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# Analysis of Citalopram and Desmethylcitalopram in Postmortem Fluids and Tissues Using Liquid Chromatography-Mass Spectrometry

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16. Abstract

Citalopram is a selective serotonin reuptake inhibitor that is a commonly prescribed drug for the treatment of depression, obsessive-compulsive disorder, panic disorder, anxiety disorder, and post-traumatic stress disorder. While the use of citalopram is relatively safe, certain side effects could negatively affect a pilot's performance and become a factor in an aviation accident. The side effects of this medication include nausea, tiredness, drowsiness, dizziness, and blurred vision. Due to the severity of aviation accidents, blood samples are often not available, so tissues must be relied upon for analysis. Therefore, understanding the distribution of a drug throughout postmortem fluids and tissues is important when trying to interpret drug impairment and/or intoxication. Our laboratory investigated the distribution of citalopram and its main active metabolite, desmethylcitalopram, in various postmortem tissues and fluids obtained from 15 fatal aviation accident cases. When available, 10 specimen types were analyzed for each case, including blood, urine, vitreous humor, liver, lung, kidney, spleen, muscle, heart, and brain. Whole blood citalopram concentrations obtained from these 15 cases ranged from 0.079 to 1.06 µg/mL. Distribution, expressed as specimen/blood ratio, for citalopram was $12 \pm 19$ in urine, $0.42 \pm 0.21$ in vitreous humor, $16 \pm 8$ in liver, $15 \pm 15$ in lung, $3.6 \pm 2.5$ in kidney, $8.1 \pm 3.7$ in spleen, $0.83 \pm 0.40$ in muscle, $2.3 \pm 1.2$ in brain, and $1.9 \pm 1.0$ in heart. Distribution coefficients obtained for citalopram had coefficient of variations (CV) ranging from 46-158%. With such large CV's, the distribution coefficients have very little use in aiding in the interpretation of citalopram-positive tissue specimens. Furthermore, no consistent citalopram/desmethylcitalopram ratio was identified within any specimen group. This study suggests that citalopram likely undergoes postmortem concentration changes.

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# CONTENTS

# ANALYSIS OF CITALOPRAM AND DESMETHYLCITALOPRAM IN POSTMORTEM FLUIDS AND TISSUES USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

## INTRODUCTION

The Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI) is responsible under Department of Transportation Orders 8020.11B and 1100.2C to "conduct toxicological analysis on specimens from … aircraft accident fatalities" and "investigate … general aviation and air carrier accidents and search for biomedical and clinical causes of the accidents, including evidence of … chemical (use)." Therefore, following an aviation accident, samples are collected at autopsy and sent to CAMI's Bioaeronautical Sciences Research Laboratory, where toxicological analysis is conducted on various postmortem fluids and tissues. Occasionally, during a toxicological evaluation, potentially impairing compounds are detected in postmortem specimens from aviation accident victims.

Citalopram, (RS)-1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1, 3-dihydroisobenzofuran-5-carbonitrile, sold under the trade names Celexa® and Cipramil® is a selective serotonin reuptake inhibitor (SSRI). The S enantiomer is sold under the name Lexapro®. As a whole, citalopram is one of the most commonly prescribed drugs for the treatment of depression, obsessive-compulsive disorder, panic disorder, anxiety disorder, and post-traumatic stress disorder.[1-3] Treatment of depression with citalopram is relatively safe; however, certain side effects of this medication, including nausea, tiredness, drowsiness, dizziness, and blurred vision could affect pilot performance and become a factor in an aviation accident.[4] For this reason, each pilot fatality received by the forensic toxicology laboratory at CAMI is screened for citalopram.

Citalopram is well absorbed following oral administration. Peak plasma concentrations are achieved within 2 to 4 hours after oral administration, and the reported elimination half-life is between 27 – 33 hours.[5-7] The predominate metabolite in the body is the pharmacologically active compound, desmethylcitalopram. The volume of distribution (Vd) for citalopram is around 20 L/kg (ranging from 12 – 36 L/kg).[5] Approximately 8 – 12% of a dose of citalopram is excreted as the unchanged drug in the urine.[5,6,8]

Scientific information concerning the postmortem distribution of citalopram is mostly limited to drug overdose cases.[9-12] Therefore, to better understand what non-fatal citalopram concentrations look like in postmortem cases, our laboratory set out to determine its distribution in various postmortem tissues and fluids. A search of our laboratory database identified 15 aviation fatalities from 15 separate aviation accidents that were reported positive for citalopram in blood and also had most biological tissues and fluids available for analysis. These specimen types included blood, urine, vitreous humor, skeletal muscle, liver, kidney, lung, spleen, brain, and heart muscle. This paper describes a method for the quantitation of both citalopram and desmethylcitalopram in postmortem fluids and tissues utilizing solid phase extraction (SPE) and liquid chromatography (LC) with atmospheric pressure chemical ionization (APCI) ion trap mass spectrometry (MS). Following method validation, fluid and tissue specimens from aviation fatalities were examined that previously screened positive for citalopram.

