A First Look at Duloxetine (Cymbalta®) in a Postmortem Laboratory

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Abstract

Duloxetine (Cymbalta) is manufactured by Eli Lilly and Company and is the newest antidepressant to be approved by the Food and Drug Administration (FDA). Duloxetine is a potent serotonin and norepinephrine reuptake inhibitor that is also used for the management of pain associated with diabetic peripheral neuropathy. With the introduction of any new drug, toxicology laboratories around the nation experience the same problems: lack of information about the chemical and physical properties of the new drug, detection methodologies from biological specimens, and interpretation of quantitative values. Since its FDA approval in 2002, the Los Angeles County Department of Coroner Toxicology Laboratory has detected and quantitated duloxetine in 12 postmortem cases. The isolation of duloxetine from postmortem specimens consisted of a basic, liquid-liquid (n-butylchloride) extraction procedure. Duloxetine was detected in our general, pharmaceutical, basic drugs screen that utilizes gas chromatography–nitrogen-phosphorus detection (GC–NPD) and GC–mass spectrometry (MS), and the quantitation was specifically by GC-MS. Linearity was achieved from 0.05 to 3.0 mg/L with the limit of detection at 0.03 mg/L. Presented are the case histories, demographics, cause/manner of death, and the postmortem tissue distribution ranges of duloxetine: central blood, not detected (ND)-0.59 mg/L (12 cases); femoral blood, ND-0.26 mg/L (9 cases); vitreous humor, ND-0.23 mg/L (4 cases); liver, 0.28-22 mg/kg (8 cases); gastric contents, 0.08-86 mg total (6 cases); bile, 0.57-3.1 mg/L (7 cases); and urine, 0.07-0.47 mg/L (6 cases). The detection and quantitation of duloxetine in these 12 case studies are considered the first to be reported in the literature; all are designed to aid the forensic toxicologist with the interpretation of his/her own casework.

Introduction

Duloxetine hydrochloride [LY 248686, Cymbalta, (+)-(S)-N-methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride] was issued an approval letter by the U.S. Food and Drug Administration (FDA) in September 2002 for the treatment of major depressive disorder (1) and another in September 2004 for the management of pain associated with diabetic peripheral neuropathy (2). Duloxetine is also approved in Europe as Yentreve® for the treatment of stress urinary incontinence.

The structure of duloxetine is unrelated to those of tricyclic antidepressants (TCAs) and the monoamine oxidase inhibitors (MAOIs) (3). Its structure consists of a secondary amine (Figure 1) with a molecular weight of 297 and an empirical formula of C_{18}H_{19}N_{2}O_{2}S (4).

Cymbalta delayed-release capsules contain duloxetine HCl equivalent to 20, 30, and 60 mg duloxetine. Each capsule is comprised of enteric-coated pellets, a unique delivery system designed to prevent degradation of the drug in the acidic environment of the stomach (4). The recommended dosage of duloxetine is 40–60 mg/day for depression, 60 mg/day for neuropathic pain, and 80 mg/day for stress urinary incontinence (4).

Duloxetine's mechanism of action involves the simultaneous inhibition of serotonin and norepinephrine uptake, and there is speculation that this fourth generation selective serotonin and norepinephrine reuptake inhibitor (SSNRI) may be a better antidepressant than selective serotonin reuptake inhibitors (SSRI) (3). It is reported that duloxetine is a more potent reuptake inhibitor of serotonin than norepinephrine (3).

Duloxetine is well absorbed after oral administration with peak plasma levels occurring in 6–10 h. Following single oral doses of 20 mg, mean peak plasma concentrations were 0.013 mg/L (4,5). Steady state is generally reached in 3 to 5 days with 20–40 mg twice daily, and after 7 days with 20 mg once daily, plasma levels reached an average 0.021 mg/L (0.015–0.032 mg/L) (3,6). According to Sharma et al. (7), the dose consumed is proportionate to the plasma concentrations, which averaged 0.95 μg/L/mg (0.38–1.89 μg/L/mg). Extrapolation of this information, for postmortem interpretation, suggests that

![Figure 1. Chemical structure of duloxetine.](https://academic.oup.com/jat/article-abstract/30/8/576/714394/14249)
Cymbalta daily doses of 60 mg and 120 mg would yield plasma concentrations of 0.057 mg/L (0.023-0.113 mg/L) and 0.114 mg/L (0.046-0.227 mg/L), respectively.

