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Artifactual formylation of the secondary amine of duloxetine hydrochloride by acetonitrile in the presence of titanium dioxide: Implications for HPLC method development

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ABSTRACT

Duloxetine hydrochloride, a secondary amine containing pharmaceutical, currently marketed as Cymbalta™, is shown to undergo N-formylation as an artifact of sample preparation prior to HPLC analysis for impurities. The reaction was discovered as a result of an investigation into variability in the levels of N-formyl duloxetine observed upon HPLC analysis. The reaction is catalyzed by sonication and/or light in the presence of titanium dioxide and is proposed to occur via a radical-initiated mechanism. The mechanism is supported by controlled sample preparation studies with deuterium-labeled acetonitrile and LC/MS studies that showed incorporation of one deuterium into N-formyl duloxetine. This discovery is broadly relevant because sonication is commonly used to aid dissolution of pharmaceuticals in acetonitrile for HPLC analysis, titanium dioxide is a commonly used excipient, the amount of light found in modern analytical laboratories is sufficient to cause the reaction to occur, and secondary amines are present in the structures of many pharmaceuticals. The artifactual reaction was effectively eliminated by changing the sample solvent to methanol and replacing sonication with shaking to aid sample dissolution.

1. Introduction

Duloxetine hydrochloride (**1**, Fig. 2) is a potent selective serotonin and norepinephrine reuptake inhibitor that is currently marketed for major depressive disorder using the trade name Cymbalta™ and is marketed under the tradename of Yentreve™ for the treatment of stress urinary incontinence. Because duloxetine hydrochloride is unstable in solution at pH values less than approximately 2.5 [1], it has been developed as enteric-coated pellets using the polymer hydroxypropylmethylcellulose acetate succinate (HPMCAS). A color coating containing titanium dioxide is applied to the pellets and these pellets are filled into gelatin capsules. Dose strengths between 20 and 60 mg per capsule are obtained by filling different amounts of the same pellets into gelatin capsule shells.

During stress and accelerated stability studies of the formulated pellets, the degradation product N-succinoyl duloxetine (**2**) was detected [2]. Additional stress studies show that N-formyl duloxetine (**3**) and N-acetyl duloxetine (**4**) also form as degradation products in this formulation (Figs. 1 and 2). The formation of N-formyl and N-acetyl derivatives of an analogous secondary amine compound resulting from degradation in a carbohydrate-containing formulation has been previously described for fluoxetine hydrochloride [3]. The origin of **3** and **4** was proposed to be from carbohydrate degradation to unknown formylating and acetylating species, possibly via Maillard chemistry.

To monitor known degradation products, including **2**–**4**, stability-indicating analytical methods were developed using HPLC with UV detection. During registration stability studies, the analytically observed levels of **3** were highly variable and were not correlated with time under either long-term (25 °C/60% RH) or accelerated (40 °C/75% RH) stability conditions. The investigation into the origin of this variability is described in this report.

2. Materials and methods

2.1. Materials

Acetonitrile-d3 was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). The house de-ionized water was used without further treatment. Bulk enteric-coated pellets were obtained from Eli Lilly and Company. Titanium dioxide, obtained from Shionogi USA, Inc. (Florham Park, NJ) was used as is. The

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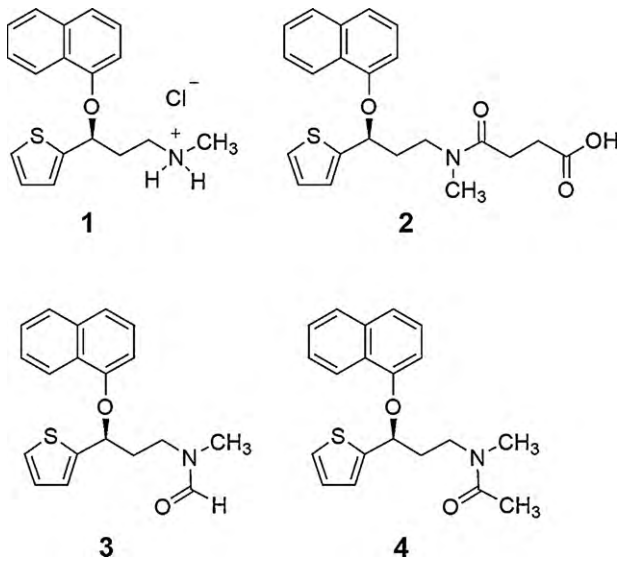


Fig. 1. Duloxetine and potential duloxetine degradation products in duloxetine capsules (**1** = duloxetine, **2** = N-succinoyl duloxetine, **3** = N-formyl duloxetine, and **4** = N-acetyl duloxetine).

pellet color coating, color mixture white, was obtained from Chr. Hansen, Inc., Mahwah, NJ. 2,2′-Azobisisobutyronitrile (AIBN) 98% was obtained from Sigma–Aldrich, St. Louis, MO. A Branson Model 8200 ultrasonic bath (Branson Ultrasonics Corp., Danbury, CT) is typical of the ultrasonic baths used for this work.

