Characterization of Impurities Formed by Interaction of Duloxetine HCl with Enteric Polymers Hydroxypropyl Methylcellulose Acetate Succinate and Hydroxypropyl Methylcellulose Phthalate

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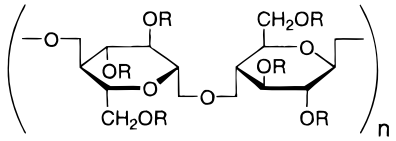
Abstract □ Duloxetine hydrochloride ((*S*)-*N*-methyl-3-(1-naphthalenyloxy)-2-thiophenepropanamine hydrochloride) has been found to react with polymer degradation products or residual free acids present in the enteric polymers hydroxypropyl methylcellulose acetate succinate (HPMCAS) and hydroxypropyl methylcellulose phthalate (HPMCP) in dosage formulations to form succinamide and phthalamide impurities, respectively. The rate of formation of the impurities is accelerated by heat and humidity. The structures were deduced using molecular weights obtained from LC–MS experiments and confirmed by comparison of UV spectra, HPLC retention times, and electrospray mass spectra to independently synthesized material. It is proposed that polymer-bound succinic and phthalic substituents can be cleaved from the polymer, resulting in the formation of either the free acids or the anhydrides. It is postulated that the reaction is enabled by migration of either (1) the free acid or anhydride or (2) the parent drug through the formulation. The formation of these impurities was minimized by increasing the thickness of the physical barrier separating the enteric coating from the drug.

Introduction

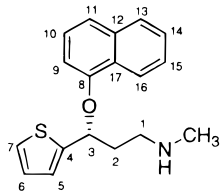
Duloxetine hydrochloride (**1**, (S)-*N*-methyl-3-(1-naphthalenyloxy)-2-thiophenepropanamine hydrochloride) is a new drug currently being developed for the treatment of major depressive disorder and urinary incontinence. It is a potent in vitro and in vivo inhibitor of serotonin and norepinephrine uptake in nerve terminals of mammalian brains and/or serotonin uptake in human platelets ex vivo.[1] Because **1** is unstable in solution at pH values less than 2.5,[2] enteric polymer-coated formulations have been developed to prevent its acid degradation in the stomach and to provide for subsequent rapid disintegration and release in the small intestine. A tablet formulation coated with the enteric polymer HPMCP was originally developed, but a formulation consisting of pellets coated with the enteric polymer HPMCAS contained in a capsule is the desired market formulation. The structures of the enteric polymers are given in Figure 1.

During the course of development of the HPMCAS-coated pellet formulation, an unknown impurity which eluted after **1** (impurity **A**) was detected by HPLC analysis of samples stressed at 60 °C for 14 days. This impurity was also detected in stability samples stored at 30 °C/60% relative humidity and 40 °C/75% relative humidity.[3] Subsequent analysis of stability samples of HPMCP-coated tablets indicated the presence of a different impurity (impurity B) that also eluted after **1**. The formation of significant levels of these unknown impurities at pharmaceutically relevant storage conditions prompted an investigation into their identity and mechanism of formation. In this report we describe the identification, synthesis, and mechanism of formation of these two impurities identified as duloxetine succinamide (**2**) and duloxetine phthalamide (**3**).



R =	HPMCAS	HPMCP
	H	CH_3
	CH_3	$CH_2CH(OH)CH_3$
	$COCH_3$	COC_6H_4COOH
	$COCH_2CH_2COOH$	
	$CH_2CH(OH)CH_3$	
	$CH_2CH(OCOCH_3)CH_3$	
	$CH_2CH(OCOCH_2CH_2COOH)CH_3$	

Figure 1—Repeating units of HPMCAS and HPMCP.



