 7 and 13 days. Control samples wrapped in aluminium foil were run simultaneously. After exposure, solid samples were dissolved and solution samples were diluted to 100 µg/mL with mobile phase, filtered and analyzed.

Thermal degradation: For thermal stress, solid drug (spread as a thin layer in a petri dish) was placed in a hot air oven at 50°C for 21 days. Samples were withdrawn at 7, 14 and 21 days, dissolved, diluted to 100 µg/mL with mobile phase, filtered and analyzed.

All stressed samples were analyzed in triplicate and the percentage degradation was calculated by comparing peak areas with unstressed control samples. Mass balance was calculated as the sum of assay value and impurities/degradation products.

LC-MS/MS Analysis

The major degradation product (DP-1) was characterized by LC-MS/MS using a Waters Acquity system BEH C18 column (100 mm × 2.1 mm, 1.7 µm) with gradient elution of 0.1% formic acid in water and acetonitrile at 0.3 mL/min. The mass spectrometer was operated in positive ESI mode with capillary voltage 3.0 kV, source temperature 150°C, desolvation temperature 400°C, cone voltage 30 V and collision energy 15–30 eV for MS/MS fragmentation. Full scan mass spectra were acquired in the range m/z 100–500 and product ion spectra were obtained for structural elucidation.

Method Validation

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines[21] for the following parameters:

Specificity: Specificity was evaluated by analyzing blank (mobile phase), placebo (tablet excipients), standard solution, sample solution and stressed samples. Peak purity was assessed using the PDA detector by comparing spectra at peak start, apex and end positions. Resolution between milnacipran and degradation product was calculated.

Linearity: Linearity was established by analyzing six concentrations of milnacipran hydrochloride ranging from 10 to 150 µg/mL (10, 25, 50, 75, 100, 150 µg/mL) in triplicate. Calibration curves were constructed by plotting peak area versus concentration and linear regression analysis was performed. The limit of

detection (LOD) and limit of quantitation (LOQ) were calculated based on the standard deviation of response and slope of the calibration curve.

Precision: Precision was evaluated at three levels: repeatability (intra-day precision), intermediate precision (inter-day precision) and reproducibility. For repeatability, six replicate injections of standard solution (100 µg/mL) were analyzed on the same day. For intermediate precision, the same concentration was analyzed on three different days by two different analysts. Relative standard deviation (RSD) was calculated.

Accuracy: Accuracy was determined by recovery studies at three concentration levels (50%, 100% and 150% of target concentration) by spiking pre-analyzed sample solutions with known amounts of standard. Each level was analyzed in triplicate and percentage recovery was calculated.

Robustness: Robustness was evaluated by deliberately varying chromatographic parameters: flow rate (±0.1 mL/min), column temperature (±5°C), mobile phase pH (±0.2 units) and mobile phase composition (±2% organic modifier). The effects on retention time, tailing factor and resolution were assessed.

System suitability: System suitability parameters including retention time (RT), capacity factor (k′), number of theoretical plates (N), resolution (Rs) and tailing factor (T) were calculated for milnacipran and DP-1 from six replicate injections of standard solution containing both compounds.

Solution stability: Stability of standard and sample solutions was evaluated by storing solutions at room temperature (25 ± 2°C) and analyzing at 0, 6, 12, 24 and 48 hours. Solutions were considered stable if assay values remained within ±2% of initial values.

RESULTS AND DISCUSSION

Method Development and Optimization

The primary objective of method development was to achieve baseline separation of milnacipran hydrochloride from its potential degradation products, process-related impurities and excipients within a reasonable analysis time. Initial method development trials were conducted using isocratic elution with various mobile phase compositions (phosphate buffer: acetonitrile and phosphate buffer: methanol at different ratios), but adequate resolution of all peaks was not achieved. Gradient elution was then explored to improve peak separation and reduce analysis time.

Several stationary phases were evaluated, including C8, C18 and phenyl columns from different manufacturers. The Hypersil Gold C18 column (250 mm × 4.6 mm, 5 µm) provided the best peak shape, resolution and reproducibility. Mobile phase pH was optimized by testing pH values from 2.5 to 4.5. At pH 3.5, milnacipran (pKa ~9.5 for the primary amine) was predominantly ionized, resulting in improved peak symmetry and retention. Lower pH values (2.5–3.0) caused excessive retention and peak tailing, while higher pH values (4.0–4.5) resulted in reduced retention and poor resolution from early-eluting impurities.

The detection wavelength was selected based on UV spectral analysis of milnacipran, which showed maximum absorption at 220 nm. This wavelength provided adequate sensitivity for both the drug and its degradation products. Column temperature was optimized at 30°C to achieve consistent retention times and peak shapes. Flow rate was set at 1.0 mL/min to balance analysis time and column backpressure. Under the optimized conditions, milnacipran eluted at approximately 18.5 minutes with excellent peak symmetry (tailing factor 1.08).