N-formyl duloxetine was prepared as follows: about 2 ml of furan in 120 ml of ethyl acetate was cooled to between −70 and −75 °C. Ozone, in air, was added to the solution, over about 2 h, using a gas dispersion tube. The ozone was sparged. An equivalent of duloxetine base (8.58 g in 12 ml of ethyl acetate) was added dropwise to the solution over 5 min. The mixture was allowed to stir for an additional 10 min (−70 to −75 °C) after which it was allowed to warm to room temperature with stirring over about 1 h. The mixture was transferred into a stirring solution of sodium dithionide (3 equiv.) in 180 ml of water which was stirred at room temperature for about 1 h. The layers were separated and the organic phase washed with 120 ml of saturated sodium bicarbonate. The organic phase was dried over sodium sulfate. Removal of the solvent yielded an oil that was purified chromatographically using a 12″ × 3″ silica column. Ethyl acetate–hexane (55:45) was used as the eluent. Collection of the product fraction followed by solvent removal yielded an oil that solidified on standing. N-formyl duloxetine was obtained in about 90% yield. The purity of the oil was determined to be 98.8% on an "as is" basis.

Table 1
Summary of method conditions.

Method parameter	Description
Column	ACT ACE C-8 75 mm × 4.6 mm id/3 μm
Column temperature	45–50 °C
Mobile phase	90:323:587 (tetrahydrofuran–methanol–pH 5.5 phosphate buffer)
Detection wavelength	230 nm at about 0.5 AUFS
Flow rate	1.5 ml/min
Injection volume	10 μl
Sample solvent	40:60 (acetonitrile–pH 8.1 phosphate buffer (aq))
Sample concentration	About 0.10 mg/ml
Typical retention time of duloxetine	About 2.6 min

2.2. HPLC–UV method

The conditions used in the HPLC determination of duloxetine degradation products are summarized in Table 1.

2.3. Sample solvent

The sample solvent buffer was prepared by dissolving about 216 mg of ammonium phosphate, monobasic and 4.5 g of potassium phosphate, dibasic, in 1 l of de-ionized water. The pH of the solution is between 7.9 and 8.3. A 40:60 solution (acetonitrile-buffer) was used as the sample solvent.

2.4. Pre-treatment of acetonitrile with radical initiator

AIBN was added to a solution of acetonitrile:water (80:20, v/v) at a concentration of 1 mg/ml and stored for 3 days at 40 °C. This aqueous acetonitrile solution was diluted using the pH 8.1 phosphate buffer solution to obtain a solution of 40% acetonitrile, 60% aqueous buffer solution.

2.5. General procedure for sample preparation

The contents of the five duloxetine capsules are transferred to a 200-ml volumetric flask. About 100 ml of sample solvent is then added and the mixture is shaken between 15 and 20 min on a wrist-action shaker. To ensure that any remaining intact pellets disintegrate and the duloxetine is dissolved, the mixture is sonicated for a maximum of 8 min. The flasks are diluted to volume with sample solvent and mixed well. Appropriate dilutions are made to obtain a final concentration of about 0.1 mg/ml duloxetine. Prior to chromatography, the final solutions are filtered using a 0.45 μm PTFE filter. Shaking was performed using a Burrell Model 75 wrist-

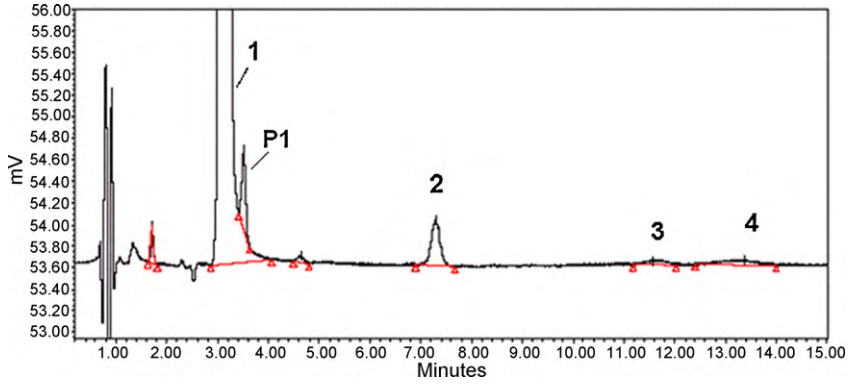


Fig. 2. Chromatogram obtained after exposure of enteric-coated pellets to 70 °C/75% RH for about 6 h (**1** = duloxetine, P1 = process impurity **2** = N-succinoyl duloxetine, **3** = N-formyl duloxetine and **4** = N-acetyl duloxetine Conditions are as described in Table 1.

Table 2
Experimental design for sonication and light experiments.

Exp. No.	Light	TiO_2	Sonication
1	Indirect window-filtered sunlight (48 h)	Yes	No
2	Light excluded (48 h)	Yes	No
3	Light excluded	Yes	30 min
4	Ambient lab lighting	Yes	30 min

action shaker while sonication was performed using a Branson Model 8200 ultrasonic bath.