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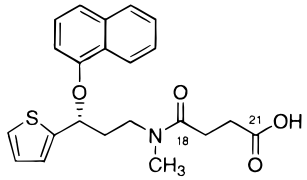

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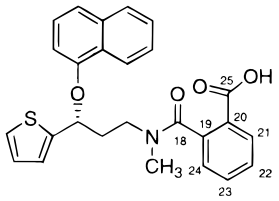

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Experimental Section

Analytical HPLC and Photodiode Array UV Detection—HPLC analyses were carried out on a Waters photodiode array system consisting of a 600E controller and pump, a 715 Ultra Wisp, and a 996 photodiode array detector. The UV spectra were obtained on the compounds as they eluted from the chromatographic column. The column, a 4.6 × 250 mm Zorbax SB−CN 5

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Table 1—NMR Chemical Shift Assignments for 1–3

	1		2		3	
site	δH	δC	δH	δC	δH	δC
1	3.07	44.99	3.43, 3.56[a]	44.30, 45.62	(3.14, 3.30) (3.56, 3.62)	43.50, 47.01
2	2.40, 2.58	34.37	2.14, 2.32[a]	36.07, 36.96	2.25, 2.44	35.32, 36.32
3	6.17	72.58	5.88, 5.97	72.75, 73.24	5.71, 5.99	73.38
4		143.56		144.18, 144.59		144.19, 144.84
5	7.28	126.07	7.23, 7.27	125.88, 125.95	7.10, 7.31	125.50, 125.77
6	6.99	126.75	6.97	126.60, 126.71	6.90, 6.99	126.68, 126.69
7	7.46	125.83	7.42	125.46, 125.49	7.44	125.37, 125.44
8		152.14		152.30, 152.40		152.10, 152.55
9	7.08	107.50	7.02, 7.05	107.27, 107.30	6.86, 7.08	106.78, 107.31
10	7.34	125.81	7.33	125.62, 125.79	7.26, 7.34	125.80, 125.98
11	7.46	120.48	7.41, 7.45[a]	120.22, 120.38	7.59	120.14, 120.20
12		134.06		134.10, 134.11		133.96, 134.11
13	7.85	127.42	7.84	127.43, 127.45	7.85	127.32, 127.44
14	7.52	125.40[b]	7.51	125.37, 125.41	7.52	125.10, 125.32[b]
15	7.52	126.40[b]	7.51	126.37, 126.42	7.52	126.34, 126.38[b]
16	8.26	121.60	8.26	121.54, 121.59	8.31	121.28, 121.65
17		125.37		125.46		125.37
18				170.52, 170.91		169.95
19			2.47	27.04, 27.76[b]		127.89
20			2.47	28.94, 29.01[b]		138.76, 139.10
21			12.01	173.90, 174.01	7.81, 7.93	129.97, 130.06
22					7.50	132.29, 132.63
23					7.62	126.64, 126.68
24					7.26	128.52, 128.32
25						166.79, 166.93
N–CH_3	2.55	32.37	2.83, 2.95	33.00, 35.09	2.62, 2.91	31.91, 36.32

[a] Proton assignments from one-bond heteronuclear multiple quantum correlation (HMQC) experiment. [b] Carbon-13 assignments may be reversed within the column.

μm (MACMOD Analytical), was operated at ambient temperature. The flow rate was maintained at 1.0 mL/min. Mobile phases **A** and **B** consisted of 80/20 and 25/75 (v/v) 25 mM KH_2PO_4 (pH 3.0)/acetonitrile, respectively. Mobile phase **B** was increased linearly from 0% to 30% at 2%/min for 15 min and then from 30% to 100% at 5.83%/min for 12 min.

MS—Nominal mass pseudo-molecular ions were determined using a Fisons Instruments VG Quattro triple quadrupole mass spectrometer equipped with a pneumatically assisted electrospray liquid chromatography/mass spectrometry interface. For the on-line LC–MS work, the flow rate was maintained at 1.0 mL/min. Mobile phases **A** and **B** consisted of 80/20/0.05 and 25/75/0.05 (v/v) water/acetonitrile/trifluoroacetic acid, respectively. Mobile phase **B** was increased linearly from 0% to 30% at 2%/min for 15 min and then from 30% to 100% at 5.83%/min for 12 min. Positive ion electron impact (EI) MS data was obtained with a VG 7070E double focusing magnetic sector mass spectrometer using a direct-insertion heated probe for sample introduction.