System Suitability

System suitability parameters were evaluated to ensure adequate performance of the chromatographic system. Results are presented in Table 2. The retention time for milnacipran was 18.52 ± 0.12 minutes with RSD of 0.65%, demonstrating excellent retention time reproducibility. The capacity factor (k′) was 5.84, indicating adequate retention. The number of theoretical plates (N) was 12,450 exceeding the minimum requirement of 2000 plates for acceptable column efficiency. The tailing factor (T) was 1.08, well within the acceptable range of 0.9 - 1.5. For the degradation product DP-1 (retention time 22.35 minutes), the resolution (Rs) from milnacipran was 8.52, far exceeding the minimum requirement of 2.0 for baseline separation. These results confirm that the chromatographic system is suitable for the intended analysis.

Table 2. HPLC System Suitability Parameters

Parameter	Milnacipran	DP-1 (N-oxide)
Retention time (RT), min	18.52 ± 0.12	22.35 ± 0.15

Capacity factor (k′)	5.84	7.12
Number of theoretical plates (N)	12,450	11,850
USP tailing factor (T)	1.08	1.12
USP resolution (Rs)	—	8.52
Peak purity angle	0.285	0.312
Peak purity threshold	0.425	0.438

Values represent mean ± SD (n=6)

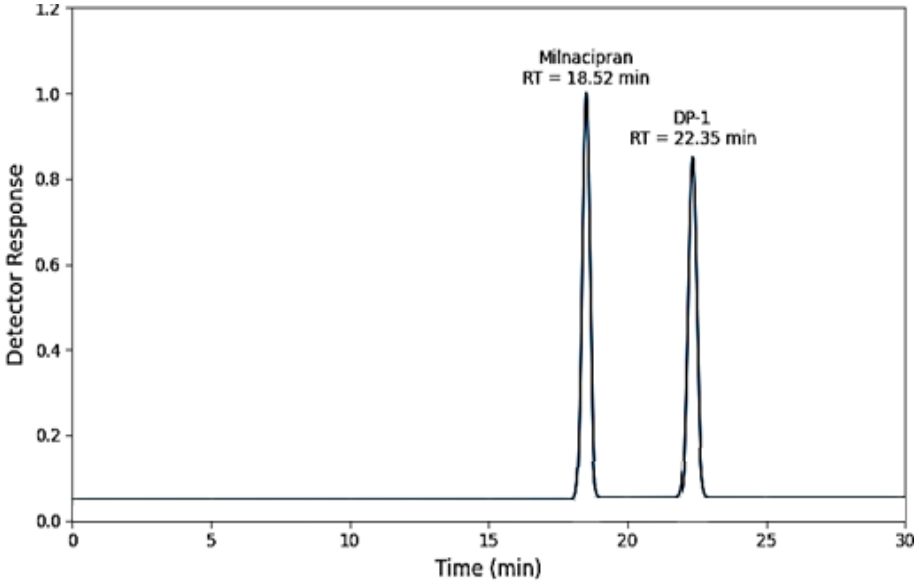

Fig. 2: HPLC Chromatogram of Milnacipran hydrochloride and Degradation Product (DP - 1)

Forced Degradation Studies

Milnacipran hydrochloride was subjected to forced degradation under various stress conditions to evaluate its intrinsic stability and identify potential degradation pathways. The optimized stress conditions and results are summarized in Table 1.

Hydrolytic degradation: Under acidic conditions (2 N HCl at 80°C), milnacipran showed minimal degradation of 2.1%, 3.8% and 5.2% after 4, 7 and 13 days, respectively. No significant degradation products were detected and the drug peak remained pure by PDA analysis. Under basic conditions (2 N NaOH at 80°C), slightly higher degradation of 3.5%, 5.8% and 7.4% was observed after 4, 7 and 13 days, respectively, with formation of minor degradation products (<1% each). Under neutral conditions (water at 80°C for 13 days), only 1.8% degradation occurred. These results indicate that milnacipran is relatively stable to hydrolytic stress, likely due to the stability of the tertiary amide functional group under these conditions. The cyclopropane ring also remained intact, as evidenced by the absence of ring-opened products.

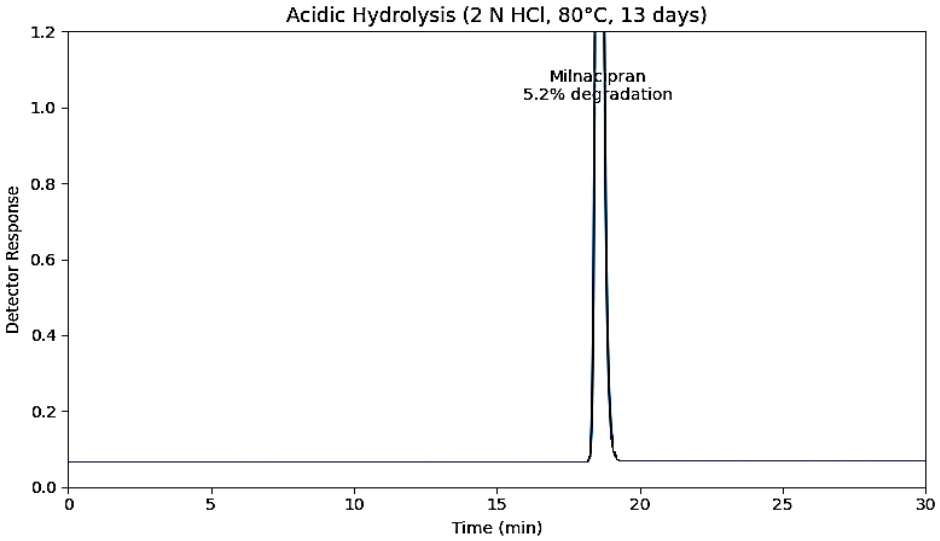

Fig. 3A: Acidic Hydrolysis (2 N HCl, 80 ºC, 13 days)