2.6. Procedure to test the effects of sonication and light

Table 2 shows the experimental design used to evaluate the impact of sonication and light upon N-formyl duloxetine formation. Duloxetine hydrochloride was dissolved in 40:60 (acetonitrile:pH 8 buffer) to yield a final concentration of 0.1 mg/ml, as duloxetine (free base). For those experiments with titanium dioxide present, 140 mg was used (a significant excess when compared to the amount that would be present in the dissolved sample from the colorant in the formulation). Twenty five milliliters aliquots of the reference standard solution were added to separate glass-stoppered flasks. Using a Gigahertz-Optik X1 Optometer, the intensity of the window-filtered indirect sunlight was determined to be 0.56 W/m^2 in the UVA region and about 3300 lux at mid-day. Experiments 3 and 4 were conducted under ambient laboratory lighting (cool white fluorescent lighting, ~1000 lux) and were sonicated immediately after preparation. Light was excluded as shown in the experimental design by wrapping the flasks with aluminum foil.

All mixtures containing titanium dioxide were filtered prior using 0.45 μm PTFE filters. The standards solutions and all tests solutions were transferred to amber vials and analyzed by the HPLC–UV method. N-formyl duloxetine levels were estimated by comparing the N-formyl duloxetine response to that of duloxetine in the reference standard solution.

2.7. LC/MS

All LC/UV/MS data were collected using an Agilent 1100 HPLC Agilent Technologies, Inc. Santa Clara, CA) combined with a Waters PDA detector and a Micromass LCT time-of-flight mass spectrometer equipped with an electrospray ionization (ESI) interface (Waters Corp, Milford, MA). Table 3 contains a list of the HPLC, ESI, and MS operating parameters.

Preliminary LC/UV/MS measurements involved collecting centroided mass data for the entire 20 min of analysis. Once the conditions were optimized for UV and MS detection, subsequent analyses utilized continuum mode MS data collection from 12 to 20 min, while UV data were still collected for the entire measurement period. This reduced collection window for MS data allowed detection in the chromatographic retention window where **3** is eluted while limiting the size of the LC/MS data file.

3. Results

During development of the formulated product, an HPLC stability-indicating method was developed (see summary of method conditions in Table 1). The composition of the sample solvent differs significantly in composition from that of the mobile phase. While the development of the mobile phase composition was dictated by specificity requirements, that of the sample solvent was dictated by the solubility of the enteric coating, duloxetine recovery, and duloxetine stability.

Table 3
HPLC, ESI, and MS operating conditions for the LC/UV/MS analysis of duloxetine and the related substance N-formyl duloxetine.

HPLC conditions	
Column	75 mm × 4.6 mm ACE 3 C_8
Flow rate (ml)	1.5
Injection volume (μl)	100
Run time (min)	20
Detection wavelength (nm)	200–400
Mobile phase (isocratic)	58.5% H_2O:32.5% MeOH:9% THF:0.01% HCl
ESI source conditions	
Capillary (V)	3171
Sample cone (V)	30
RF lens (V)	195
Extraction cone (V)	5
Source temp. (°C)	105
Desolvation temp. (°C)	400
Nebulizer gas flow (L/h)	90
Desolvation gas flow (L/h)	850
ESI flow rate (μl/min)	375 (4:1 split from LC)
MS conditions	
Scan range (amu)	100–800
Scan duration (ms)	900
Interscan delay (ms)	100
Mass resolution (m/Δm)	5000

Analysis of samples during routine stability studies revealed a high level of variability in the measured levels of N-formyl duloxetine (**3**). An investigation of this observed analytical variability was therefore undertaken.

Fig. 3 illustrates the quantified levels and the magnitude of the variability observed in the determination of **3** in duloxetine product stored under room temperature conditions for a period of up to 24 months. As can be seen in the figure, there was no correlation between storage time and quantified level. Based upon the validation data obtained for the method, the intermediate precision for this analyte should have been about 0.02% absolute standard deviation. Therefore, the observed variability appeared to exceed what would have been predicted based upon the validation data. Levels of **3** in excess of the 0.2% ICH qualification limit (total daily intake of 120 mg) [4] were observed in some samples.

The precise origin of **3** was unknown and was presumed to be the result of a reaction of duloxetine with an excipient or an excipient impurity or degradation product (e.g., formic acid) [5]. This presumption was supported by stress studies conducted using duloxetine pellets. The degradation product levels obtained by stress testing pellets for up to 22 h at 70 °C/75% RH are provided in Fig. 4. The apparent delay (ca. 8 days) in the increase in impurities was hypothesized to be the result of time required for the pellets to equilibrate with ambient moisture levels. Fig. 5 shows the levels of

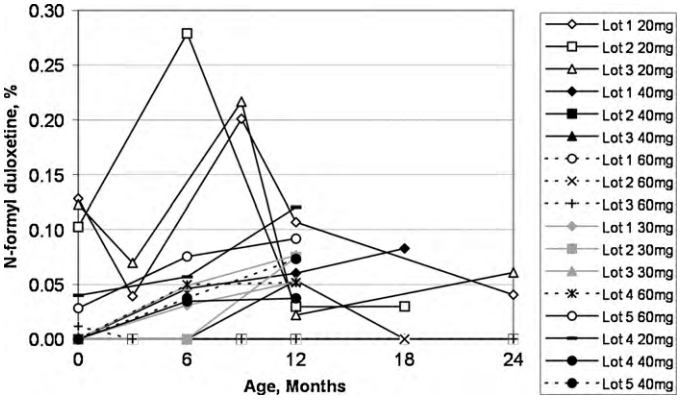


Fig. 3. N-Formyl duloxetine levels averaged across packages and capsule strengths (24 months, 25 °C/60% RH).