NMR—Proton and carbon-13 NMR spectra were recorded at 299.957 and 75.432 MHz, respectively, using a Varian Unity spectrometer equipped with a 5-mm Nalorac indirect detection probe and a Matrix shim system. Samples consisted of 20 mg/mL **1**, 27 mg/mL **2**, and 20 mg/mL **3** dissolved in dimethyl sulfoxide-d_6. Chemical shifts are reported in parts per million relative to dimethyl sulfoxide-d_6 (2.50 ppm for ^{1}H, 39.50 for ^{13}C). All spectra were recorded in a nonspinning mode.

IR—Fourier transform IR spectra were collected on a Nicolet 60SXB spectrometer. 1024 scans were coadded at 4 cm^{-1} resolution from 3775 to 625 cm^{-1} using Happ–Geutzal apodization.

Synthesis of 2 and 3—The synthesis of **2** was accomplished by allowing duloxetine HCl to react with a slight molar excess of succinic anhydride in chloroform containing a trace of triethylamine. HPLC analysis of the reaction mixture after 15 min at 40 °C confirmed nearly complete conversion of duloxetine to **2**. Approximately 200 mg of crude **2** was purified by preparative reverse phase HPLC for characterization. The synthesis of **3** was accomplished in the same manner as **2** using phthalic anhydride in place of succinic anhydride. It was also purified by preparative reverse phase HPLC.

Characterization of Synthesized Impurities—Both impurities were characterized using NMR, MS, and IR. See Table 1 for NMR assignments. **2**: EI MS *m/z* (fragment) 397 ($[M]^+$), 297 ($[M]^+$ of **1**), 254 ($C_{12}H_{16}NO_3S$), 152 ($C_8H_{10}NS$), 123 (C_7H_5S), 44 (CO_2); IR 1730 (C=O), 1644 (amide I), 1599 (aryl-CH), 1595 (aryl-CH), 1396 (aryl-CH), 1237 (aryl-O), 1095 (O–CH), 1079 (C=C), 837 cm^{-1} (HC=CH). **3**: EI MS *m/z* (fragment) 297 ($[M]^+$ of **1**), 266 ($C_{17}H_{14}OS$), 237 ($C_{15}H_9OS$), 144 ($C_{10}H_8O$), 76 (C_6H_5); IR 1713 (C=O), 1610 (amide I), 1596 (aryl-CH), 1398 (aryl-CH), 1237 (aryl-O), 1095 (O–CH), 1066 (C=C), 834 cm^{-1} (HC=CH).

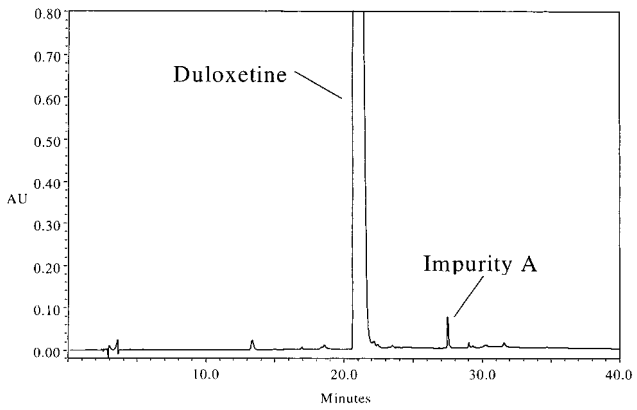

Figure 2—HPLC chromatogram of duloxetine hydrochloride pellet formulation stressed at 60 °C for 14 days.

Results and Discussion

Identification of Impurities A and B—Pellets of **1** coated with the enteric polymer HPMCAS were stressed at 60 °C for 14 days. HPLC analysis of the stressed sample (Figure 2) revealed a late-eluting impurity (impurity **A**) at a level of approximately 0.5%. The UV spectra of **1** and impurity **A** (Figure 3), obtained by PDA detection, were similar. Electrospray LC–MS analysis of the impurity peak showed a $[M + H]^+$ ion at *m/z* 398, suggesting a