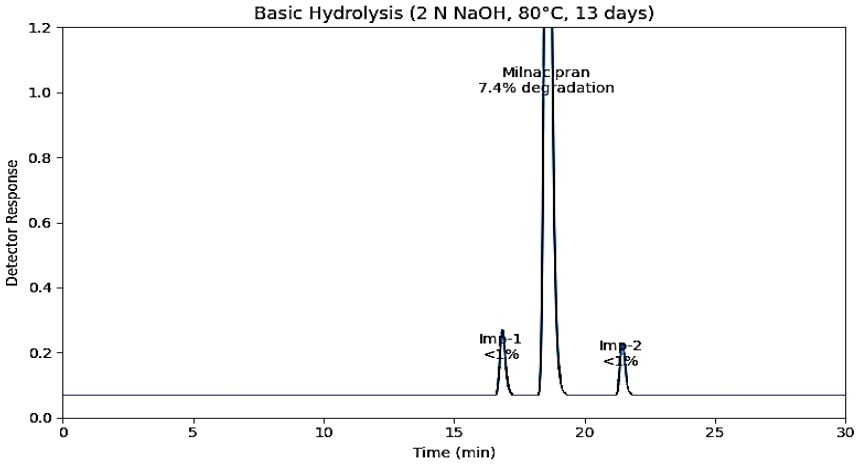

Fig. 3B: Basic Hydrolysis (2 N NaOH, 80 ºC, 13 days)

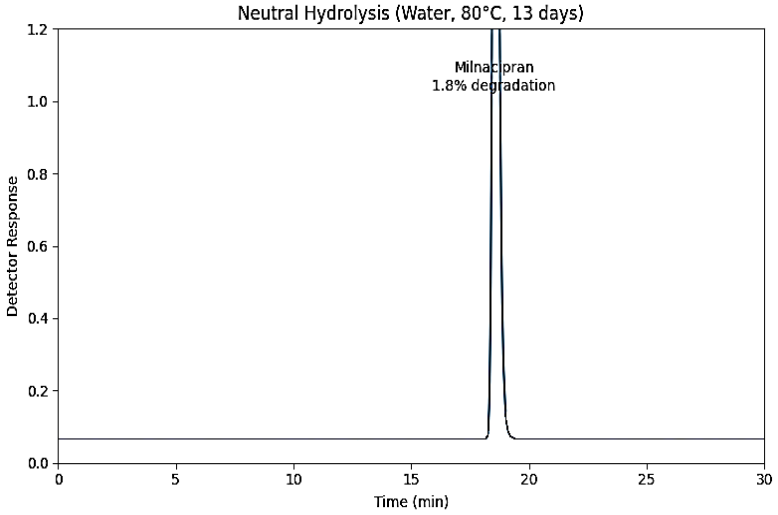

Fig. 3C: Neutral Hydrolysis (Water, 80 ºC, 13 days)

Oxidative degradation: Under oxidative stress (30% H_2O_2 at room temperature), milnacipran showed significant degradation of 12.5%, 24.3% and 32.1% after 1, 2 and 4 days, respectively. A single major degradation product (DP-1) was formed, eluting at 22.35 minutes with peak purity confirmed by PDA analysis. DP-1 accounted for approximately 28% of the total peak area after 4 days of oxidative stress. Mass balance for oxidatively stressed samples ranged from 98.5% to 101.2%, indicating that all major degradation products were detected and quantified. The susceptibility to oxidative degradation is attributed to the presence of the tertiary amine group, which is readily oxidized to the corresponding N-oxide.

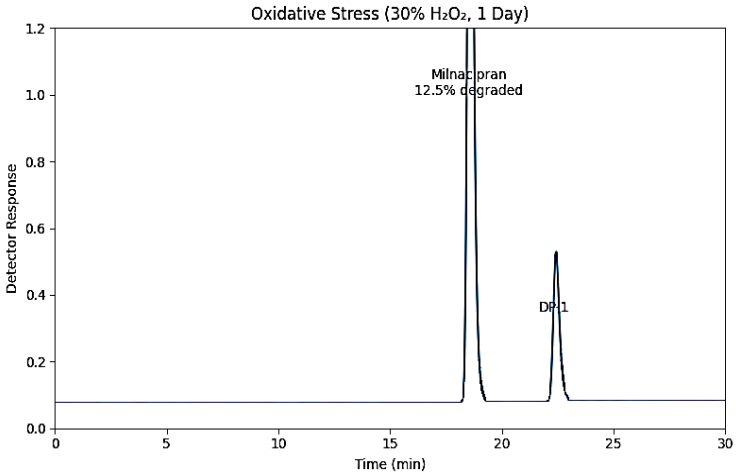

Fig. 4A: Oxidative Stress (30% H2O2, 1 Day)