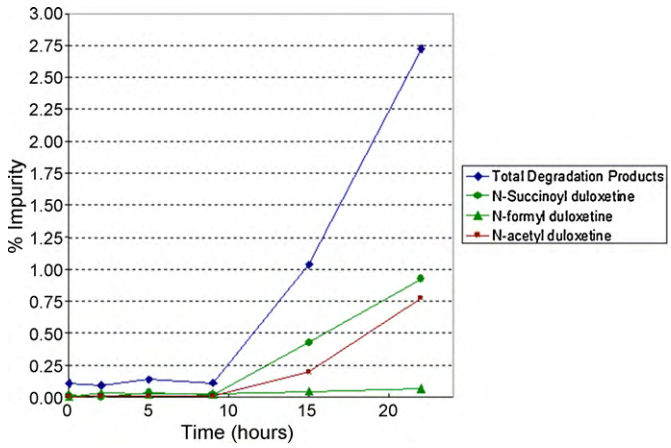


Fig. 4. Duloxetine pellets stored up to 22 h at 70 °C/75% RH.

3 scaled to accentuate the observed changes in this impurity. Levels of **3** are observed to increase under stress conditions; however, the levels observed are significantly below those of either **2** or **4**.

During the testing of clinical trial materials, only **2** and **4** had been observed at quantifiable levels under room temperature (25 °C/60% RH) or accelerated (40 °C/75% RH) storage. Furthermore, **2**, the peak identified as the major degradation through stress testing, was below the 0.20% for packaged material stored at 25 °C/60% RH. Therefore, the high and variable levels of **3** shown in Fig. 3 were entirely unexpected.

To evaluate the possibility that the variable levels of **3** observed under long-term storage conditions (i.e., 25 °C/60% RH) were due to instability of **3**, an experiment to determine the solid-state stability of **3** was carried out. A reference sample of **3** was exposed to the stress conditions of 70 °C/75% RH for the same period of time (about 22 h) studied above. The chromatography of the stressed sample showed no additional peaks over the course of the study and, relative to a reference solution, no decrease in the levels of **3** were observed. Therefore, the observed variability cannot be attributed to instability of **3**.

To investigate the variability in the levels of **3** observed in the stability test results, a control sample was introduced into the laboratory conducting the registration stability studies. The control sample was a recently manufactured drug product lot that would not be expected to contain measurable levels of **3**. Because elevated levels of **3** had only been observed in a single laboratory, initial testing of this lot was conducted in an independent laboratory. The test results confirmed the presence of very low levels

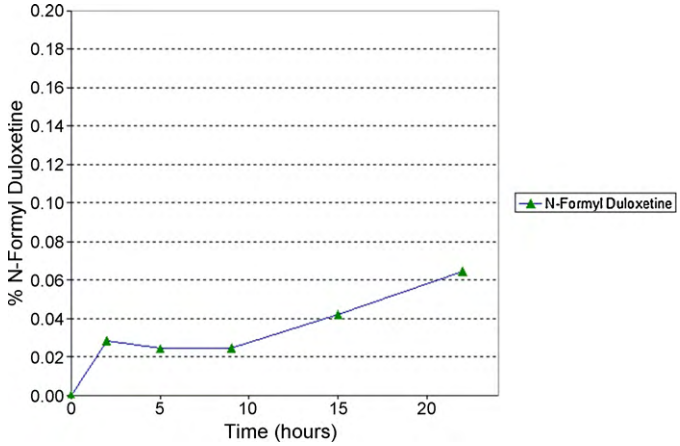


Fig. 5. N-Formyl levels for pellets stored at 70 °C/75% RH (expanded view).

of **3**. Subsequently, each time duloxetine stability samples were tested, the control sample was also tested. The results obtained demonstrated that the levels of **3** observed in the control sample varied substantially in excess of what would have been predicted based upon method precision. Thus, it was hypothesized that the N-formylation reaction might be, at least in part, artifactual in nature and that possibly electrophilic impurities in the acetonitrile used in the sample solvent to aid dissolution might be the artifactual formylating species.

