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Dissolution Method for Milnacipran Hydrochloride Capsules: Development, Validation, and Study of Changes in Dissolution Rate after Storage

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ABSTRACT

A dissolution test for milnacipran hydrochloride capsules was developed and validated according to international guidelines. After selection of the best conditions, the method was validated using USP Apparatus 1 (baskets), 50-rpm rotation speed, 900 mL of 0.01 N HCl, and test time of 60 min. The drug released was determined by both LC–UV (PDA) and UV–D^2 methods. The kinetic parameters of drug release (mathematical models, $t_{80\%}$, and dissolution efficiency) were investigated, and the stability of the dosage form was evaluated by analyzing changes in the dissolution rate of milnacipran hydrochloride capsules during storage at 40 °C and 75% RH for different periods.

INTRODUCTION

Dissolution testing can provide information not only on the rate and extent of drug absorption in the body but also on the effects of drug biopharmaceutical properties and formulation principles on the release properties of a pharmaceutical product (*1*). Therefore, in vitro dissolution tests are usually used to assess the lot-to-lot quality of a drug product, guide development of new formulations, an d ensure continued product quality and performance after certain changes such as formulation, manufacturing process, site of manufacture, and the scale-up of the manufacturing process (*2*). The dissolution procedure requires an apparatus, a dissolution medium, and test conditions that provide a method that is discriminating yet sufficiently rugged and reproducible for day-to-day operation and capable of being transferred between laboratories. With regard to stability, the dissolution test should appropriately reflect relevant changes in the drug product caused by temperature, humidity, photosensitivity, and other stresses over time (*3*).

Milnacipran hydrochloride (MNC), [101152-94-7], $C_{15}H_{22}N_2O{\cdot}HCl$, molecular weight 282.81 g/mol (Figure 1), is a racemic mixture with the chemical name (±)-[1R(S),2S(R)]-2-(aminomethyl)-N,N-diethyl-1-phenylcyclopropanecarboxamide hydrochloride, and its solubility in water is 19 mg/mL (*4–6*). MNC is a selective serotonin and norepinephrine reuptake inhibitor (SNRI) indicated as an antidepressant and for the management of fibromyalgia. It shows preferential blockade of norepinephrine reuptake over serotonin and minimal activity at other receptors or transporters (*6–8*). MNC is well absorbed after oral administration with maximum concentrations reached within 2–4 h, and its absorption is not affected by food. It presents an absolute bioavailability of approximately 85–90% (*9*). The solubility and absolute bioavailability data for this drug classify it as Class I (high solubility and high permeability) based on the Biopharmaceutical Classification System. This is the case where the drug is well absorbed, and for immediate-release dosage forms that dissolve very rapidly, the absorption rate is controlled by the gastric emptying rate, and some correlation with dissolution rate is expected only if the dissolution is slower than gastric emptying (*10*).

Methods for quantitation of milnacipran in combination with other antidepressants and their metabolites in biological fluids have been proposed (*11–17*). However, there is no compendial method to assay milnacipran hydrochloride in pharmaceutical dosage forms. A stability-indicating liquid chromatographic method with UV detection (LC–UV) and a second-order derivative UV spectroscopic method (UV–D^2) for quality control of milnacipran in capsules were developed and validated by the authors (*18*) according to guidelines (*19–21*). Therefore, the purpose of this work was to develop and validate a dissolution test for MNC in capsules (50 mg) based on its physicochemical characteristics and apply the LC–UV and UV–D^2 methods to quantify the drug released from the capsules during the dissolution procedure. The kinetic parameters of drug release were investigated, and the stability of the dosage form was evaluated by analyzing changes in the dissolution rate of MNC capsules over time and in various storage conditions.