## MATERIALS AND METHODS

### Chemicals and Reagents

Citalopram and desmethylcitalopram were purchased from Cerilliant (Cerilliant Corp., Round Rock, TX) at a concentration of 1.00 mg/mL in methanol. D$_6$-citalopram was purchased from Cerilliant at a concentration of 100 µg/mL in methanol. Potassium phosphate dibasic, sodium fluoride, ammonium hydroxide, acetic acid, methanol, and ethyl acetate were purchased from Fisher Scientific (Fisher Scientific, Inc.; Pittsburgh, PA) in the highest possible purity. Formic acid (97%) was purchased from ICN (ICN Biomedicals, Inc., Irvine, CA). Double deionized water (DDW) was obtained using an ELGA, PURELAB Ultra water system (ELGA, Inc.; Lowell, MA). The pH of all solutions was measured using a Corning model 430 pH meter connected to a Corning 3-in-1 model pH electrode.

Fifty mM formic acid constituted the aqueous portion of the HPLC mobile phase and was adjusted to pH 5.00 with conc. ammonium hydroxide. The formic acid buffer was mixed with acetonitrile in a 98:2 (v:v) ratio, respectively, to help prevent the growth of microbes. This mixture was filtered through a vacuum filtering apparatus that incorporated a 0.45 µm GH polypro 47 mm hydrophilic, polypropylene membrane filter obtained from Pall Gelman laboratory (Pall Corp., East Hills, NY). The primary organic component of the mobile phase was

HPLC grade acetonitrile, which was filtered prior to use through a vacuum filter apparatus that incorporated the same type of membrane filter described above.

## Sample Selection and Storage

Our laboratory identified 15 citalopram-positive fatalities from separate aviation accidents that occurred between 2007-2010. These particular cases were selected because they each had a majority of the desired biological tissues and fluids available for this study (blood, urine, vitreous humor, lung, liver, kidney, spleen, muscle, heart, and brain). In each case, blood was stored at -20°C in tubes containing 1.00% (w/v) sodium fluoride/potassium oxalate prior to analysis. All other specimen types were stored without chemical preservation at -20°C until analysis. Whole-blood citalopram concentrations determined in this study agreed well with those previously determined by our laboratory, verifying that no significant deterioration had occurred under these storage conditions.

## Instrumentation and LC/MS Method

All analyses were performed using a LC-MS, which consists of an Agilent 1200 series LC with a LTQ XL Mass Spectrometer. Chromatographic separation was achieved using an Ascentis® Express C18 column (10cm, 4.6mm, 2.7 μm). The HPLC was operated in an isocratic mode with a flow rate of 1.00 mL/min. The mobile phase ratio employed was 80:20 (acetonitrile:buffer). The sample injection volume was held constant at 5 μL. The HPLC column was routinely equilibrated for 2-3 hours prior to use. Following use, the column was stored in 100% acetonitrile.

Initially, precursor ions were identified for all compounds. Following [M+H]+ ion identification, ionization conditions were optimized by infusing each analyte directly into the mobile phase, which was then introduced into the mass spectrometer at a flow rate of 1.00 mL/min. Tuning the MS for the desired ions was then accomplished using the autotune feature of the Xcalibur™ software. Each sample analysis consisted of 1 data collection segment. This segment collected data for all 3 analytes. The operating conditions for the data collection segment were as follows: APCI capillary temperature, 225°C; APCI vaporizer temperature, 450°C; source discharge current, 5.00 μA; sheath gas flow (nitrogen), 25; auxiliary gas flow (nitrogen), 20; capillary voltage, 9.0 V; tube lens, 70.0 V; multipole 00 offset, -4.5 V; lens 0, -4.00 V; multipole 0 offset, -4.75 V; lens 1, -9.00 V; gate lens, -54.00 V; multipole 1 offset, -10.0 V; front lens, -5.5 V; and 1 micro-scan having a maximum ion injection time of 30 msec. This segment was further split into 4 separate scan events.