3.1. Addition of potential impurities in the sample solvent

The possibility that specific electrophilic impurities present in acetonitrile might lead to artifactual N-formylation during sample preparation was considered. Experiments were conducted by spiking the sample solvent with formamide, formic acid and formaldehyde (all viewed as potential impurities in acetonitrile that might lead to N-formylation of duloxetine, though a reaction with formaldehyde would involve an additional oxidation step to result in the formation of **3**). These solutions were prepared by separately mixing 0.1 ml of formamide, formic acid, and formaldehyde solution (37%) with 1 l of sample solvent. These spiked sample solvent solutions were used to prepare duloxetine capsules for analysis as described in the procedure (Section 2.5).

None of these spiking experiments resulted in enhanced levels (e.g., >0.1%) of **3**. From these experiments it was concluded that formic acid, formamide, and formaldehyde were not directly involved in the observed high and variable levels of **3**.

3.2. Potential role of excipients

The role of excipients present in the pellets was also considered. The outer pellet layer is a white color coat, and one of the components of the color coat is titanium dioxide. Because titanium dioxide is known to catalyze oxidative reactions [6,7] an experiment involving the color coating was undertaken.

The color coating was added to a solution of the duloxetine reference standard and shaken and sonicated to mimic sample preparation. Both the original reference standard solution and the spiked standard solution were analyzed using the conditions in Table 1. No detectable levels of **3** were observed in the reference standard solution (see chromatograms shown in Fig. 6a); however, about 0.13% was observed in the spiked solution (see chromatograms shown in Fig. 6b), implicating the involvement of the color coating in the formation of **3**.

To confirm that titanium dioxide is the component of the color coat responsible for the formation of **3**, an amount of titanium dioxide equivalent to the levels present in the drug product was added to a flask containing duloxetine reference standard. Sample solvent was added and the mixture sonicated, diluted to volume and analyzed using the conditions of Table 1. The resulting chromatogram (Fig. 7) shows a large peak at ~720 s (relative retention time (RRT) of 3.7 defined relative to duloxetine) corresponding to the formylated product, **3**.

These data suggest that the titanium dioxide is serving as a catalyst in the formation of **3**. Because titanium dioxide is known to catalyze oxidative reactions through the formation of hydroxyl radicals via either exposure to light or sonication [8–11], the potential role of radicals in the formation of **3** was considered. An experiment was conducted to specifically introduce radicals into the system using the radical initiator, AIBN (2,2′-azobisisobutyronitrile). Thus, AIBN was dissolved in the sample solvent system (see Section 2) and allowed to age for 3 days at 40 °C. Samples prepared using this sample solvent were analyzed using the HPLC conditions in Table 1; the results of this analysis showed the presence of high levels of **3** (between 2 and 4%).

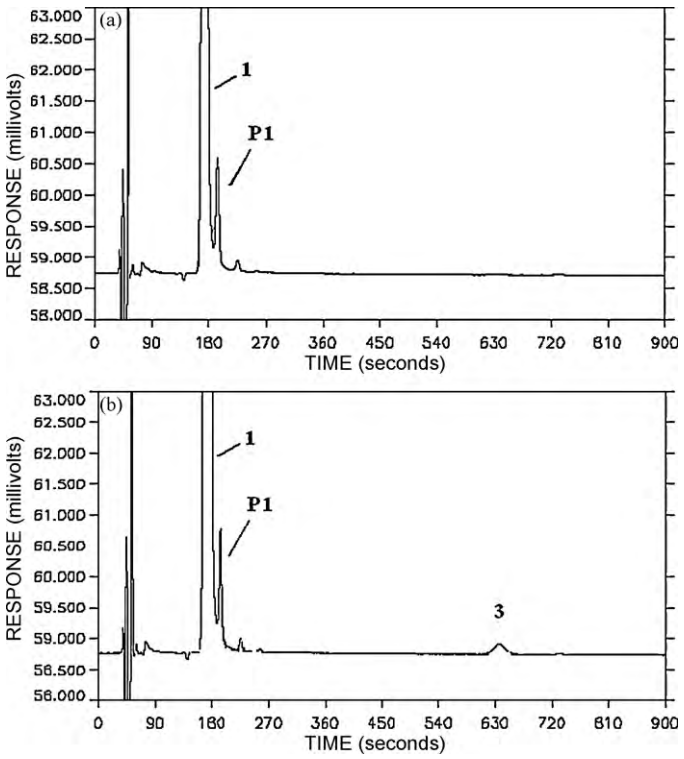


Fig. 6. Reference standard solution without (a, upper trace) and with (b, lower trace) color coating exposure.

Collectively, these experimental results indicate that titanium dioxide is catalyzing the decomposition of the acetonitrile [12] used in the sample solvent via a radical reaction to a reactive impurity that can react with duloxetine during sample preparation, leading to the artifactual formation of **3** in solution. Thus, the formyl carbon of **3** (when formed artifactually during sample preparation) must be derived from one of the two carbons in acetonitrile.