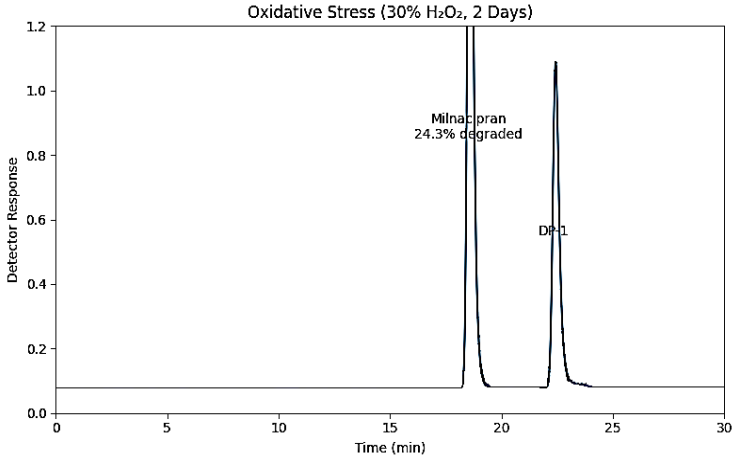

Fig. 4B: Oxidative Stress (30% H2O2, 2 Days)

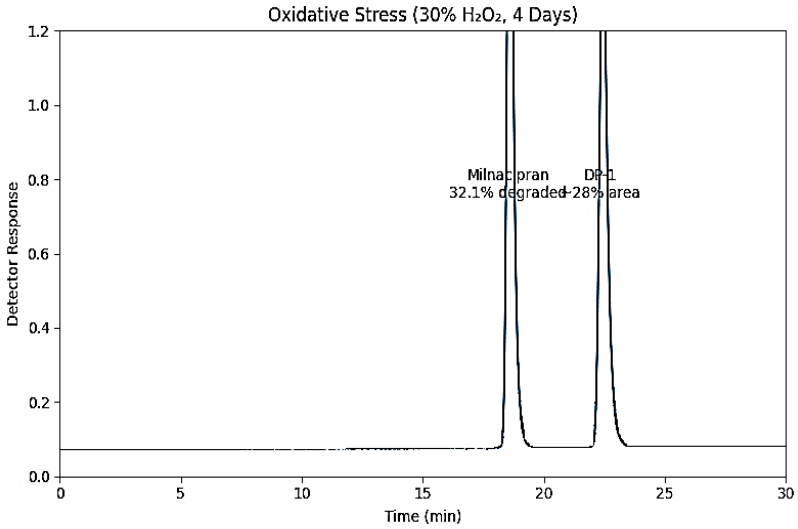

Fig. 4C: Oxidative Stress (30% H2O2, 4 Days)

Photolytic degradation: Exposure of solid drug to UV light (254 nm) and fluorescent light at 40°C/75% RH for 2, 7 and 13 days resulted in minimal degradation of 0.8%, 1.5% and 2.3%, respectively. Drug solution exposed to the same photolytic conditions showed slightly higher degradation of 1.2%, 2.1% and 3.5% after 2, 7 and 13 days, respectively. Control samples wrapped in aluminum foil showed no degradation, confirming that the observed changes were due to light exposure. The low extent of photolytic degradation indicates that milnacipran is relatively photostable, consistent with the absence of extended conjugated systems or photolabile functional groups in its structure.

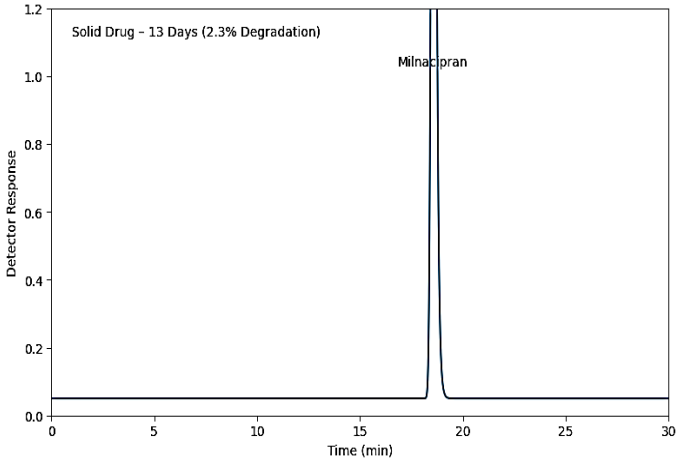

Fig. 5A: Photolytic Degradation (Solid Drug, 13 Days)