Scan event 1 conducted a full scan from $m/z$ 100-340, from which citalopram, desmethylcitalopram, and $D_6$-citalopram precursor, [M+H]+, ions at $m/z$ 325, 311.1, and $m/z$ 331 (respectively) were obtained. Scan event 2 collected the citalopram product ions between 100-340 following collision-induced dissociation (CID) of the precursor ion ($m/z$ 325) using a collision energy of 26%. Scan event 3 collected the desmethylcitalopram product ions between 100-340 following CID of the precursor ion ($m/z$ 311.1) using a collision energy of 24%. Scan event 4 collected the $D_6$-citalopram product ions between 100-340 following CID of the precursor ion ($m/z$ 331) using a collision energy of 25%.

The MS/MS spectra of citalopram provided 3 predominant ions consisting of $m/z$ 307.2, 262.1, and 280.1. The MS/MS spectra of desmethylcitalopram provided 3 predominant ions at $m/z$ 293.1, 262.1, 280.1. The MS/MS spectra of $D_6$-citalopram provided 3 predominant ions at $m/z$ 313.2, 262.1, 280.1. The MS/MS ions $m/z$ 307.2, 293.1, and 313.2 were used for quantitative purposes for their respective compounds.

Acceptability criteria employed for analyte identification were as follows: (1) compound confirmation was conducted with MS/MS spectral matching; and (2) the analyte was required to have a retention time within ± 2.00% of the average retention time for each respective calibrator used to construct the calibration curve for that analyte. Analytes not meeting these criteria were reported as either negative or inconclusive.

## Calibrator and Control Preparation

Calibration curves were prepared at concentrations ranging from 1.56 to 6400 ng/mL. A minimum of 6 calibrators were used to construct each calibration curve. Controls were prepared at concentrations of 10, 100, and 1000 ng/mL and extracted with each batch of unknowns to verify the accuracy of the established calibration curve.

Calibration curves for citalopram and desmethylcitalopram were prepared by serial dilution, utilizing bovine whole blood as the diluent. Controls were prepared in a similar manner but from a separate manufacturer's lot of drug standard. An aqueous internal standard solution, $D_6$-citalopram, was prepared at a final concentration of 1000 ng/mL.

The linear dynamic range (LDR), limit of detection (LOD), and limit of quantitation (LOQ) were determined using the prepared calibration curve described above. Our laboratory defines LOD as the lowest concentration of analyte having a minimum quantitation ion signal-to-noise ratio (S/N) of 10, in addition to meeting the MS/MS spectral "fingerprint" identification and retention time criteria. The LOQ was defined as meeting all LOD

criteria, plus having an experimentally determined value within ± 20% of its prepared concentration.

*Sample Preparation and Extraction Procedure*

Calibrators, controls, and postmortem specimens were prepared by the following procedure. Tissue samples were diluted with a 1.00% NaF solution in a 1:2 (tissue:1% NaF solution) dilution prior to being homogenized. All tissue samples were homogenized using an Omni post-mounted homogenizer (Omni International; Kennesaw, GA). One mL aliquots of each calibrator, control, postmortem fluid, and 3.00 g aliquots of each tissue homogenate (1 g wet tissue) were transferred into individual 16 x 150-mm screw-top tubes. To each test tube, 100 μL of internal standard (100 ng) was added and allowed to stand for 5 min. Next, 6.00 mL of potassium phosphate buffer (pH 6.00) was added to each tube and mixed vigorously. The tubes were then centrifuged at 1600 x *g* for 20 min providing for removal of proteins and cellular debris. Following centrifugation, the extracts were transferred to Bond Elute Certify® SPE columns (Varian, Inc.), which had been pre-conditioned with 2 mL methanol followed by 2 mL of 0.10 M phosphate buffer (pH 6.00). Care was taken to prevent the columns from drying prior to sample addition. Column flow rates of 1-2 mL/min were maintained in each SPE step using a Varian Cerex® 24-port, positive-pressure extraction manifold with a nitrogen pressure of 2 psi. After each sample passed through its respective column, all SPE columns were washed with 1 mL of 1.0 M acetic acid and dried for 5 min with 25 psi of nitrogen. Once dry, the columns were again washed by adding 2 mL of methanol to each. The columns were again dried with nitrogen at 25 psi for 5 min. The analytes of interest were eluted from the columns with 3 mL of 2% ammonium hydroxide in ethyl acetate and evaporated to dryness in a TurboVap™ concentration workstation (Caliper Life Sciences; Hopkinton, MA) set at 40°C under a stream of dry nitrogen. Once dried, the residue was reconstituted in 50 μL of acetonitrile and transferred to autosampler vials for analysis. All specimens were analyzed at one time to avoid inter-assay variations. Specimens with analyte concentrations above the associated calibration curves were diluted by an appropriate factor and re-extracted.