EXPERIMENTAL

Chemicals

Milnacipran hydrochloride was purchased from Synfine Research (Canada). The pharmaceutical dosage form

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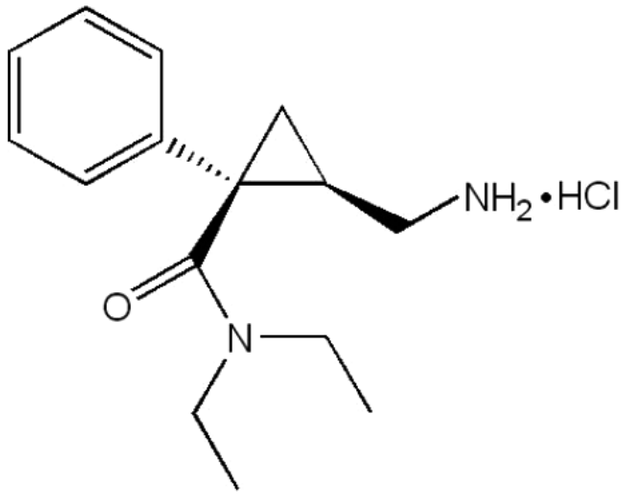

Figure 1. Structure of milnacipran hydrochloride.

(50 mg) containing the inactive ingredients dibasic calcium phosphate, povidone, carboxymethylcellulose calcium, colloidal silicon dioxide, magnesium stearate, and talc was commercially available (Pierre Fabre Médicament, Boulogne, França). All chemicals used were of analytical grade, and all solvents were of LC grade. Ultrapure water was from a Millipore system (Milli-Q Plus, Milford, MA, USA).

Apparatus

The development and validation of the dissolution test was performed using a VANKEL VK 8000 dissolution autosampling station consisting of a bidirectional peristaltic pump, VK 750D digitally controlled heater/circulator, and a VK 7010 multibath ($n = 8$) dissolution testing station with automated sampling manifold.

The LC system consisted of a Shimadzu (Kyoto, Japan) LC-20AT liquid chromatograph, SPD-M10A$_{VP}$ photodiode array detector (PDA), SIL-20A auto sampler, DGU-20A$_3$ degasser, CBM-20A system controller, and Class-VP data system equipped with a reversed phase column (Nucleosil C_8 endcapped, 150 × 4.6 mm, 100 Å, 5 µm) from Macherey–Nagel (MN). The mobile phase was 70:30:0.085 acetonitrile/water/triethylamine (v/v/v) at a constant flow rate of 1.5 mL/min at room temperature (23 ± 1 °C). The pH of the aqueous phase was adjusted to 7.5 with phosphoric acid. Aliquots of 20 µL were injected. The detector was set at a wavelength of 210 nm.

A UV–vis spectrophotometer (SHIMADZU, Japan) Model UV-1601 PC was used for spectroscopic measurements. The software employed was UVPC personal spectroscopy software, version 3.9. For all the tested solutions, the second derivative spectra (D^2) were recorded over the range 280–250 nm in a 1-cm quartz cell, fixing $\Delta\lambda$ at 4 nm and scaling factor of 200. The amplitude values of D^2 were measured at 268 nm for MNC, zero-crossing of inactive ingredients.

An Ultrabasic potentiometer (Denver, Colorado, USA) was used for pH determinations, and sample filtration was carried out using Vankel filters (qualitative, 35 µm).

Sink Conditions

The solubility was determined by dissolving the highest unit dose of the drug, in this case 50 mg, in 250 mL of each medium tested (i.e., 0.1 N HCl and pH 4.1 and 7.4 USP buffers) at 37 °C (*2*). Aliquots of 1 mL were removed after 15 min, transferred into 10-mL volumetric flasks, diluted with mobile phase (20 µg/mL), and analyzed ($n = 2$) by the LC method previously described.

Dissolution Testing Conditions

Dissolution testing was performed in compliance with *USP* (*3*) using baskets (Apparatus 1) at 50 rpm, in 900 mL of 0.01 N HCl at 37 ± 0.5 °C. Manual sampling aliquots (5 mL) were withdrawn at 5, 10, 15, 30, and 60 min and immediately filtered. No replacement of the medium was done. The percentage of drug dissolved was determined using both LC–UV and UV–D^2 methods for the same samples to compare the results, using dissolution medium as a blank.