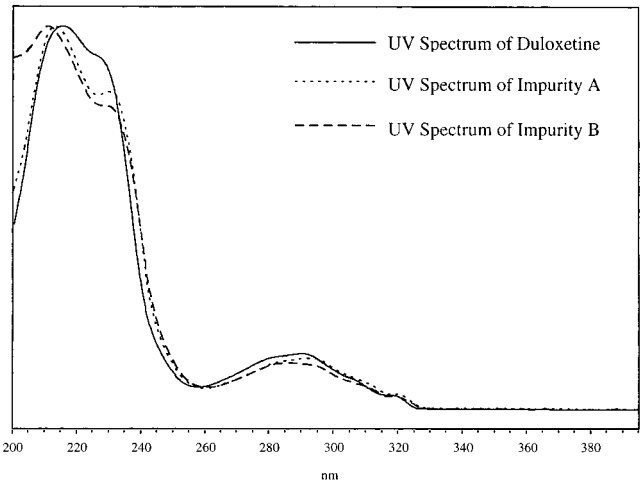

Figure 3—UV spectra of **1** and impurities **A** and **B**.

molecular weight of 397. From these results the source of the impurity was postulated to result from a reaction of the secondary amine of **1** with a UV-transparent component of the formulation.

The list of ingredients used in the manufacture of the pellets was examined for compounds that could possibly react with **1**. The enteric coating used for the pellets, HPMCAS, is a hydroxypropyl methylcellulose polymer substituted with acetate and succinate groups, which is known to contain small amounts of residual free succinic acid.[4] The secondary amine of **1** could potentially react with the succinic acid (or an active succinoyl derivative) to form a duloxetine succinamide (**2**) that would have a molecular weight of 397.

This hypothesis was tested by reacting **1** with succinic anhydride in chloroform (see the Experimental Section). HPLC analysis of the reaction product revealed that **1** had been consumed and a new peak had appeared that matched the retention time, UV spectrum, and electrospray mass spectrum (Figure 4) of impurity **A**. The structure of the synthesized material was shown to be that of **2** (see below).

The discovery of **2** in duloxetine pellets coated with the enteric polymer HPMCAS prompted an investigation of duloxetine tablets coated with the enteric polymer HPMCP. HPMCP is a hydroxypropyl methylcellulose polymer substituted with phthalate groups and is known to contain small amounts of residual free phthalic acid.[4] One would expect that the same type of condensation reaction leading to **2** in duloxetine pellets might also occur in duloxetine tablets, resulting in the formation of a duloxetine phthalamide (**3**), which would have a molecular weight of 445. HPLC analysis of a sample of enteric-coated duloxetine tablets stressed at 40 °C/75% relative humidity for 2 months (Figure 5) detected a late-eluting impurity (impurity **B**). Comparison of the UV spectrum of impurity **B** to that of **1** (Figure 3) again suggested that the impurity was related to **1**. Electrospray LC–MS results on impurity peak **B** showed a $[M + H]^+$ ion at *m/z* 446, suggesting a molecular weight of 445. From this information, the structure of impurity **B** was proposed to be **3**.

Structure **3** was therefore prepared by reaction of phthalic anhydride with **1** in chloroform (see the Experimental Section). Impurity **B** was shown to be **3** by comparison of the HPLC retention time, UV spectrum, and electrospray mass spectrum (Figure 4) to that of synthetically prepared **3**.

Structure Elucidation of 2 and 3—High-resolution FAB MS yielded $[M + H]^+$ ions of *m/z* 398 and 446, corresponding to molecular formulas of $C_{22}H_{23}NO_4S$ and $C_{26}H_{24}NO_4S$ for **2** and **3**, respectively.[5] Accurate mass electron impact (EI) MS, IR, and NMR data supported the presence of the duloxetine moiety; however, the proton and carbon-13 NMR spectra exhibited the spectral complexity typically observed for amides in solution.[6] Amides in solution can exist in two interconverting conformers, *cis* and *trans*, of unequal population leading to complex proton and carbon-13 NMR spectra because each magnetically inequivalent site may show two resonances. To establish that the spectral complexity observed was attributable to changes in molecular configuration, the proton NMR spectra of **2** were recorded as the temperature was raised from 20 to 100 °C (Figure 6). As the temperature increased, the separation between resonance pairs decreased and the line widths broadened until at 90 °C the proton resonances of position 3 collapsed and the line width began to sharpen. These observations are consistent with the proposed amide *cis–trans* isomerism.