*Extraction Efficiency*

The extraction efficiency for citalopram and desmethyl-citalopram was determined using a procedure commonly employed in our laboratory.[13] Two control groups, X and Y, were prepared using negative whole blood diluent and extracted in the same manner as described immediately above. Group X was spiked with a precisely known amount of citalopram and desmethylcitalopram prior to extrac-

tion, and group Y was spiked with the same precisely known amount of citalopram and desmethylcitalopram following solid phase extraction. Upon analysis, the average response factor obtained from group X was divided by the average response factor obtained from group Y to yield the percent recovery value.

*Matrix Effect*

The evaluation of ion suppression was conducted by infusing a constant flow of analyte into the mobile phase (at the source) and simultaneously injecting an extracted matrix specimen while monitoring the quatitation ion for each analyte of interest. The drug standard at 10 μg/mL was infused into the HPLC mobile phase at 3 μL/min using the built-in LC/MS syringe pump.

## RESULTS AND DISCUSSION

*Analysis of Citalopram*

Both citalopram and desmethylcitalopram were eluted from the analytical column in approximately 1 min. By using atmospheric pressure chemical ionization, MS spectra were produced consisting predominantly of the protonated [M+H]+ ion. The precursor ions were *m/z* 325.2, 311.1, 331.1 for citalopram, desmethylcitalopram, and $D_6$-citalopram, respectively. The ion trap collected and subsequently fragmented each of these precursor ions. The MS/MS spectra of citalopram provided 3 predominant ions consisting of *m/z* 307.2, 262.1, and 280.1. The MS/MS spectra of desmethylcitalopram provided 3 predominant ions at *m/z* 293.1, 262.1, 280.1, and $D_6$-citalopram provided 3 predominant ions at *m/z* 313.2, 262.1, 280.1. The full-scan MS/MS spectra for citalopram and desmethylcitalopram provided "fingerprints" used for analyte identification and confirmation

Product ions *m/z* 307.2, 293.1, and 313.2 were used for quantitative purposes for their respective compounds. While ions *m/z* 262.1 and 280.1 were present in each of the analyte product spectra, each of these ions does indeed originate from the unique precursor ion. We verified this by injecting 1000 μg on-column of each compound separately and found no "bleed over" from one precursor ion to a different analytes' product spectra.

Ion suppression was evaluated by monitoring the quantitation ion for each analyte of interest while infusing a constant flow of analyte into the mobile phase at the source and simultaneously injecting an extracted negative blood specimen. Ion suppression would cause a decrease in the monitored ion intensity. No decrease in the analyte's expected retention time range was observed.

Basic acceptability criteria consisted of retention times and MS/MS spectral matching. Analyte retention times obtained from postmortem specimens were required to

be within ± 2.0% of the average calibrator retention time. Typical retention times were around 0.85 min for citalopram and desmethylcitalopram. The full scan MS/MS spectra for citalopram and desmethylcitalopram provided "fingerprints" used for analyte identification and confirmation. Any peak not matching these criteria was reported as negative. The LDR, LOD, and LOQ were determined by analysis of a calibration curve that contained calibrators ranging in concentration from 0.78 – 6400 ng/mL. The LDR for citalopram and desmethylcitalopram was determined to be 3.13 – 3200 ng/mL and 1.56 – 3200 ng/mL, respectively. The correlation coefficient for these calibration curves exceeded 0.99 when a weighting factor of 1/X was used. The LOD and LOQ determined for citalopram and desmethylcitalopram are listed in Table 1. The LOD and LOQ for citalopram when extracted from whole blood were determined to be 1.56 ng/mL and 3.13 ng/mL, respectively. The LOD and LOQ for desmethylcitalopram were 1.56 ng/mL and 1.56 ng/mL, respectively.

Care was taken to ensure that instrumental carryover from one sample to the next was not a factor. An acetonitrile blank, injected following a 6400 ng/mL calibrator, showed no carryover contamination. Subsequently, blanks were used following each postmortem specimen in the sample sequence to verify that no sample-to-sample contamination occurred.

Intra-day (within day) and inter-day (between days) accuracy and precision were performed using whole blood controls at concentrations of 10, 100, and 1000 ng/mL. These values were chosen because they are distributed throughout the extensive LDR of these compounds. These controls were prepared in 50 mL quantities on Day 1 of the experiment and stored at 4°C until extracted.

For intra-day analyses, a calibration curve was extracted along with 5 replicates of each control concentration on Day 1 of the experiment. Inter-day accuracy and precision were evaluated by extracting 5 replicates of each of the 3 control concentrations on Days 3 and 5 and generating quantitative values by utilizing the calibration curve originally prepared on Day 1.