The reference substance solution was prepared using an amount of powder equivalent to 10 mg of MNC that was transferred to a 10-mL volumetric flask and diluted to volume with 0.01 N HCl (1.0 mg/mL). A 3.0-mL aliquot of this solution was transferred to a 50-mL volumetric flask and diluted with dissolution medium, for a final concentration of 60 µg/mL.

Dissolution Method Validation

The proposed method was validated using both LC–UV and UV–D^2 methods to assay the dissolved drug. Specificity, linearity, accuracy, and precision were evaluated (*2*, *3*).

Stability of Standard and Sample Solutions

The stability of MNC reference substance stock solution and capsule solutions in dissolution media was evaluated after preparation and collection ($n = 3$) at room temperature (23 ± 1 °C) and after 72 h stored in a refrigerator (8 ± 2 °C).

Specificity

The usual concentration of excipients contained in the pharmaceutical formulation was based on the literature (*22*). An amount equivalent to one capsule was transferred to a vessel with 900 mL of medium at 37 ± 0.5 °C and stirred for 1 h at 50 rpm using USP Apparatus 1. Aliquots of this solution were filtered before LC and UV–D^2 analysis.

Linearity

Aliquots of MNC standard solution (200 µg/mL) in 0.01 N HCl were transferred to 20-mL volumetric flasks and diluted with dissolution medium to achieve five different concentrations: 1.0, 30.0, 60.0, 90.0, and 120.0 µg/mL for LC analysis and 5.0, 30.0, 60.0, 90.0, and 120.0 µg/mL for UV–D^2. The solutions were analyzed in triplicate every day for three consecutive days. Linearity was evaluated by linear regression analysis using analysis of variance (ANOVA).

Table 1. Mathematical Models to Represent the Drug Dissolution Profiles

Zero-order kinetics	$Q_t = Q_0 + K_0 t$
First-order kinetics	$\log Q_t = \log Q_0 + (K_1 t) / 2.303$
Higuchi model	$f_t = K_H t^{1/2}$
Hixson–Crowell model	$W_0^{1/3} - W_t^{1/3} = K_s t$

Q_t: amount of drug dissolved in time t.
Q_0: initial amount of drug in solution.
K_0 and K_1: zero-order and first-order release constants, respectively.
f_t: amount of drug released in time t by surface unity.
K_H: Higuchi dissolution constant.
W_0: initial amount of drug in the pharmaceutical dosage form.
W_t: amount of drug in the pharmaceutical dosage form at time t.
K_s: constant incorporating the surface–volume relationship.

Accuracy and Precision

Accuracy was evaluated by the recovery of known amounts of MNC reference substance added to the placebo. Aliquots of 0.45, 5.4, and 9 mL of the standard solution (10 mg/mL) plus an amount of excipients equivalent to one capsule were added to the vessels (900 mL) containing dissolution medium at 37 ± 0.5 °C and agitated for 60 min with baskets at 50 rpm. The final concentrations were 5.0, 60.0, and 100.0 µg/mL. The analyses were done in duplicate on three different days. Repeatability (intraday) and intermediate precision (interday) were evaluated based on RSD from the recovery data.

Release profiles comparison

Student's *t* test was used to verify whether the two methods (LC–UV and UV–D^2) are equivalent for the determination of drug release. The difference factor (f_1) and similarity factor (f_2) were used to compare the dissolution profiles, using the LC–UV method as the reference:

$$f_1 = \left\{ \left[\sum_{t=1}^{n} |R_t - T_t| \right] \Big/ \left[\sum_{t=1}^{n} R_t \right] \right\} \times 100$$

$$f_2 = 50 \cdot \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where n is the number of time points, R is the dissolution value obtained using the reference method at time t, and T is the dissolution value obtained using the test method at the same time.