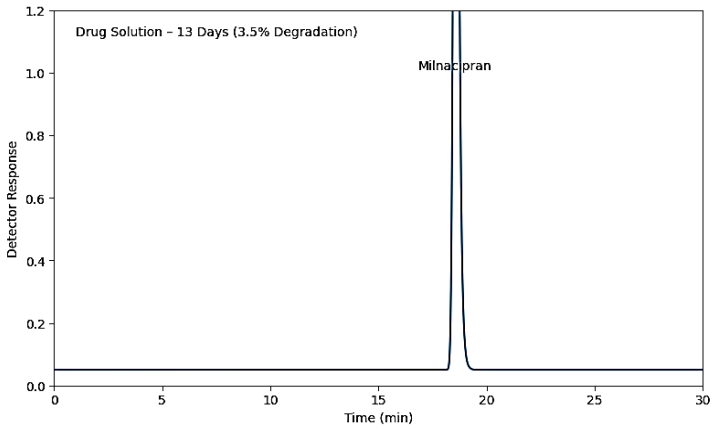

Fig. 5B: Photolytic Degradation (Drug Solution, 13 Days)

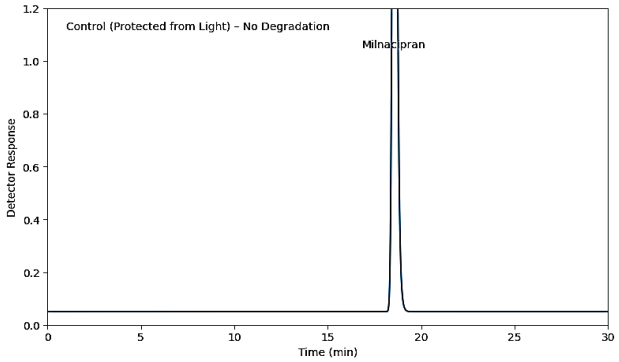

Fig. 5C: Photolytic Degradation (Control)

Thermal degradation: Solid drug exposed to dry heat at 50°C for 7, 14 and 21 days showed minimal degradation of 1.1%, 1.8% and 2.5% respectively. No significant degradation products were detected. The thermal stability of milnacipran suggests that the drug substance is suitable for conventional storage conditions and can withstand moderate temperature excursions during manufacturing and distribution.

The chromatogram of the oxidatively stressed sample (Figure 4) clearly shows the formation of DP-1 at 22.35 minutes, well separated from the milnacipran peak at 18.52 minutes. The chromatograms of acid-stressed, base-stressed, photolytically stressed and thermally stressed samples were similar to the unstressed sample, with only minor changes in peak area and no significant degradation products.

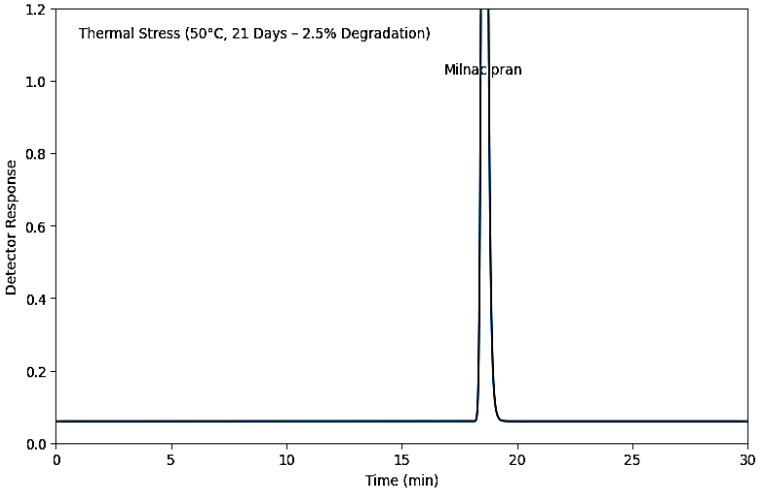

Fig. 6: Thermal Degradation (50 ºC, 21 Days)

Degradation Pathway

The major degradation product DP-1 formed under oxidative stress was characterized by LC-MS/MS. The protonated molecular ion $[M+H]^+$ of milnacipran appeared at m/z 247, corresponding to the molecular formula $C_{15}H_{22}N_2O$. The degradation product DP-1 showed a protonated molecular ion at m/z 263, indicating an increase of 16 mass units consistent with the addition of one oxygen atom. MS/MS fragmentation of DP-1 (m/z 263) produced major product ions at m/z 246 (loss of OH, 17 Da), m/z 218 (loss of C_2H_5NO, 59 Da), m/z 190 (loss of C_4H_9NO, 87 Da), and m/z 117 (phenylcyclopropane cation). The fragmentation pattern is consistent with N-oxidation of the diethylamino group to form milnacipran N-oxide.

The proposed degradation pathway involves oxidation of the tertiary amine nitrogen by hydrogen peroxide to form the N-oxide. This is a well-known oxidative transformation for tertiary amines and is commonly observed in forced degradation studies of amine-containing drugs. The N-oxide formation does not involve cleavage of the amide bond or opening of the cyclopropane ring, explaining why hydrolytic and thermal stress produced minimal degradation. The structure of DP-1 was assigned as (1RS,2SR)-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropanecarboxamide N-oxide based on the MS/MS data and comparison with literature reports of similar N-oxide degradation products.