Complete NMR chemical shift assignments (Table 1) for **2** and **3** were based upon comparison with the chemical shifts of **1** and the use of single and multiple bond heteronuclear correlation experiments optimized for three-bond C–H coupling constants. No efforts were made to make the assignments *cis* or *trans* specific, although in solution the *trans* conformer is preferred.[6] The connectivity of the succinoyl and phthaloyl moieties to the *N*-methyl nitrogen was established by the long-range coupling observed between the *N*-methyl protons and the carbonyl carbon at position 18 for both **2** and **3**. Starting with the *N*-methyl protons and carbon, which can be readily assigned on the basis of their corresponding chemical shifts, the carbons at positions 2 and 3 can be assigned by their long-range couplings to the *N*-methyl protons. Assignment of the resonances attributable to the thiophene ring can also be readily sorted out. Only one carbon from the thiophene ring shows a long-range coupling to the proton at position 3 and it is assigned to position 5. The assignments of the carbon resonances to positions 6 and 7 were based upon the observed long-range couplings to positions 5 and 7 and positions 5 and 6, respectively. The naphthalenyloxy resonances are similarly assigned using observed long-range couplings. Of particular interest are the assignments of positions 13 and 16 which were made based upon the coupling observed between positions 11 and 13 and positions 12 and 16.

Proposed Mechanism of Formation—In both the pellet and tablet formulations the duloxetine-containing layers are separated from the enteric coating by a subcoat layer consisting primarily of hydroxypropyl methylcellulose and other ingredients. This raises the question about how the impurities form since the duloxetine layer is *physically separated* from the enteric polymers. Two possibilities can be envisioned: (1) the impurities are not actually present in the formulation but rather form during sample preparation or (2) migration of formulation components is occurring that allows **1** to come in contact and react with either the enteric polymers or an active succinoyl derivative present in the polymer.

To test whether **2** was actually present in the formulation or if it was formed during the extraction procedure, two experiments were conducted. In the first experiment, pellets containing **2** were extracted with both methanol and methanol containing approximately 4% (v/v) methylamine.[7] If **2** were formed during the extraction procedure, methylamine, which is a primary amine and a better nucleophile than **1**, should compete with **1** for the active succinoyl derivative and diminish the amount of **2** formed. This, however, was not the case as the same levels of **2** were measured in both samples.

In the second experiment, placebo pellets were stressed in the same manner as pellets containing **1**. Both the placebo and the duloxetine pellets were extracted in the