Kinetic Parameters

Four mathematical models were applied to evaluate the kinetics of drug release: zero-order, first-order, Higuchi, and Hixon–Crowell according to equations described in Table 1. Only one point greater than 80% drug released was used. The mathematical model that best expressed the dissolution profile of MNC capsules was selected based on the coefficient of determination (R^2) or on the adjusted coefficient of determination (R_{adj}^2) values, when comparing models with different numbers of parameters. The dissolution efficiency (%*DE*) and sampling time for ≥80% of drug dissolution ($t_{80\%}$) were used to characterize the drug-release profile. Frequently, pharmacopoeias use this parameter as an acceptance criterion for the quantity of active ingredient dissolved expressed as a percentage of the labeled content (*Q*) (*3*, *23*).

Table 2. Solubility Test of Milnacipran Hydrochloride

Medium (37 °C)	pH	% Drug Dissolved[a,b] (15 min)	RSD
0.1 N HCl	1.0	101.2	0.55
Acetate Buffer	4.1	101.4	0.55
Phosphate Buffer	7.4	101.9	0.69

[a] 10 mg of drug in 50 mL of medium with magnetic shaking.
[b] Analyzed by LC method (n = 2).

Stability of the Pharmaceutical Dosage Form

Drug release from capsules may change because of the reaction of the capsule shells with the contents as well as aging. Capsules prepared from gelatin are physically unstable when water content is outside the range of 12–18%. Excipients and water content of dosage forms can affect the dissolution characteristics of drug product over time, and can affect its functional stability. Higher water content generally causes a larger change in dissolution behavior (*24*).

Changes in the dissolution rate of MNC capsules past their expiration date of 12 months after production, capsules stored at room temperature, and capsules within their expiration date after storage at 40 °C and 75% RH for two and four weeks were evaluated. In the second case, samples were stored in desiccators, using saline solution (16.7% NaCl) to maintain humidity.

RESULTS AND DISCUSSION

Dissolution Testing Conditions

Dissolution testing should be carried out under physiological conditions, if possible, allowing interpretation of dissolution data with respect to the in vivo performance of a drug product. However, it is critical that dissolution methods developed for use as quality control (QC) tools consistently deliver reliable test results and assess drug product quality attributes (e.g., particle size, polymorphic form, or excipients). For QC purposes in general, the simplest dissolution medium is preferred whenever possible, regardless of the dosage form (*1*, *2*).

The results from the solubility test (Table 2) show that MNC was soluble in all media tested (0.1 N HCl, pH 4.1 and 7.4 USP buffer solutions), suggesting that sink condition was achieved over the pH range evaluated. Since MNC presents high solubility, it could exhibit a fast dissolution rate in any medium. Therefore, the capsules were tested in 900 mL of 0.01 N HCl (pH 2.0), a dissolution medium that

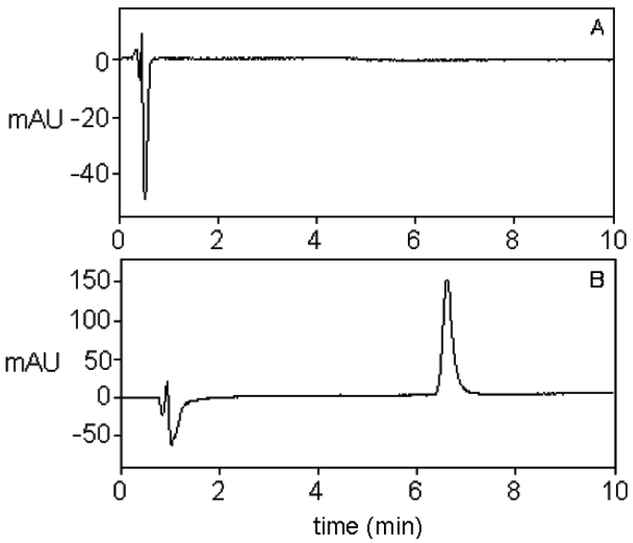

Figure 2. Specificity chromatograms of (A) placebo and (B) standard solution (60 µg/mL) of milnacipran hydrochloride in dissolution medium (0.01 N HCl).

reflects gastric pH, and the most likely site of dissolution for an immediate-release solid oral dosage form.