Accuracy of this test was evaluated by looking at the relative error, i.e., the percentage difference between the measured value and the target value. The precision was measured as the coefficient of variation (CV). Intra-day relative errors in the 10, 100, and 1000 ng/mL control groups were ≤ 7% for either compound. Furthermore, the intra-day CV values were ≤ 4% for citalopram and desmethylcitalopram. The inter-day relative errors for Days 3 and 5 for either analyte at each control concentration were ≤ 8%, and the CVs were ≤ 6%. Accuracy and precision results show that this method is both accurate and precise over a 5-day period (Table 2).

**Table 1.** LDR, LOD, LOQ and recovery for citalopram and desmethylcitalopram.

| Compound | LDR (ng/mL) | LOD (ng/mL) | LOQ (ng/mL) | Extraction Efficiency (%) ± sd* | | |
| --- | --- | --- | --- | --- | --- | --- |
| | | | | 10 ng/mL | 100 ng/mL | 1000 ng/mL |
| Citalopram | 3.13 - 3200 | 1.56 | 3.13 | 87 ± 8 | 93 ± 6 | 87 ± 6 |
| Desmethyl- | 1.56 - 3200 | 1.56 | 1.56 | 95 ± 9 | 87 ± 8 | 74 ± 8 |

* n=4 for all measurements vitreous humor

**Table 2.** Intra and inter-day accuracy and precision.

| | | Day 1 | | | Day 3 | | | Day 5 | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Target (ng/mL) | Mean (ng/mL) | CV | %E | Mean (ng/mL) | CV | %E | Mean (ng/mL) | CV | %E |
| Citalopram | 10 | 9.6 ± 0.2 | 2 | 4 | 9.6 ± 0.3 | 3 | 4 | 9.9 ± 0.3 | 3 | 1 |
| | 100 | 98.8 ± 0.8 | 1 | 1 | 98 ± 3 | 3 | 2 | 98 ± 2 | 2 | 2 |
| | 1000 | 947 ± 41 | 4 | 5 | 963 ± 23 | 2 | 4 | 983 ± 14 | 1 | 2 |
| Desmethyl- | 10 | 9.6 ± 0.2 | 2 | 4 | 10 ± 0.2 | 2 | 0 | 9.2 ± 0.4 | 5 | 8 |
| | 100 | 101 ± 1 | 1 | 4 | 96 ± 3 | 3 | 4 | 101 ± 5 | 5 | 1 |
| | 1000 | 1071 ± 37 | 3 | 7 | 1060 ± 17 | 2 | 6 | 1038 ± 58 | 6 | 4 |

n=5 for all measurements
Accuracy measured as relative error (%E) from target concentration.
Precision measured as CV in replicate measurements.

The stability of citalopram and desmethylcitalopram in whole blood was determined by evaluating the control concentrations obtained on Day 5 of the inter-day experiment (Table 2). Neither compound showed apparent decrease in concentration after 1 week of storage at 4°C at concentrations of 10, 100, and 1000 ng/mL. These results demonstrated that whole blood specimens should be stable for 5 days when stored at 4°C. However, as good laboratory practice and to ensure the highest quality analytical data, we recommend that biological specimens always be analyzed promptly after thawing.

The extraction efficiency for citalopram and desmethylcitalopram at various concentrations obtained from this SPE procedure was high. As can be seen in Table 1, the extraction efficiency of citalopram ranged from 87 – 93% over a wide range of concentrations. The extraction efficiency of desmethylcitalopram ranged from 74 – 95%.

*Postmortem Concentrations of Citalopram and Desmethylcitalopram*

As previously stated, in fatal aviation accidents, specimens from accident victims are routinely sent to CAMI for toxicological analysis. Postmortem fluid and tissue samples obtained from 15 separate aviation fatalities that had previously screened positive for citalopram were re-examined using the current method. The fluid and tissue specimens examined from each victim, if available, included: blood, urine, vitreous humor, skeletal muscle, liver, kidney, lung, spleen, brain, and heart muscle.

The pharmacology, pharmacokinetics, and pharmacodynamics of citalopram and its active metabolite are beyond the scope of this paper. These topics are, however, extensively covered elsewhere.[5,6,14-18] Since $D_6$-citalopram was used as the internal standard in this study, citalopram values obtained from various fluid/tissue types were considered very reliable. However, even though $D_6$-citalopram is structurally very similar to desmethylcitalopram, the interpretation of quantitative desmethylcitalopram data obtained from specimen types other than blood should be scrutinized due to possible variations in extraction efficiency between specimen types.

The National Transportation Safety Board (NTSB) is responsible for determining the cause(s) of all fatal civil aviation accidents in the United States. The NTSB has issued final Probable Cause for 12 of the 15 cases presented here. In their assessment, citalopram was not listed as either a cause or a factor in any of these aviation accidents.