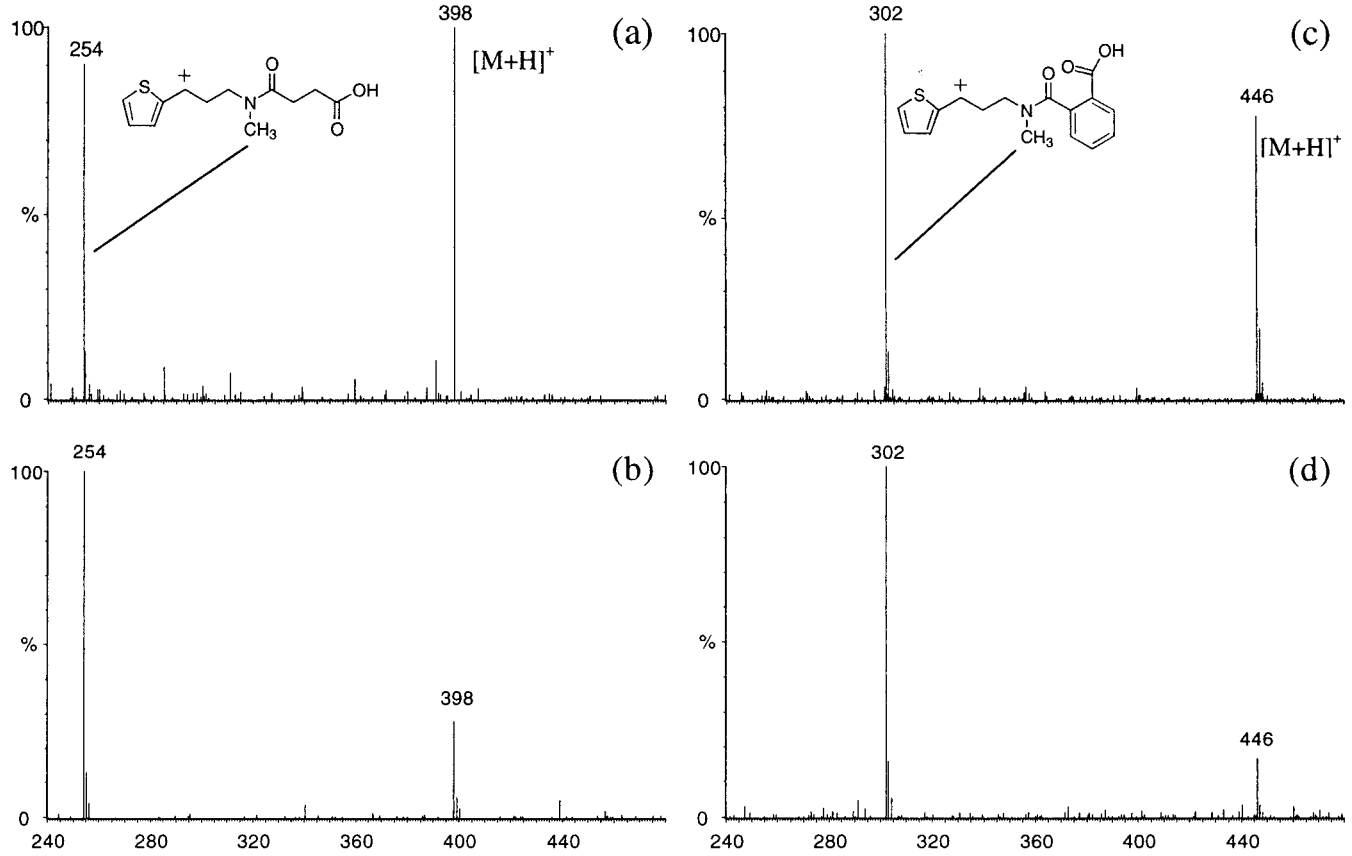

Figure 4—Positive ion electrospray mass spectra of (a) impurity **A**, (b) synthesized **2**, (c) impurity **B**, and (d) synthesized **3**. The spectra of impurities **A** and **B** were obtained from LC–MS analysis of stressed duloxetine formulations while the spectra of **1** and **2** were obtained from LC–MS analysis of solutions of the synthesized products.

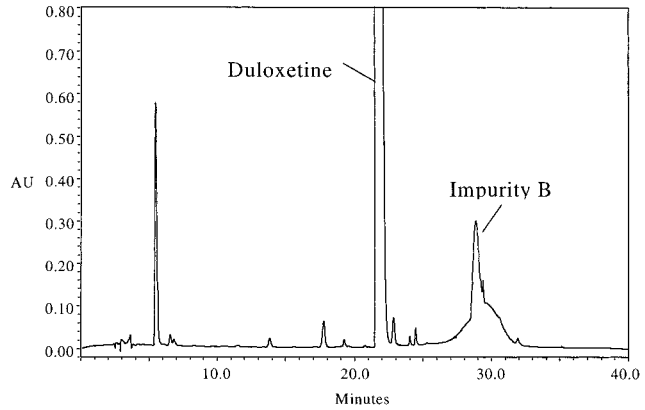

Figure 5—HPLC chromatogram of duloxetine hydrochloride tablet formulation stressed at 40 °C/75% relative humidity for 2 months.