Dissolution Method Validation

MNC remained stable after 2 h at room temperature as well as for 72 h under refrigeration in standard and sample solutions in 0.01 N HCl subjected to stability testing. The results ranged from 99.8 ± 0.22% to 100.2 ± 0.22% for solutions at room temperature, and from 100.0 ± 0.21% to 100.4 ± 0.21% under refrigeration (average ± RSD). No degradation was observed, and the MNC peak remained pure through PDA analysis.

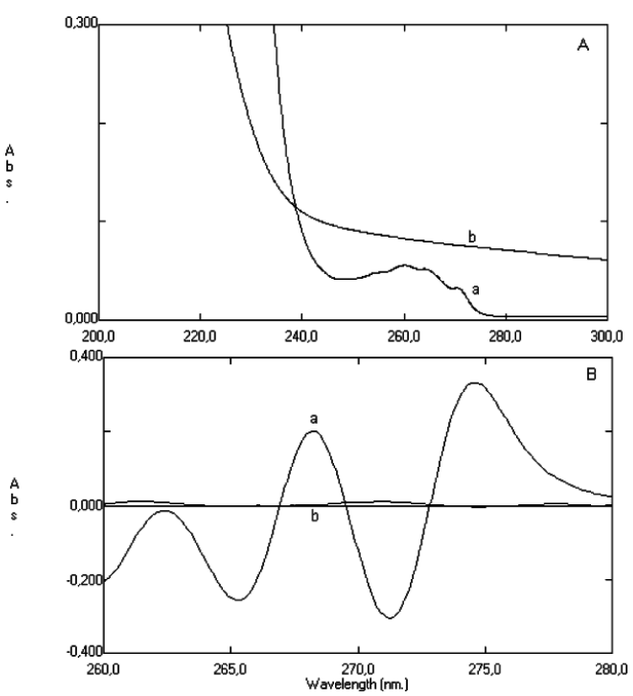

Figure 3. (A) UV-zero-order spectra and (B) UV–D² spectra of (a) milnacipran hydrochloride standard solution (60 µg/mL) and (b) placebo solution in 0.01 N HCl.

Table 3. Accuracy of the Dissolution of Milnacipran in Capsules

Standard solution (µg/mL)	Mean Recovery % (*n* = 6)	
	LC method	UV–D²
5	97.3	99.5
60	98.2	97.9
100	98.0	97.9
Mean absolute recovery (%) ± SE (*n* = 18)	97.8 ± 0.32	98.4 ± 0.50

The specificity of the LC–UV dissolution test method using a PDA detector demonstrated no excipient interference (Figure 2). The same analysis was done using the UV–D² method. The results suggest that the spectroscopic method could also be used for MNC assay in dissolution testing, since the formulation excipients had no interference when using second derivative UV at 268 nm, $\Delta\lambda = 4$ nm, and a scaling factor of 200 (Figure 3), considering the results of the stability test.

Linearity was suitable for both methods at the concentration ranges of 1–120 µg/mL (LC) and 5–120 µg/mL (UV–D²). Correlation coefficients (*r*) were greater than 0.999 for both methods. The average equations for three calibration curves were $y = 36153\,x + 8059$ (LC) and $y = 0.0032x - 0.0016$ (UV–D²). ANOVA showed significant linear regression and no significant deviation from linearity ($p < 0.05$). The measured recovery was typically 95–105% of the amount added (*3*). The accuracy of the methods was considered adequate in the range of 95.53–104.51% for MNC (Table 3). Repeatability and intermediate precision were evaluated at three different concentration levels (5.0, 60.0, and 100.0 µg/mL) over three days. The low RSD values (≤5%) demonstrate the good precision of both methods. Results are presented in Table 4.