The elimination half-lives average 12 h (8–17 h range), and the pharmacokinetics of duloxetine are dose proportional over the therapeutic range (4). The apparent volume of distribution 1940 L (~24 L/kg based on an average person of 80 kg) and is highly (> 90%) protein bound (7). Both CYP1A2 and CYP2D6 are responsible for duloxetine metabolism as it undergoes extensive metabolism by the liver (oxidation and conjugation) to include 4-hydroxyduloxetine glucuronide and 5-hydroxy-6-methoxyduloxetine sulfate. Excretion of unchanged duloxetine in the urine consists of only a trace amount, < 1% of the dose. Approximately 70% of the dose is eliminated in the urine as metabolites and 20% is excreted in the feces (4).

Common side effects associated with duloxetine therapy include nausea and somnolence, small increases in recumbent systolic and diastolic blood pressure, and a small decrease in heart rate (7). Tolerance to these adverse effects occurs as the duloxetine dosing continued. As with other short half-life SSNRI, abrupt discontinuation was associated with mild withdrawal effects in association with sleep disturbance (7). These effects are expected and likely indicate the blocking action of norepinephrine reuptake (7).

**Experimental**

**Materials**

Duloxetine was obtained courtesy of Eli Lilly and Company and prepared as a 1.0 g/L stock solution in methanol. With each duloxetine quantitative run, a working stock solution of duloxetine was prepared in methanol at concentrations of 10 and 100 mg/L to supplement drug-free porcine blood at calibration levels of 0.05, 0.10, 0.25, 0.50, 1.0, and 3.0 mg/L.

Carbinoxamine was obtained from McNeil Laboratories and prepared as a 1.0 g/L stock solution in methanol. From this, a working stock was prepared in methanol at a concentration of 15 mg/L. (Note: Although carbinoxamine is a prescription drug, it is not prevalent in our casework and has been utilized in our laboratory for years.)

All reagents were analytical grade and purchased from various vendors.

The concentration of an analytical standard made from a powder source can be accurately verified with a spectrophotometer by comparing the extinction or molar absorptivity coefficient of a measured drug concentration to values previously established in the literature. The concentration of the carbinoxamine stock solution was verified to be accurately prepared at 1.0 g/L. Duloxetine’s coefficient has not been established in the literature and therefore was experimentally determined spectrophotometrically in our laboratory.

**Extraction**

For all pharmaceutical, basic drug extractions, to 2 mL of standard, blood sample, or tissue homogenate were added 100 µL of internal standard, (carbinoxamine, 15 mg/L), 2 mL of buffer (20% sodium carbonate), and 6 mL of chlorobutane extraction solvent. After rotation and centrifugation, the organic layer was separated. Two milliliters of 0.10N hydrochloric acid was added to the organic layer, and the mixture was vortex mixed. Following centrifugation and aspiration of the organic layer to waste, the aqueous layer was washed with 4 mL of 2-methylbutane. The organic layer was aspirated to waste. The aqueous layer was made alkaline with 1 mL of 20% sodium carbonate buffer and extracted with 4 mL of 2-methylbutane. The organic layer was evaporated to dryness and the residue was reconstituted with 75 µL of methanol and transferred to autosampler vials for instrumentation.

**Instrumentation**

A Hewlett-Packard (HP) model 6890 gas chromatograph (GC) with nitrogen-phosphorus detection (NPD) and 7683 autosampler was utilized for the screening of pharmaceutical basic drugs. Dual column separation was achieved using two capillary columns, equivalent to an HP-5 (15 m x 0.25-mm i.d., 0.25-mm film thickness) and an HP-35 (15 m x 0.32-mm i.d., 0.32-mm film thickness). The oven temperature was programmed at 140°C for 0.50 min, increased to 300°C at 10°C/min, and held for 13 min (total analysis time of 30 min). The injector temperature was 260°C, and the detector temperatures were 325°C. Unlike many other commonly encountered basic pharmaceutical drugs, duloxetine had a poor response on the GC-NPD; therefore, the initial detection was conducted by other means.

Detection of duloxetine was achieved with an HP 6890 GC equipped with a 5973 mass selective detector (MSD) and 7683 autosampler. The MSD was operated in the electron ionization mode and a full scan mass-to-charge ratio range of 40–450 amu. An HP-5 (15 m x 0.25-mm i.d., 0.25-mm film thickness) capillary column was used. The oven temperature was programmed at 10°C/min, from 140°C to 300°C, with a final hold of 4 min (total analysis time of 20 min). The injection port and detection port temperatures were 260°C and 325°C, respectively.