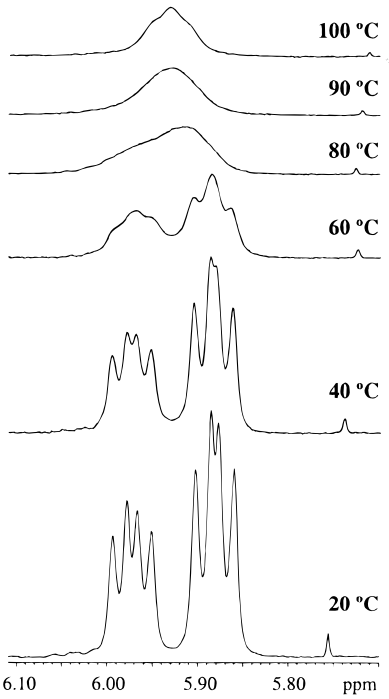

Figure 6—Variable temperature NMR spectra of the proton at position 3 of **2**.

same manner except that **1** was added to the extraction solvent used for the placebo sample. If **2** were formed during the extraction procedure it would be detected in the placebo extract. Analysis of the two extracts revealed significant quantities of **2** present in the duloxetine pellet sample but not in the placebo extract.

The results of these two experiments suggest that **2** is formed in the pellet formulation and not during the extraction procedure. For this to occur **1** must come in contact with the active succinoyl derivative either by migration of one or both of the reactants through the subcoat layer or by the breakdown of the subcoat layer. Thus, to minimize the formation of **2**, the thickness of the subcoat layer was increased in the proposed commercial formulation. Stability tests conducted on this formulation have shown that the levels of **2** are negligible (<0.1%) following storage at 25 °C/60% relative humidity for 18 months.

In summary, we propose that **2** and **3** are formed from reaction of succinic and phthalic anhydrides with **1**, respectively. Intimate contact required for reaction could

be facilitated by either migration of the anhydrides or of **1** between the physically separated layers. The anhydrides could form as a result of either dehydration of the residual free succinic and phthalic acids present in the enteric polymer or an intramolecular attack of the free acid on the ester linkage to the polymer backbone, cleaving to form free anhydride. It does not appear likely that **1** is reacting directly with the succinic and phthalic ester moieties on the intact polymer, since one would also expect the formation of a duloxetine acetamide degradation product, which has not been observed. We cannot rule out the possibility that the free acids are reacting directly with **1**; however, the reaction of carboxylic acids with amines is not a favored reaction (in contrast to the reaction of amines with the corresponding anhydrides). Further investigation would be required to conclusively define the active reactants leading to **2** and **3**.

Concluding Remarks

This study suggests that new impurities in formulations can result from reactions between enteric polymer substituents and drugs containing nucleophilic functional groups even when they are physically separated in the formulation. Two impurities detected in formulations of **1** upon aging were determined to be the result of the reaction of **1** with phthaloyl and succinoyl moieties present in the enteric polymers HPMCP and HPMCAS, respectively. Because the enteric polymers are physically separated from **1** by a subcoating, the formation of these impurities indicates that either **1** or the phthaloyl or succinoyl moieties are migrating through the subcoating to enable physical contact and reaction. The amount of impurity **2** formed in the commercial formulation of **1** has been minimized by increasing the thickness of the physical barrier separating the reacting species.

References and Notes

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2. Bopp, R.J.; Breau, A. P.; Faulkinbury, T. J.; Heath, P. C.; Miller, C.; Stephan, E. A.; Weigel, L. O.; Wong, D. T. The Rearrangement of Duloxetine Under Mineral Acid Conditions. Presented at the 206th National American Chemical Society Meeting, San Diego, CA; March 13, 1993; ORGN 111.
3. The level of the impurity was measured to be 0.3% in a sample stored at 30 °C/60% relative humidity for 12 months and 3.6% in a sample stored at 40 °C/75% relative humidity for 6 months.
4. Information obtained from product literature supplied by the manufacturer of HPMCAS. According to the manufacturer's certificate of analysis, the HPMCAS contained 0.06% free succinic acid and the HPMCP contained 0.57% free phthalic acid.
5. **2** (calculated 398.1426, found 398.1458); **3** (calculated 446.1426, found 446.1416)
6. Dorman, D. E.; Bovey, F. A. *J. Org. Chem*. **1973**, *38*, 1719−1721.
7. This corresponded to a molar ratio of methylamine to duloxetine of ∼450:1.

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