Table 4. Repeatability and Intermediate Precision of the Dissolution of Milnacipran in Capsules

	Mean observed value (%) ± SE		RSD (%)	
Intraday (*n* = 6)	LC	UV–D²	LC	UV–D²
day I	97.2 ± 0.48	98.1 ± 0.93	1.22	2.33
day II	97.4 ± 0.47	98.9 ± 1.19	1.19	2.94
day III	98.9 ± 0.48	98.3 ± 0.39	1.16	0.98
Interday (*n* = 3)	97.8 ± 0.53	98.4 ± 0.25	0.94	0.45

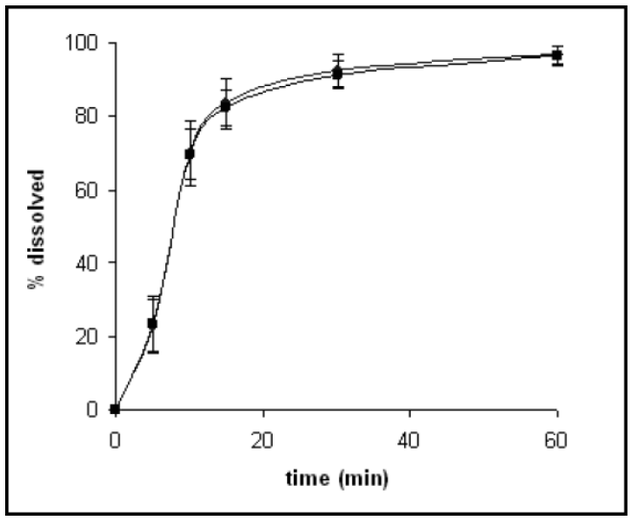

Figure 4. Dissolution profiles of 50-mg milnacipran hydrochloride capsules (n = 12). The values correspond to an average of 12 determinations (±SD) by (▲) UV–D²and (■) LC–UV.

Kinetic Parameters and Release Profiles Comparison

Drug-release kinetics was evaluated through the dissolution profiles (Figure 4). According to the R^2 or R_{adj}^2 valu es, dissolution profiles were best described by the Hixson–Crowell model (Table 5). This model assumes that the release rate is limited by drug particle dissolution rate (*23*).

The sampling times for 80% of drug dissolution ($t_{80\%}$) using the Hixson–Crowell model were 13.3 min (K_S = 0.1449) by LC–UV and 12.9 min (K_S = 0.1488) by UV–D². According to the acceptance limits for highly soluble and rapidly dissolving drug products, Q = 80% in 60 min or less is sufficient as a routine quality control test (*2*, *3*). The dissolution efficiency (% ± SE) of the pharmaceutical dosage form, defined as the area under the dissolution curve up to a certain time expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time (*23*), was 79.8 ± 1.06% by LC–UV and 80.5 ± 1.25% by UV–D².

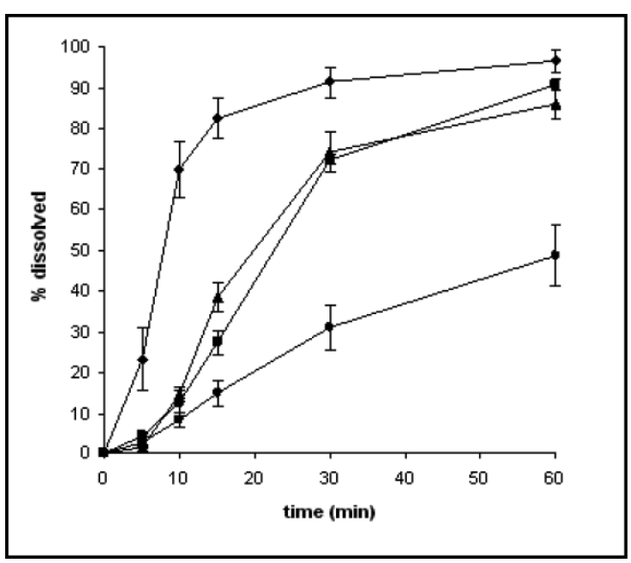

Figure 5. Dissolution profiles of milnacipran hydrochloride capsules (♦) before storage, (•) after storage at room temperature and post expiration date, and after storage at 40 °C and 75% RH for (▲) 2 weeks and (■) 4 weeks.