3.3. Experiments involving deuterium-labeled acetonitrile

To test the hypothesis that the formyl carbon of **3** can be derived from one of the carbons of acetonitrile in the sample solvent, an experiment was conducted using deuterated acetonitrile (CD_3CN). Thus, an aliquot of **1** was dissolved in the method sample solvent prepared from CD_3CN and the pH 8 buffer, and titanium dioxide was added to this solution in order to mimic the sample preparation conditions and enhance the formation of **3**. After exposure to ambient room temperature and light (~1000 lux, cool white fluorescent light) for about 72 h, this mixture was analyzed for levels of **3**. An impurity identified as **3** by retention time comparison to authentic **3** was present in the solution at a level of about 2.5%. This sample was then analyzed by LC/UV/MS (method in Table 1). The goals of the analysis included (a) confirmation of the formation of **3** and (b) determination whether a deuterium atom was incorporated into **3**. The incorporation of a deuterium atom would confirm that the formylating carbon was derived from the deuterated methyl of acetonitrile, while the lack of deuterium incorporation would implicate the cyano carbon. For comparison, a 1.25 mg/ml solution of authentic **3** in non-deuterated acetonitrile pH 8 buffer was analyzed using the same LC/UV/MS conditions.

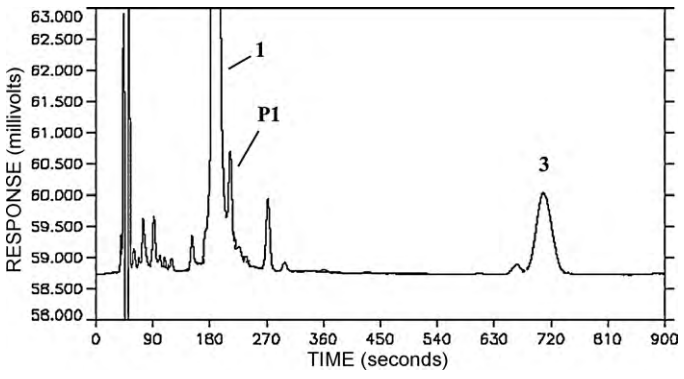


Fig. 7. Representative chromatogram showing formation of N-formyl duloxetine (**3**) after exposure to titanium dioxide in the presence of acetonitrile/buffer.

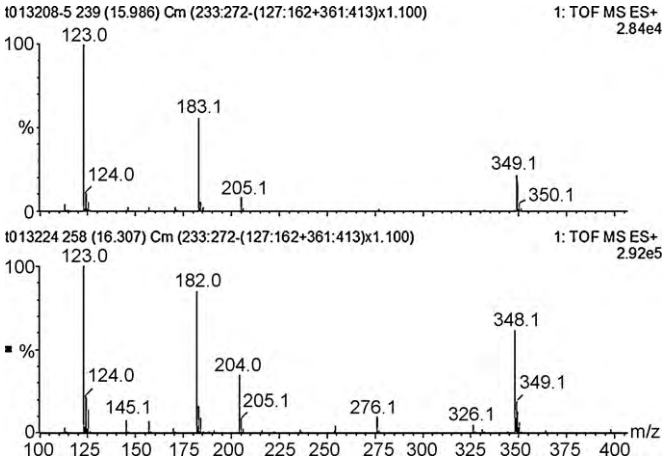


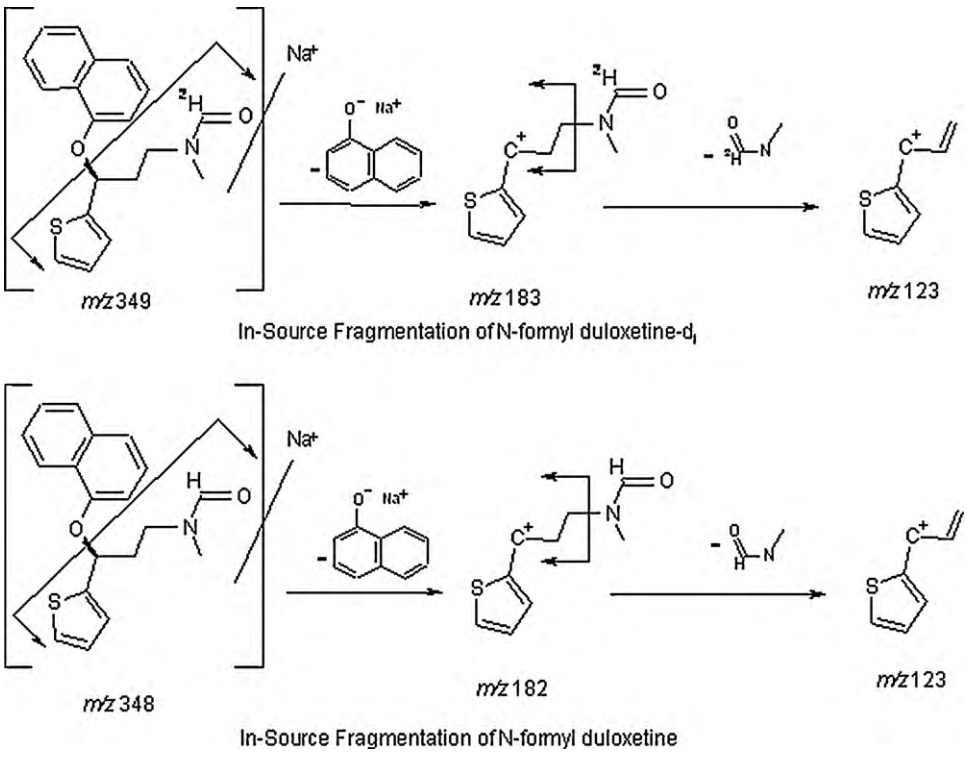
Fig. 8. LC/ESI/MS spectra of (top) N-formyl duloxetine(d_1) from the deutero-acetonitrile experiment and (bottom) authentic N-formyl duloxetine (**3**).