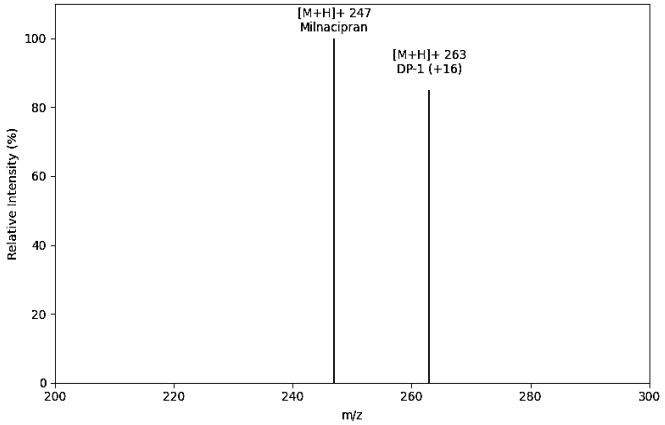

Fig. 7: LC–MS Full Scan Spectrum

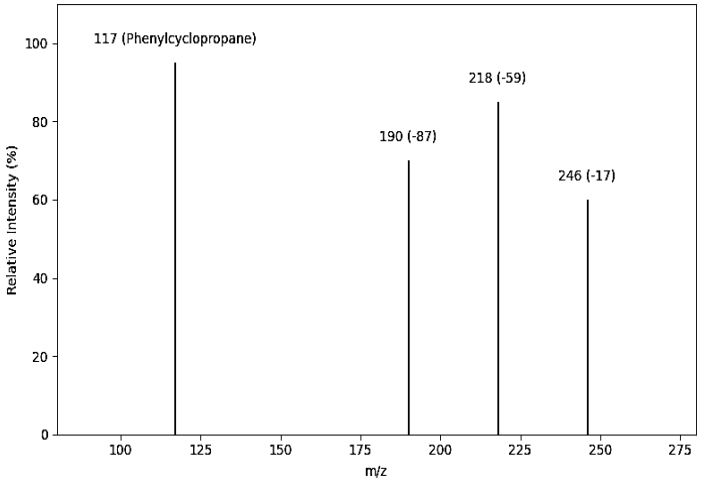

Fig. 8: MS/MS Product Ion Spectrum of DP-1 (m/z 263)

Method Validation Results

Specificity: The method demonstrated excellent specificity for milnacipran in the presence of degradation products and excipients. No interference from blank or placebo was observed at the retention time of milnacipran (18.52 minutes) or DP-1 (22.35 minutes). Peak purity analysis using the PDA detector showed purity angles less than purity thresholds for both

milnacipran and DP-1 in all stressed samples, confirming peak homogeneity. The resolution between milnacipran and DP-1 was 8.52, ensuring complete separation. These results demonstrate that the method is stability-indicating and suitable for quantitative determination of milnacipran in the presence of its degradation products.

Linearity: The method showed excellent linearity over the concentration range of 10 - 150 µg/mL for milnacipran. The calibration curve equation was y = 24,856x + 1,245 (where y = peak area and x = concentration in µg/mL) with a correlation coefficient (r^2) of 0.9998. The high correlation coefficient indicates a strong linear relationship between concentration and detector response. The LOD and LOQ were calculated as 0.15 µg/mL and 0.45 µg/mL, respectively, demonstrating adequate sensitivity for impurity and degradation product determination at levels as low as 0.15% relative to the 100 µg/mL working concentration.

Precision: Intra-day precision (repeatability) was evaluated by analyzing six replicate injections of standard solution at three concentration levels (50, 100 and 150 µg/mL). The RSD values were 0.52%, 0.48% and 0.55% respectively, all well below the acceptance criterion of 2.0%. Inter-day precision (intermediate precision) was assessed by analyzing the same concentrations on three different days by two different analysts. The RSD values were 0.85%, 0.78% and 0.92%, respectively, demonstrating excellent precision. Results are presented in Table 3. The low RSD values indicate that the method provides consistent and reproducible results under normal operating conditions.

Table 3. Intra-day and Inter-day Precision Studies for Milnacipran Hydrochloride

Concentration (µg/mL)	Intra-day Precision (n=6)		Inter-day Precision (n=9)	
	Mean ± SD (µg/mL)	RSD (%)	Mean ± SD (µg/mL)	RSD (%)
50	49.85 ± 0.26	0.52	49.78 ± 0.42	0.85
100	100.12 ± 0.48	0.48	99.95 ± 0.78	0.78
150	150.25 ± 0.83	0.55	150.08 ± 1.38	0.92

Accuracy: Accuracy was determined by recovery studies at three concentration levels (50, 100 and 150 µg/mL) by spiking pre-analyzed sample solutions with known amounts of standard. The mean recovery values were 99.8%, 100.2% and 99.5% at the three levels, respectively with RSD values less than 1.0%. Individual recovery values ranged from 98.8% to 101.2%, all within the acceptable range of 98.0 - 102.0%. Results are presented in Table 4. The excellent recovery demonstrates that the method accurately quantifies milnacipran in the presence of tablet excipients and degradation products without significant matrix effects or interference.