Therapeutic blood concentrations range from 0.020 to 0.200 μg/mL.[19] Toxic levels of citalopram have been reported at concentrations as low as 0.500 μg/mL.[19]

Previous studies have found that concentrations of citalopram in non-citalopram related deaths significantly overlapped concentrations found in citalopram-related deaths.[7,20] Blood concentrations observed in the current study ranged from 0.079 to 1.06 μg/mL, representing mid-therapeutic to possible toxic levels. However, since the site from which the blood was collected at autopsy is unknown for each of these cases, and due to postmortem redistribution or other factors, these blood concentrations may not be representative of the levels observed prior to death.

The concentration of citalopram and desmethylcitalopram in each postmortem specimen analyzed from these 15 cases can be seen in Tables 3 and 4. On average, the highest concentrations of citalopram and desmethylcitalopram present in each victim were found in liver and lung. High concentrations in the liver were expected as liver is the main site of metabolism, and a prevalent route of excretion for both analytes is the feces.[8] The general trend for highest concentration to lowest concentration of both drugs was liver, lung, spleen, urine, kidney, heart, brain, blood, muscle, and vitreous humor. With a moderately high Vd for citalopram, around 20 L/kg, we expected it to be high in tissues.

We evaluated the desmethylcitalopram/citalopram ratio within each of the specimen types. In almost every instance, citalopram was at higher concentrations than its metabolite. However, no significant correlation between citalopram and desmethylcitalopram concentrations existed within or between any of the specimen types analyzed.

The mean distribution coefficients for citalopram and desmethylcitalopram, expressed as specimen concentration/blood concentration, are listed in Tables 6 and 7. No consistent distribution patterns between cases were observed. The large CV values associated with the distribution coefficients were not completely unexpected, as many unknown variables exist in these cases. The large CV's could result from numerous factors, such as differing blood collection sites at autopsy, postmortem interval, postmortem redistribution, contamination, time between citalopram consumption and death, citalopram dosage, age of the victim, and health of the victim, i.e., renal and hepatic function. The blood collection site and postmortem interval for these cases are unknown. However, in most of the cases we receive for analysis in which the collection site is reported, the blood typically is noted as having been collected from the chest cavity. Alkaline compounds readily undergo postmortem redistribution in the interval between death and specimen collection. This redistribution could account for some of the larger CV values obtained.

**Table 3.** Citalopram concentrations obtained from 15 pilot fatalities.[*]

| Case | Blood | Urine | VH | Liver | Lung | Kidney | Spleen | Muscle | Brain | Heart |
|------|-------|-------|-----|-------|------|--------|--------|--------|-------|-------|
| 1 | 0.468 | — | 0.224 | 10.64 | 4.22 | 0.316 | 6.04 | 0.185 | 0.933 | 2.01 |
| 2 | 0.431 | — | — | 6.07 | 3.51 | 1.04 | 3.06 | 0.364 | 0.925 | 1.16 |
| 3 | 0.213 | — | — | 3.30 | 9.66 | 0.756 | 2.39 | 0.265 | 0.427 | 0.390 |
| 4 | 1.06 | 2.87 | — | 12.40 | 15.93 | 2.47 | 7.21 | 0.783 | 1.42 | 2.40 |
| 5 | 0.650 | 0.883 | 0.072 | 3.34 | 6.68 | 0.708 | 1.56 | 0.178 | 0.407 | 0.758 |
| 6 | 0.581 | 1.05 | 0.365 | 7.03 | 3.62 | 1.34 | 3.83 | 0.379 | 1.40 | 1.38 |
| 7 | 0.842 | — | — | 14.52 | 4.80 | 1.94 | 3.73 | 0.274 | 0.554 | 0.790 |
| 8 | 0.309 | 0.459 | 0.102 | 6.48 | 2.86 | 1.24 | 2.39 | 0.305 | 1.16 | 1.37 |
| 9 | 0.800 | 2.11 | — | 6.69 | 6.85 | 2.44 | 4.54 | 0.729 | 2.34 | 1.15 |
| 10 | 0.244 | 4.25 | — | 5.25 | 2.17 | 2.03 | 3.44 | 0.354 | 1.36 | 1.40 |
| 11 | 0.917 | — | — | 8.00 | 5.55 | 1.14 | 3.94 | 0.339 | 2.28 | 1.20 |
| 12 | 0.163 | — | — | 5.69 | 8.95 | 1.40 | 1.81 | 0.236 | 1.11 | 1.22 |
| 13 | 0.079 | — | — | 2.17 | 2.12 | 0.583 | 1.31 | 0.101 | 0.378 | 0.378 |
| 14 | 0.486 | 28.12 | 0.329 | 5.68 | 5.19 | 1.59 | 3.90 | — | 2.01 | 3.91 |
| 15 | 0.607 | — | — | 5.15 | 9.28 | 1.89 | 3.99 | 0.458 | 1.50 | 1.64 |