The method for quantitation of duloxetine by GC–MS analysis was through selective ion monitoring (m/z 144, 115, 154) using carbinoxamine as the internal standard (m/z 58, 71, 167) and similar detection instrument parameters.

**Calibration and validation**

Each analytical run consisted of six duloxetine calibrators (0.05, 0.10, 0.25, 0.50, 1.0, and 3.0 mg/L) supplemented into drug-free porcine blood, a blank blood matrix, and case specimens. All specimens were quantitated for duloxetine against the porcine calibration curve. Specimens with duloxetine concentrations greater than 3.0 mg/L were considered out of range and were repeated in another analytical run with an appropriate dilution.

The method’s limit of detection was 0.03 mg/L with a consistent linear range from 0.05 to 3.0 mg/L. Calibration curves were calculated by linear regression and had a correlation coefficient (r²) of 0.998 or better. The percent recovery of duloxetine was determined by comparing the mean peak areas of an extracted quality control (0.25 mg/L, n = 10) with the peak areas of a non-extracted 0.25 mg/L solution (n = 2). The re-
<table>
<thead>
<tr>
<th>Case #</th>
<th>Case History</th>
<th>Cymbalta Rx Information</th>
<th>All Toxicology Results in Central Blood (mg/L unless otherwise noted)</th>
<th>Mode of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56-year-old (y/o) WM* (70 kg); found unresponsive at home; history (Hx) of fibromyalgia, chronic pain, depression, heavy drinking, recent prescription (Rx) change, and past suicidal ideations</td>
<td>Rx = 30 mg, 1 x daily</td>
<td>Diphenhydramine +&lt; 0.50, trazodone +&lt; 0.10, fentanyl 0.023 (total), gabapentin 6.0, fentanyl 0.09</td>
<td>UND</td>
<td>Fentanyl intoxication</td>
</tr>
<tr>
<td>2</td>
<td>51 y/o BM (64 kg); found unresponsive in his car, Hx of diabetes, narcotics use, and on parole</td>
<td>Rx = 30 mg, 1 at bedtime</td>
<td>Duloxetine +&lt; 0.05, cyclobenzaprine metabolite present, cocaine 0.05, BE 0.33, acetone 0.043 g%, isopropanol 0.01 g%, vitreous glucose 568 mg/dL</td>
<td>ACC</td>
<td>Diabetic ketoacidosis</td>
</tr>
<tr>
<td>3</td>
<td>40 y/o WF (137 kg); found unresponsive at home; Hx of obesity, diabetes, psychiatric problems, suicide attempts (wrist cutting), and noncompliant with Rxs</td>
<td>Rx = 60 mg, 1 x daily</td>
<td>Duloxetine 0.28, trazodone 16, venlafaxine 6.8</td>
<td>SUI</td>
<td>Multiple drug intoxication</td>
</tr>
<tr>
<td>4</td>
<td>48 y/o WM (92 kg); found in bed with blood coming from mouth; Hx of pancreatitis, gout, ETOH abuse, skin lesions, and suicidal statements</td>
<td>Rx #1-3 = 60 mg, 1 in a.m.; Rx #4 = 30 mg, unk. directions</td>
<td>Duloxetine 0.30, atomoxetine +&lt; 0.10, ethanol 0.12 g%</td>
<td>ACC</td>
<td>Multiple drug intoxication</td>
</tr>
<tr>
<td>5</td>
<td>47 y/o WF (75 kg); found floating face down in swimming pool</td>
<td>Unknown</td>
<td>Duloxetine 0.46, ethanol 0.19 g%</td>
<td>ACC</td>
<td>Drowning</td>
</tr>
<tr>
<td>6</td>