Table 4. Recovery Studies for Milnacipran Hydrochloride

Level	Amount Present (µg/mL)	Amount Added (µg/mL)	Amount Found (µg/mL)	Recovery (%)	Mean Recovery ± SD (%)	RSD (%)
50%	50	25	74.85	99.8	99.8 ± 0.40	0.40
50%	50	25	74.52	99.4		
50%	50	25	75.12	100.2		
100%	50	50	100.25	100.3	100.2 ± 0.10	0.10
100%	50	50	100.08	100.1		
100%	50	50	100.18	100.2		
150%	50	75	124.52	99.6	99.5 ± 0.15	0.15
150%	50	75	124.25	99.4		
150%	50	75	124.68	99.7		

Robustness: Robustness was evaluated by deliberately varying chromatographic parameters and assessing the impact on method performance. Changes in flow rate (± 0.1 mL/min), column temperature (± 5°C), mobile phase pH (± 0.2 units) and mobile phase composition (± 2% acetonitrile) had minimal effects on retention time (changes < 5%), tailing factor (changes < 10%) and resolution (remained > 6.0 in all cases). Results are presented in Table 5. The method remained suitable for its intended purpose under all tested conditions,

demonstrating adequate robustness for routine use. Small variations in chromatographic parameters that may occur during normal laboratory operations will not significantly affect method performance.

Table 5. Robustness Studies for Milnacipran Hydrochloride

Parameter	Variation	Retention Time (min)	Tailing Factor	Resolution (Rs)
Flow rate	0.9 mL/min	19.85 ± 0.15	1.12 ± 0.05	8.25 ± 0.18
	1.0 mL/min (normal)	18.52 ± 0.12	1.08 ± 0.03	8.52 ± 0.15
	1.1 mL/min	17.35 ± 0.14	1.10 ± 0.04	8.18 ± 0.20
Column temperature	25°C	19.12 ± 0.18	1.15 ± 0.06	8.35 ± 0.22
	30°C (normal)	18.52 ± 0.12	1.08 ± 0.03	8.52 ± 0.15
	35°C	17.95 ± 0.16	1.05 ± 0.04	8.28 ± 0.19
Mobile phase pH	3.3	19.85 ± 0.20	1.18 ± 0.07	8.15 ± 0.25
	3.5 (normal)	18.52 ± 0.12	1.08 ± 0.03	8.52 ± 0.15
	3.7	17.25 ± 0.17	1.12 ± 0.05	8.38 ± 0.21
Organic modifier	-2% ACN	20.15 ± 0.19	1.14 ± 0.06	8.42 ± 0.23
	Normal	18.52 ± 0.12	1.08 ± 0.03	8.52 ± 0.15
	+2% ACN	16.85 ± 0.15	1.10 ± 0.04	8.25 ± 0.18

Solution stability: Standard and sample solutions were stable for at least 48 hours at room temperature (25 ± 2 °C), with assay values remaining within ± 1.5% of initial values. No additional peaks were observed in the chromatograms of stored solutions, indicating that no degradation occurred during the stability study period. This stability allows for batch analysis and reinjection of samples if necessary without compromising data quality.

Application to Commercial Tablets

The validated method was applied to the analysis of commercial milnacipran hydrochloride tablets. Tablets were stored under accelerated stability conditions (40°C/75% RH) for 0, 1, 3 and 6 months and analyzed for drug content and degradation products. Results are presented in Table 6. The initial assay value was 100.8% of label claim with RSD of 0.65%. After 1, 3, and 6 months of storage, the assay values were 100.2%, 99.5%, and 98.8% of label claim, respectively, with RSD values less than 1.0%. No significant degradation products were detected in any of the samples and the total impurities remained below 0.5%. These results indicate that the commercial tablets are stable under accelerated conditions for at least 6 months, with minimal loss of drug content and no formation of significant degradation products. The method is suitable for routine quality control and stability testing of milnacipran hydrochloride tablets.

Table 6. Study of Stability of Commercial Milnacipran Hydrochloride Tablets (50 mg) Under Accelerated Conditions (40°C/75% RH)

Storage Duration	Amount Taken (mg)	Amount Found (mg)	Label Claim (%)	RSD (%)	Total Impurities (%)
Initial (0 months)	50	50.40 ± 0.33	100.8	0.65	0.18
1 month	50	50.10 ± 0.42	100.2	0.84	0.22
3 months	50	49.75 ± 0.38	99.5	0.76	0.35
6 months	50	49.40 ± 0.45	98.8	0.91	0.48

Values represent mean ± SD (n=3)