[*] All concentrations shown in units of $\mu$g/mL or $\mu$g/g
— Specimen type not available for analysis


**Table 4.** Desmethylcitalopram concentrations obtained from 15 pilot fatalities.[*]

| Case | Blood | Urine | VH | Liver | Lung | Kidney | Spleen | Muscle | Brain | Heart |
|------|-------|-------|-----|-------|------|--------|--------|--------|-------|-------|
| 1 | 0.041 | — | 0.008 | 0.972 | 0.286 | 0.074 | 0.454 | 0.019 | 0.026 | 0.230 |
| 2 | 0.020 | — | — | 0.779 | 0.903 | 0.0170 | 0.288 | 0.023 | 0.039 | 0.089 |
| 3 | 0.002 | — | — | 0.439 | 0.509 | 0.072 | 0.177 | 0.008 | 0.045 | 0.003 |
| 4 | 0.283 | 0.818 | — | 5.29 | 5.25 | 0.834 | 0.706 | 0.098 | 0.454 | 0.695 |
| 5 | 0.035 | 0.730 | 0.018 | 2.70 | 3.80 | 0.883 | 0.940 | 0.044 | 0.111 | 0.435 |
| 6 | 0.233 | 0.808 | 0.172 | 3.43 | 0.329 | 1.14 | 1.48 | 0.025 | 0.220 | 0.450 |
| 7 | 0.179 | — | — | 8.62 | 2.30 | 1.20 | 2.43 | 0.045 | 0.133 | 0.302 |
| 8 | 0.058 | 1.01 | 0.018 | 1.71 | 0.475 | 0.370 | 0.530 | 0.048 | 0.083 | 0.122 |
| 9 | 0.318 | 1.04 | — | 2.71 | 2.73 | 0.991 | 1.16 | 0.186 | 0.225 | 0.123 |
| 10 | 0.072 | 3.71 | — | 4.11 | 1.89 | 2.08 | 2.06 | 0.133 | 0.306 | 0.758 |
| 11 | 0.395 | — | — | 6.87 | 3.49 | 0.821 | 2.10 | 0.164 | 0.311 | 0.365 |
| 12 | 0.051 | — | — | 1.91 | 2.60 | 0.701 | 0.544 | 0.053 | 0.096 | 0.250 |
| 13 | 0.058 | — | — | 2.01 | 2.11 | 0.841 | 1.24 | 0.085 | 0.112 | 0.303 |
| 14 | 0.120 | 1.44 | 0.188 | 2.12 | 1.76 | 0.715 | 1.30 | — | 0.177 | 1.68 |
| 15 | 0.146 | — | — | 1.88 | 3.02 | 0.761 | 0.841 | 0.108 | 0.111 | 0.285 |

[*] All concentrations shown in units of $\mu$g/mL or $\mu$g/g
— Specimen type not available for analysis

**Table 5.** Ratio of desmethylcitalopram to citalopram in postmortem specimens.

| Case | Blood | Urine | VH* | Liver | Lung | Kidney | Spleen | Muscle | Brain | Heart |
|------|-------|-------|------|-------|------|--------|--------|--------|-------|-------|
| 1 | 0.088 | — | 0.036 | 0.091 | 0.068 | 0.234 | 0.075 | 0.103 | 0.028 | 0.115 |
| 2 | 0.046 | — | — | 0.128 | 0.258 | 0.163 | 0.094 | 0.063 | 0.042 | 0.077 |
| 3 | 0.009 | — | — | 0.133 | 0.053 | 0.095 | 0.074 | 0.030 | 0.105 | 0.008 |
| 4 | 0.268 | 0.285 | — | 0.427 | 0.329 | 0.337 | 0.098 | 0.125 | 0.321 | 0.289 |
| 5 | 0.054 | 0.827 | 0.250 | 0.805 | 0.570 | 1.247 | 0.603 | 0.247 | 0.273 | 0.574 |
| 6 | 0.401 | 0.771 | 0.471 | 0.487 | 0.091 | 0.854 | 0.386 | 0.066 | 0.158 | 0.325 |
| 7 | 0.213 | — | — | 0.594 | 0.479 | 0.619 | 0.651 | 0.164 | 0.240 | 0.382 |
| 8 | 0.188 | 2.203 | 0.176 | 0.264 | 0.166 | 0.297 | 0.222 | 0.157 | 0.072 | 0.089 |
| 9 | 0.398 | 0.494 | — | 0.405 | 0.398 | 0.406 | 0.256 | 0.255 | 0.096 | 0.107 |
| 10 | 0.295 | 0.872 | — | 0.783 | 0.874 | 1.025 | 0.597 | 0.376 | 0.225 | 0.540 |
| 11 | 0.431 | — | — | 0.859 | 0.628 | 0.720 | 0.532 | 0.484 | 0.136 | 0.305 |
| 12 | 0.313 | — | — | 0.336 | 0.291 | 0.502 | 0.301 | 0.225 | 0.086 | 0.205 |
| 13 | 0.734 | — | — | 0.924 | 0.991 | 1.443 | 0.945 | 0.842 | 0.296 | 0.802 |
| 14 | 0.247 | 0.051 | 0.571 | 0.373 | 0.339 | 0.450 | 0.333 | — | 0.088 | 0.431 |
| 15 | 0.241 | — | — | 0.351 | 0.326 | 0.403 | 0.211 | 0.236 | 0.074 | 0.173 |
| Mean | 0.262 | 0.786 | 0.301 | 0.464 | 0.391 | 0.586 | 0.358 | 0.241 | 0.149 | 0.295 |
| s.d. | 0.180 | 0.643 | 0.195 | 0.264 | 0.269 | 0.386 | 0.249 | 0.205 | 0.093 | 0.214 |
| CV | 69 | 82 | 65 | 57 | 69 | 66 | 69 | 85 | 63 | 72 |