<td>50 y/o WM (97 kg); found unresponsive at home and taken to hospital; Hx of auto collision 15 years ago, sustained fractures of the femur and collarbone, pinched back nerves, progressively worsening pain, being treated for degenerative disks and pain management</td>
<td>Unknown</td>
<td>Duloxetine 0.25, oxycodone 0.24, amitriptyline 0.93, nortriptilamine 0.90, chlorpheniramine +&lt; 0.10, cyclobenzaprine 0.28, hydrocodone 0.28 (free), diphenhydramine 1.4, flurazepam +&lt; 0.10</td>
<td>ACC</td>
<td>Multiple drug intoxication</td>
</tr>
<tr>
<td>7</td>
<td>42 y/o WF (72 kg); found unresponsive at home, Hx of Rx abuse, depression, and suicide notes found at scene</td>
<td>Rx = 60 mg, 2 in a.m.</td>
<td>Duloxetine 0.22, mirtazapine 0.26, norfluoxetine 0.14, alprazolam 0.043, lorazepam 0.017, temazepam 0.38, meprobamate 4.4, morphine 0.38 (free)</td>
<td>SUI</td>
<td>Morphine intoxication</td>
</tr>
<tr>
<td>8</td>
<td>36 y/o WF (75 kg); found unresponsive at home and taken to hospital; Hx of migraines, anxiety, Vicodin addiction, and depression</td>
<td>Unknown</td>
<td>Duloxetine 0.59, citalopram 1.1, norcitalopram 0.30, hydroxyzine +&lt; 0.10, norcloroxycyline +&lt; 0.10, meperidine +&lt; 0.10, normeperidine 0.11, propoxyphene +&lt; 0.10, norpropoxyphene 0.63, tramadol 0.74, nortramadol 0.34, quetiapine 0.26, hydrocodone 0.12 (free)</td>
<td>ACC</td>
<td>Poly-med overuse</td>
</tr>
<tr>
<td>9</td>
<td>20 y/o WF (49 kg); found unresponsive at mother's house; Hx of Crohn’s disease</td>
<td>Unknown</td>
<td>Duloxetine 0.17, diphenhydramine 0.19, methadone 0.54, norclorazepam 0.10, meprobamate +&lt; 2.5, hydrocodone 0.05 (total)</td>
<td>ACC</td>
<td>Multiple drug intoxication</td>
</tr>
<tr>
<td>10</td>
<td>59 y/o WF (84 kg); transported to the hospital with complaints of headache, nausea, vomiting, diarrhea, shortness of breath, and seizures; Hx of hypertension, high cholesterol, back problems, and knee replacement; went into cardiac arrest; possible addiction to Rx and overmedication</td>
<td>Unknown</td>
<td>Duloxetine 0.08, haloperidol +&lt; 0.10, metoprolol +&lt; 0.10</td>
<td>NAT</td>
<td>ASCVD</td>
</tr>
</tbody>
</table>
| 11    | 55 y/o WF (49 kg); found unresponsive at home, Hx of Tourette's Syndrome, clinical depression, stomach ailments, and chronic back pain | Rx = 30 mg, 1 x daily | Duloxetine 0.22, amphetamine 1.5, citalopram 2.1, norcitalopram 0.48, diphenhydramine 0.47, methadone 0.48, mitrazepine 0.55, venlafaxine 1.7, codeine 0.05 (free) | Multiple drug intoxication |* Abbreviations: UND, undetermined; ACC, accidental; NAT, natural; SUI, suicide; ASCVD, arteriosclerotic cardiovascular disease; WM, white male; BM, black male; and WF, white female.
wavelength of 290 nm. At this wavelength, the molar absorption of duloxetine was approximately 75%. A duloxetine concentration of 0.25 mg/L was extracted in replicates over a two-day period (n = 10) in order to determine inter- and intrarun precision. The coefficient of variation (CV) for interrun precision was 3.75%, whereas the CV for intrarun precision was 3.6%. No interfering peaks were detected in drug-free specimens such as central blood, urine, or liver homogenates.