Under the mobile phase conditions used for LC/MS, **3** eluted at about 17 min (relative retention time of 5.3). The TIC was essentially featureless due to the high level of background. Mass spectral analysis of the TIC at ~17 min (RRT 5.3 (from the deutero-acetonitrile experiment) resulted in the data illustrated in Fig. 8 (top spectrum). The major ions observed in this spectrum include *m/z* 123, 183, and 349, where *m/z* 349 corresponds to [N-formyl duloxetine(d_1) + Na]$^+$. Closer inspection of the mass spectrum shows that there is a low-abundance ion at *m/z* 348, which corresponds to [N-formyl duloxetine + Na]$^+$. Based on the height of the extracted ion chromatograms for *m/z* 182, 183, 348 and 349, the deuterated version accounts for more than 90% of the total. Considering all isotopes, the percentage of deuterated **3** is approximately 98%. This correlates well with the purity of the CD_3CN used in the experiment.

The bottom spectrum illustrated in Fig. 8 shows the corresponding LC/MS analysis of authentic (non-deuterated) **3**. As expected, ions of *m/z* of 123, 182, and 348 are observed, reflecting the absence of the deuterium atom.

Scheme 1 illustrates the fragmentation pathway for deuterated and non-deuterated N-formyl duloxetine. The elimination of sodiated naphthol most probably occurs due to the presence of 0.01% HCl in the mobile phase. Further elimination of acetamide from the resulting ion, which occurs due to excess internal energy imparted to ions in the vacuum interface of the ESI source, results in a carbonium ion (*m/z* 123) that is resonance stabilized by the thiophene ring. This helps to explain why *m/z* 123 is the base peak in both spectra. In the non-deuterated **3**, elimination of acetamide results in an ion whose mass difference is 59, i.e., 182–123, while the mass difference is 60 for acetamide elimination from deuterated **3**. This could only be true if the eliminated acetamide contained one deuterium atom, as shown in Scheme 1.

The mass spectral analysis of **3** that was produced using deuterated acetonitrile (CD_3CN) provides strong evidence that the methyl



Scheme 1. Proposed fragmentation pathways of deuterated and non-deuterated **3**.

group of acetonitrile was the carbon source for the formyl group of artifactually produced N-formyl duloxetine, as determined from the incorporation of one deuterium into the structure.

3.4. Experiments to test the role of light and sonication

Experiments were conducted to test the effects of both sonication and light on the formation of **3** when duloxetine is dissolved in the sample solvent, in the presence of titanium dioxide (see Section 2.6 and Table 2 for experimental details). Table 4 shows the results of these experiments. As shown in the table, exposure of the solutions to indirect sunlight (over the course of a weekend, experiments 1 and 2) resulted in the formation of measurable levels of **3**. When titanium dioxide is present in the sample solvent, the indirect filtered sunlight exposure resulted in dramatic increases in observed levels of **3** (experiment 1). Sonication for 30 min in the presence of titanium dioxide resulted in increased levels of **3** (experiment 3), but when laboratory light was present for just 30 min (experiment 4), greater levels of **3** were observed. In the absence of light and sonication, only low levels of **3** are observed (experiment 2). These experiments clearly show either or both sonication and exposure to light during the sample preparation can induce the artifactual formylation of duloxetine.