\* vitreous humor
— Specimen type not available for analysis

**Table 6.** Postmortem tissue distribution coefficients for citalopram.

| | Urine/ Blood | VH*/ Blood | Liver/ Blood | Lung/ Blood | Kidney/ Blood | Spleen/ Blood | Muscle/ Blood | Brain/ Blood | Heart/ Blood |
|------|------|------|------|------|------|------|------|------|------|
| n | 7 | 5 | 15 | 15 | 15 | 15 | 14 | 15 | 15 |
| Mean | 12 | 0.42 | 16 | 15 | 3.6 | 8.1 | 0.83 | 2.3 | 1.9 |
| s.d. | 19 | 0.21 | 7.7 | 15 | 2.5 | 3.7 | 0.40 | 1.2 | 1.0 |
| CV | 158 | 50 | 48 | 100 | 69 | 46 | 48 | 52 | 53 |

\* vitreous humor

**Table 7.** Postmortem tissue distribution coefficients for desmethylcitalopram.

| | Urine/ Blood | VH*/ Blood | Liver/ Blood | Lung/ Blood | Kidney/ Blood | Spleen/ Blood | Muscle/ Blood | Brain/ Blood | Heart/ Blood |
|------|------|------|------|------|------|------|------|------|------|
| n | 7 | 5 | 15 | 15 | 15 | 15 | 14 | 15 | 15 |
| Mean | 16 | 0.67 | 44 | 42 | 11 | 17 | 1.0 | 3.0 | 4.7 |
| s.d. | 16 | 0.49 | 50 | 63 | 10 | 21 | 0.95 | 5.3 | 4.2 |
| CV | 100 | 73 | 114 | 150 | 91 | 124 | 95 | 177 | 89 |

\* vitreous humor

Drug concentrations determined from a blood specimen can aid in determining impairment and/or cause of death. However, due to the destructive nature of aviation accidents, our laboratory receives blood in only approximately 70% of the cases examined. If a distribution coefficient has a relatively small CV, it may be possible, with caution, to use a tissue or fluid distribution coefficient to roughly estimate a blood concentration in cases where blood is not available for analysis. However, the results obtained from our limited number of cases show that citalopram blood concentrations cannot be estimated, even crudely, from other tissue/fluid concentrations.

## SUMMARY AND CONCLUSION

The use of citalopram is widespread. Thus, the possible occurrence of undesirable side effects is of concern in the aviation community. With this in mind, a method for the identification and quantitation of citalopram and desmethylcitalopram has been developed that is rapid, reliable, and sensitive. By utilizing SPE, a clean extract was achieved with minimal solvent use. Additionally, the extraction provided excellent analyte recovery. The method described in this paper exemplifies the effectiveness of applying LC/MS-APCI technique to forensic samples. This methodology was demonstrated to be highly effective for the identification and quantitation of citalopram and desmethylcitalopram in various postmortem fluid and tissue specimens. A total of 131 tissue and fluid samples from 15 aviation fatalities were measured to determine citalopram and desmethylcitalopram concentrations. The results obtained from these cases suggest that citalopram is readily absorbed by all tissues and fluids in the body. The CV values obtained for the calculated distribution coefficients were extraordinarily large, suggesting these compounds likely undergo significant postmortem concentration changes.

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