Table 5. Coefficient of Determination (R^2) and Adjusted Coefficient of Determination (R^2_{adj}) of the Mathematical Models

	LC–UV		UV–D²	
Model	R^2	R_{adj}^2	R^2	R_{adj}^2
Zero-order kinetics	0.9581	0.8953	0.9590	0.8975
First-order kinetics	0.8181	0.5453	0.8230	0.5575
Higuchi model	0.8947	0.8245	0.8899	0.8165
Hixson–Crowell model	0.9651	0.9128	0.9652	0.9130

The *t*-test was used (t = 2.03 for 34 degrees of freedom) to compare both methods. Nonsignificant difference was found at the 95% confidence level (p = 0.05), with the t statistic (1.06) being less than the critical value. The drug-release profiles obtained by LC–UV and UV–D² were compared using the difference factor (f_1 = 0.67) and similarity factor (f_2 = 92.52). The results indicate that the curves are similar because f_1 was less than 15 and f_2 was greater than 50.

Stability of Pharmaceutical Dosage Form

Pharmaceutical dosage forms are complex systems composed of active pharmaceutical ingredients and various excipients that may undergo both chemical and physical degradation. It is generally accepted that the dissolution rate at room-temperature storage cannot be predicted from shorter-term storage under accelerated conditions of high temperature and humidity. On the other hand, some examples suggest that stability evaluation by accelerated tests may be possible. It is difficult to describe changes in dissolution or drug-release rates during storage by kinetic equations because of the complicated and varied mechanisms involved. However, some attempts have been made, and various empirical relationships noted (*24*). There was a decrease in dissolution rate for MNC capsules stored at 40 °C and 75% RH for two and four weeks, as well as for capsules after the expiration date (Figure 5), and the sampling times for 80% of drug dissolution were calculated, as shown in Table 6. As mentioned before, these changes are well known and have been previously reported. However, large changes in drug dissolution characteristics on long-term storage of the dosage form indicate that functional changes are occurring in the drug product and may compromise the in vivo performance (*24*).

Table 6. Sampling Time for 80% Drug Dissolution after Stability Studies

	Hixson–Crowell model (LC–UV)	
Storage	**$t_{80\%}$ (min)**	**K_S**
40 °C and 75% RH (2 weeks)	42.1	0.0458
40 °C and 75% RH (4 weeks)	62.6	0.0308
Room temperature (after expiration)	119.0	0.0162

CONCLUSIONS

A dissolution method for milnacipran hydrochloride capsules was developed and validated as a quality control test. The best condition for dissolution testing is a dissolution medium of 0.01 N HCl (37 ± 0.5 °C) in baskets at 50 rpm. The LC–UV and UV–D^2 methods were used to analyze the percentage of drug dissolved versus time, and both presented acceptable specificity, linearity, precision, and accuracy. The kinetic analysis of the dissolution process is best described by the Hixson–Crowell model. The kinetic parameters (K_S and $t_{80\%}$) estimated by the model and DE (%) show that the formulation performed according to the proposed acceptance criteria.

Dissolution characteristics of milnacipran hydrochloride capsules subjected to different storage conditions were studied to observe changes in dissolution rate, and a slower dissolution profile was observed. Even considering the high solubility and fast absorption of MNC, the observed changes in dissolution profile were pronounced, which may affect in vivo performance, especially with capsules approaching the expiration date. However, it is important to note that the method is sensitive to these changes.

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