4. Discussion

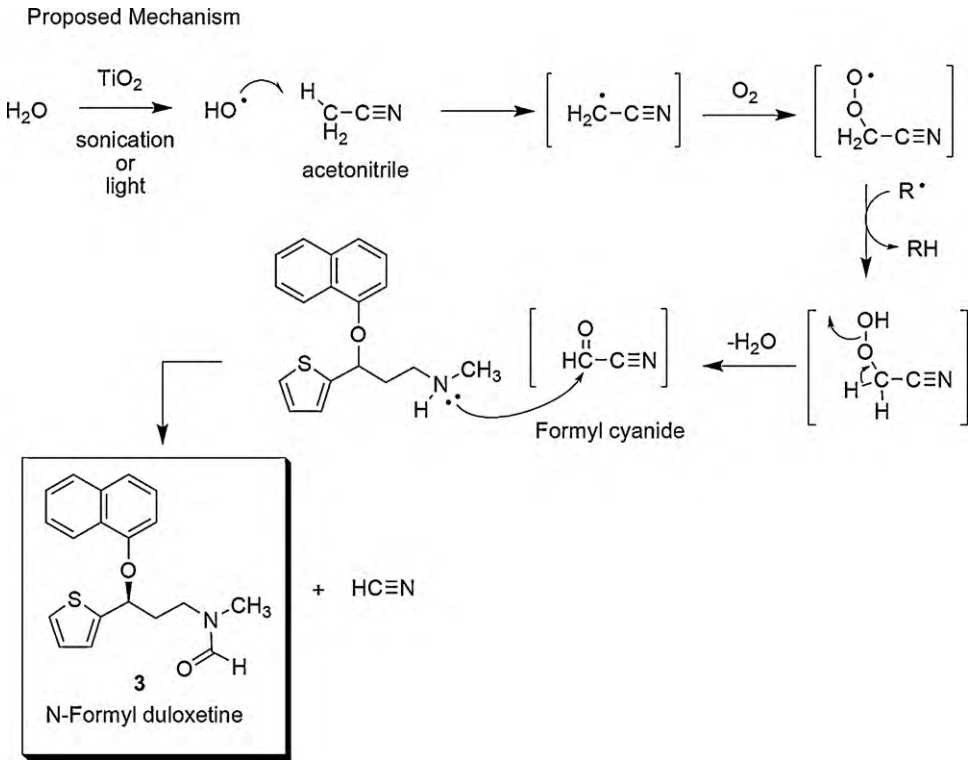
The introduction of the control sample into the testing plan for the drug product stability provided critical evidence that the elevated levels of N-formyl duloxetine measured analytically using HPLC were an artifact of the sample preparation rather than a reflection of the stability of the drug product. Because acetonitrile that has been exposed to the radical-initiator AIBN causes rapid formylation of the secondary amine of duloxetine (**1**) and because titanium dioxide can catalyze the production of radicals via either light or sonication, it is likely that decomposition of acetonitrile in the presence of titanium dioxide also proceeds via a free radical mechanism.

A proposed mechanism for the formation of **3** from duloxetine during the analytical sample preparation step is shown in Scheme 2. Since titanium dioxide is known to produce hydroxyl radicals upon exposure to either light or sonication [8–10], Scheme 2 shows the formation of hydroxyl radicals as the initiating step in the oxidative decomposition of acetonitrile. Hydroxyl radical, which is known to be an aggressive oxidant, is proposed to abstract a hydrogen atom from the methyl group of acetonitrile. In the presence of dissolved oxygen, reaction with the carbon-centered radical would be rapid, yielding a peroxy radical. This peroxy radical could undergo radical decomposition or, upon abstraction of a hydrogen atom, could form the corresponding peroxide. This peroxide would be expected to be unstable, and loss of water would give formyl nitrile, a reactive species that is known to readily react with water to form formic acid and HCN [13]. The reactive formyl cyanide would be subject to nucleophilic attack, e.g., by the secondary amine of duloxetine, resulting in the elimination of the cyano group to yield **3**.

The LC/MS studies conducted using deuterated acetonitrile in the sample dissolution solvent for the drug product analysis clearly

Table 4
Results of sonication and light experiments.

Exp. No.	Light	Titanium dioxide	Sonication	N-formyl duloxetine (%)
1	Indirect filtered sunlight (48 h)	Yes	No	75.1
2	Light excluded (48 h)	Yes	No	0.09
3	Light excluded	Yes	30 min	1.08
4	Ambient lab lighting	Yes	30 min	1.32



Scheme 2. Proposed mechanism for the artifactual formation of N-formyl duloxetine (**3**) during sample preparation.

showed that the origin of the carbon incorporated into the N-formyl duloxetine structure was the methyl group of acetonitrile by the incorporation of one deuterium; this result supports the proposed mechanism. On the basis of these data, it is established that either sonication or exposure to light, when titanium dioxide is present, can cause degradation of acetonitrile that leads ultimately to artifactual N-formylation of the secondary amine of duloxetine hydrochloride during sample preparation.

5. Conclusion

The physicochemical properties of acetonitrile including low UV cutoff, comparatively low viscosity, and general lack of reactivity has made acetonitrile one of the most frequently used organic modifiers for reversed-phase HPLC separations. The results described in this report demonstrate that while acetonitrile is often considered inert, under certain circumstances acetonitrile is not inert and can react with pharmaceutical compounds to form impurities during sample preparation. Specifically, exposure of solutions of acetonitrile in the presence of titanium dioxide to either sonication or to ambient laboratory light was shown to induce the N-formylation of duloxetine hydrochloride, a secondary amine. The reaction is proposed to occur via a radical-initiated mechanism and this proposal is supported by stable-isotope labeling studies with deuterated acetonitrile. The artifactual reaction was effectively eliminated by changing the sample solvent to methanol and replacing sonication with shaking to aid sample dissolution. This discovery is broadly relevant because sonication is commonly used to aid dissolution of pharmaceuticals for HPLC analysis, titanium dioxide is a commonly used excipient, the amount of light found in modern analytical laboratories is sufficient to cause the photocatalysis, and secondary amines are common functionalities in the structure of many pharmaceuticals. It is also possible that other nucleophilic functions groups (e.g., primary amines, hydroxyls) could form artifactual impurities via this pathway during sample preparation.

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