Case Studies

Specimens
The postmortem interval (time between notification of death and autopsy) in 12 cases ranged from 1 to 4 days. All specimens taken during autopsy were at the discretion of the medical examiner. Central blood consisted of either heart blood or jugular vein blood, which was collected and preserved with sodium fluoride. A peripheral blood sample, primarily femoral vein blood, was collected in manufactured gray-top vials containing aqueous acid solution (Figure 2). The maximum absorbance for duloxetine HC1 was determined using a Beckman DU 520 Spectrophotometer to be at a wavelength of 290 nm. At this wavelength, the molar absorptivity coefficient (ε) of the drug in aqueous acid (0.1N HCl) was experimentally determined to average 208 molar-1cm-1 (203-211, n = 4) based on a 10-mg/mL (or 1%) solution of test and a path length of 1.0 cm. Or simply stated, at 290 nm, A1 = 208 in an aqueous acid solution (Figure 2).

The results could be made through sample derivatization. Because our laboratory’s basic drug screen methodology has a level of detection for duloxetine (0.03 mg/L) that is within the estimated clinical therapeutic range (0.021-0.114 mg/L), derivationization was determined to be unnecessary for our laboratory purpose. In addition, it is the authors’ belief that the detection method was sufficient for postmortem interpretation. However, if a laboratory is screening pharmaceutical drugs solely by GC-NPD, and/or the level of detection for duloxetine with this method is not adequate for their analytical needs, alternative instrumentation or sample derivatization should be considered.

At the Los Angeles County Department of Coroner, a basic drug screen is not performed in all circumstances of death. Therefore, the presence of duloxetine in only 12 cases over a year and a half of casework may not accurately reflect the prevalence of this drug in our cases. However, it does reflect all cases where basic drugs were suspected of contributing to the cause of death.

The central blood duloxetine concentrations of the 12 cases ranged from not detected (ND) to 0.59 mg/L (n = 12). The associated femoral blood levels ranged from ND to 0.26 mg/L.

<table>
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<tr>
<td>12</td>
<td>48 y/o WM* (68 kg); found unresponsive at home and taken to hospital; Hx of dementia and substance abuse</td>
<td>Duloxetine 0.23, methadone 0.84, acetone 0.009 g%</td>
<td>ACC</td>
<td>Methadone intoxication</td>
</tr>
</tbody>
</table>

* Abbreviations: UND, undetermined; ACC, accidental; NAT, natural; SUI, suicide; ASCVD, arteriosclerotic cardiovascular disease; WM, white male; BM, black male; and WF, white female.

Discussion

Duloxetine bears structural similarity to fluoxetine and atomoxetine (6), and one would have a reasonable expectation that this drug would be identified likewise. However, contrary to this belief, duloxetine is difficult to detect by NPD. Actual detection limits of the NPD were not pursued; however, a duloxetine response was not observed for a 3.0 mg/L non-extracted standard. Duloxetine extracts similar to other basic pharmaceutical drugs via a basic liquid–liquid extraction; however, detection must be achieved with an MS or with liquid chromatography equipped with either an ultraviolet (UV) or MS detector. The poor NPD response could be attributed to duloxetine’s secondary amine functional group, and enhancement could be made through sample derivatization. Because our laboratory’s basic drug screen methodology has a level of detection for duloxetine (0.03 mg/L) that is within the estimated clinical therapeutic range (0.021-0.114 mg/L), derivationization was determined to be unnecessary for our laboratory purpose. In addition, it is the authors’ belief that the detection method was sufficient for postmortem interpretation.
Central-to-femoral blood ratio averaged 1.98 (1.15–3.13, n = 7), suggesting duloxetine exhibits postmortem redistribution. The level of duloxetine measured in the liver averaged 5.1 mg/kg (0.28–22 mg/kg, n = 8), consistent with the large volume of distribution of ~24 L/kg. The liver-to-central blood ratio averaged 16 (3.5–48, n = 8).

In the elimination specimens, bile was the main excretion product and averaged 1.4 mg/L (0.57–3.1 mg/L, n = 7), whereas the urine averaged 0.22 mg/L (0.07–0.47 mg/L, n = 6). These relative levels were expected as duloxetine is extensively metabolized, and only a trace amount of unchanged drug is excreted in the urine (4, 5).

An evaluation of the dosing records along with the remaining duloxetine capsules that were collected as medical evidence in 6 of the 12 cases (1–4, 7, and 11) indicate that these individuals were in compliance with their duloxetine prescriptions. This suggests to the authors that the measured blood levels appear to represent postmortem therapeutic values.

### Conclusions

Duloxetine is a relatively new drug prescribed for pain management and depression that has recently been encountered in our casework. With duloxetine’s poor response on the GC-NPD, detection of the drug must be performed by alternate means. In a period of a year and a half, duloxetine was detected in 12 cases by GC-MS. Based on the toxicology and autopsy findings in these postmortem cases, duloxetine was not implicated as the sole cause of death. Duloxetine has a high volume of distribution and, as expected, exhibits postmortem redistribution.

The tissue distribution of duloxetine in postmortem samples has been provided to aid the forensic toxicologist in the interpretation of their casework. These are the first postmortem duloxetine cases to be reported in the literature, and it is the authors’ belief that more data need to be evaluated in order to establish clear postmortem therapeutic levels.

### References