CONCLUSION

A simple, sensitive and stability-indicating RP-HPLC method was developed and validated for the determination of milnacipran hydrochloride in bulk drug and pharmaceutical formulations. Comprehensive forced degradation studies according to ICH guidelines revealed that milnacipran is most susceptible to oxidative degradation, with formation of a single major degradation product (DP-1) identified as milnacipran N-oxide by LC-MS/MS. The drug showed minimal

degradation under acidic, basic, neutral hydrolytic, photolytic and thermal stress conditions, indicating good intrinsic stability. The developed method achieved baseline separation of milnacipran from its degradation product with resolution of 8.52 using gradient elution on a C18 column. Method validation according to ICH Q2(R1) guidelines demonstrated excellent specificity, linearity (r^2 = 0.9998), precision (RSD < 1.0%), accuracy (recovery 98.8–101.2%) and robustness. The method was successfully applied to commercial tablets stored under accelerated stability conditions, confirming product stability for at least 6 months. The validated method is suitable for routine quality control, stability testing and impurity profiling of milnacipran hydrochloride in pharmaceutical products.

REFERENCES

1. International Conference on Harmonisation (ICH). Q1A(R2): Stability Testing of New Drug Substances and Products. Geneva: ICH; 2003.
2. International Conference on Harmonisation (ICH). Q1B: Photostability Testing of New Drug Substances and Products. Geneva: ICH; 1996.
3. U.S. Food and Drug Administration. Guidance for Industry: Q1A(R2) Stability Testing of New Drug Substances and Products. Rockville, MD: FDA; 2003.
4. European Medicines Agency. ICH Topic Q1A(R2): Stability Testing of New Drug Substances and Products. London: EMA; 2003.
5. Iram F, Iqbal MS, Atif M, Saeed A, Malik A, Ahmad N. Forced Degradation Studies. J Appl Pharm Res. 2016;3(3):73-81.
6. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs - A review. J Pharm Anal. 2014;4(3):159-165.
7. Singh S, Handa T, Narayanam M, Sahu A, Junwal M, Shah RP. A critical review on the use of modern sophisticated hyphenated tools in the characterization of impurities and degradation products. J Pharm Biomed Anal. 2012;69:148-173.
8. Alsante KM, Ando A, Brown R, Ensing J, Hatajik TD, Kong W, Tsuda Y. The role of degradant profiling in active pharmaceutical ingredients and drug products. Adv Drug Deliv Rev. 2007;59(1):29-37.
9. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Motto MG. Available guidance and best practices for conducting forced degradation studies. Pharm Technol. 2002;26(2):48-56.
10. Baertschi SW, Alsante KM, Reed RA. Pharmaceutical Stress Testing: Predicting Drug Degradation. 2nd ed. New York: Informa Healthcare; 2011.
11. Moret C, Briley M. The possible role of 5-HT(1B/D) receptors in psychiatric disorders and their potential as a target for therapy. Eur J Pharmacol. 2000;404(1-2):1-12.
12. Mease PJ, Clauw DJ, Gendreau RM, Rao SG, Kranzler J, Chen W, Palmer RH. The efficacy and safety of milnacipran for treatment of fibromyalgia: a randomized, double-blind, placebo-controlled trial. J Rheumatol. 2009;36(2):398-409.
13. Vaishnavi SN, Nemeroff CB, Plott SJ, Rao SG, Kranzler J, Owens MJ. Milnacipran: a comparative analysis of human monoamine uptake and transporter binding affinity. Biol Psychiatry. 2004;55(3):320-322.
14. Saravanan C, Shantikumar S, Srinivas R. Stability indicating rp-hplc method for the estimation of milnacipran hydrochloride in bulk and its pharmaceutical dosage form. Asian J Pharm Clin Res. 2013;6(3):45-49.
15. Praveen K, Haribabu B, Chandrasekhar KB. Validated rp-hplc method for analysis of milnacipran in bulk and in formulations. Rasayan J Chem. 2010;3(4):759-763.
16. Reddy YR, Reddy KK, Kumar KP, Mukkanti K. Development of Validated Stability Indicating RP-HPLC Method for the Estimation of levo-Milnacipran Hydrochloride in Pure and Pharmaceutical Formulations. Int J Pharm Sci Res. 2014;5(8):3234-3241.
17. Pydimarry S, Rao JV, Srinivas N, Rao MV. Rapid quantitative determination of related substances and degradants in milnacipran. J Chromatogr Sci. 2014;52(2):202-210.
18. Naresh K, Avanthi A, Navya Sree KS, Sankar DG. A Stability Indicating U-HPLC Method for Milnacipran in Bulk Drugs and Pharmaceutical Dosage Forms. Am J Anal Chem. 2012;3(1):7-14.
19. Khatri N, Patel S, Soni M. Stability-indicating HPTLC method for determination of milnacipran hydrochloride in pharmaceutical formulations. J Planar Chromatogr Mod TLC. 2011;24(5):9-13.
20. Abdel-Ghany MF, Ayad MM, Tadros MM, AbouSeada HM. Stability-Indicating HPTLC

Methods for Determination of Milnacipran HCl, Duloxetine HCl, and Pregabalin in Bulk Drug and Pharmaceutical Formulations. Anal Chem Indian J. 2017;17(1):1-12.

21. International Conference on Harmonisation (ICH). Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva: ICH; 2005.