Amanda J. Jenkins,[1] *Ph.D. and Krista Gubanich,*[1] *M.S.*

Disposition of Citalopram in Biological Specimens from Postmortem Cases*

REFERENCE: Jenkins AJ, Gubanich K. Disposition of citalopram in biological specimens from postmortem cases. J Forensic Sci 2002;47(1):159–164.

ABSTRACT: Citalopram is a bicyclic phthalate compound approved in 1998 by the U.S. Food and Drug Administration for the treatment of depression. It is a highly selective serotonin reuptake inhibitor that appears to have little effect on noradrenaline or dopamine reuptake. Since this drug has only recently been released on the U.S. market, information regarding therapeutic, toxic, and lethal concentrations is sparse. This study reports the detection of citalopram in 22 postmortem cases. Citalopram was identified and quantitated by capillary column gas chromatography with nitrogen phosphorus detection after basic liquid-liquid extraction. Confirmation was achieved by full scan electron impact gas chromatography/mass spectrometry. In the 22 cases studied, heart blood citalopram concentrations ranged from 0.09 to 1.64 mg/L ($n = 22$, mean $\pm$ $SD = 0.51 \pm 0.43$, median = 0.34); femoral blood concentrations ranged from 0.09 to 0.76 mg/L ($n = 14$, mean $\pm$ $SD = 0.34 \pm 0.23$, median = 0.28); and urine concentrations ranged from 0.05 to 276.00 mg/L ($n = 13$). Liver was analyzed in three cases with citalopram concentrations ranging from 2.22 to 8.08 mg/kg. The average heart blood/femoral blood ratio was 1.26 (range 0.75 to 1.98, $n = 14$). In each case, the cause of death was not considered to be related to citalopram toxicity. These data may therefore provide a basis for establishing post mortem citalopram concentrations following therapeutic doses.

KEYWORDS: forensic science, citalopram, antidepressant, postmortem drug disposition

Citalopram ($C_{20}H_{21}FN_2O$, Celexa®) (Fig. 1) was approved in 1998 by the U.S. Food and Drug Administration for use in the treatment of depression. It may also be useful in the treatment of obsessive compulsive disorder. It is a selective serotonin reuptake inhibitor (SSRI) which appears to exhibit little effect on noradrenaline or dopamine reuptake (1). It is available under the tradename Celexa® as the hydrobromide salt as a racemate in doses of 20 and 40 mg (base equivalent) tablets for oral administration (1). The oral bioavailability of citalopram is 80 to 95% (2). Oral doses of 30 to 60 mg per day result in serum concentrations of 0.009 to 0.200 mg/L. The time to peak levels ranges from 2 to 4 h after oral dosing with a plasma half life of 30 h (3). Citalopram is metabolized by hepatic cytochrome P450 isozymes (CYP2C19, 3A4, 2D6) to form N-demethylated metabolites, desmethylcitalopram, and didesmethylcitalopram. These metabolites have pharmacological activity, with the parent S-isomer possessing the most antidepressant activity (4). Citalopram also undergoes deamination to a propionic acid derivative, and N-oxidation to citalopram-N-oxide. Oral doses of 30 to 60 mg per day result in serum concentrations of 0.009 to 0.105 mg/L of the desmethyl metabolite. A pharmaockinetic drug interaction has been reported between citalopram and oxcarbazepine. In two patients, carbamazepine was substituted with oxcarbazepine which resulted in a 3 to 8 fold increase in plasma citalopram concentrations. In one of the patients an increase in anxiety and tremors was observed (5). Adverse effects of citalopram administration include nausea, sweating, dry mouth, migraine, diarrhea, and insomnia. More serious adverse effects include tachycardia and hypotension (1). Citalopram is considered to have a low potential for convulsions and extrapyramidal effects.

Several assays for citalopram and its metabolites have been published. These include an isocratic reversed phase HPLC method with diode array detection and C18 solid phase extraction columns (6), fluorescence detection with direct plasma injection (7), and gas chromatography and gas chromatography/mass spectrometry (8). Citalopram is also detectable by thin layer chromatography with a limit of detection of 2 ug/mL in urine using the Ansys Diagnostics, Inc. Toxi-Lab A system (9). Parent drug has an Rf of 0.39 and exhibits a yellow color at Stage I to negative to blue to brown through Stages II to IV. Since this drug has been recently released into the U.S. market, information on serum and blood concentrations in clinical and postmortem specimens is sparse. This study therefore reports the qualitative and quantitative determination of citalopram in postmortem specimens and also the detection of the desmethyl metabolite. Unfortunately, at the time this study was conducted a reference standard of this metabolite was unavailable and therefore, unequivocal identification and quantitation was not possible.

Materials and Methods

Materials

Citalopram was obtained courtesy of Forest Pharmaceuticals, Inc. (St. Louis, Missouri). Metabolites were unavailable. The internal standard, promazine, was purchased from Sigma Chemical Co. (St Louis, Missouri). ACS grade sodium chloride, ammonium chloride, ammonium hydroxide, sodium hydroxide, hydrochloric acid, and sulfuric acid were obtained from Mallinckrodt Chemical

[1] The Office of the Cuyahoga County Coroner, Cleveland, OH.

* Presented in part at the annual meeting of the American Academy of Forensic Sciences, February 2000, Reno, NV.

Received 24 Feb. 2001; and in revised form 14 May 2001; accepted 22 May 2001.

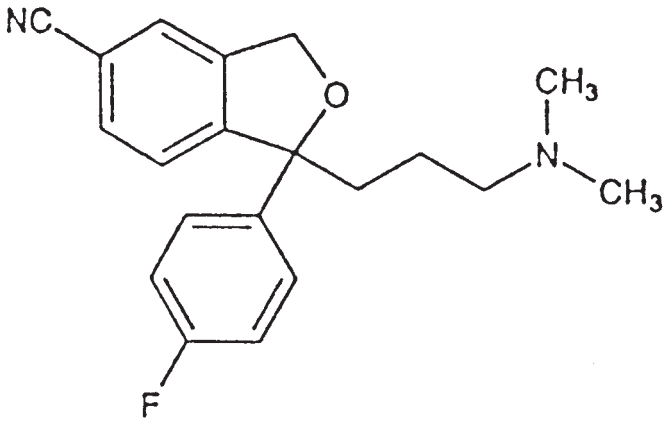

FIG. 1—*Chemical structure of citalopram.*

Company (Paris, Kentucky). HPLC grade hexane, ethyl acetate, and methanol were supplied by American Burdik and Jackson Laboratories Inc. (Muskegon, Michigan). Trizma base was purchased from the Sigma Chemical Co. (St Louis, Missouri).

Buffers and dilute acid and basic solutions were prepared with deionized water: Tris buffer, 1.2 M, pH 9.2: 145.3 g of Trizma base was dissolved in 1 L of water and the pH adjusted to 9.2 with concentrated hydrochloric acid.

Hydrochloric acid/methanol 1%: 100 µL of concentrated hydrochloric acid was q.s. to 100 mL with methanol.

Ammonium chloride buffer, pH 9.2: ammonium chloride was added to 1 L of water to saturate. The pH was adjusted to pH 9.2 with ammonium hydroxide.

A stock standard reference solution of citalopram (100 mg/L) was prepared in methanol. Dilutions of the stock solution were prepared as calibrators in drug free blood at the following concentrations: 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00, and 2.00 mg/L. Another stock reference solution was prepared in methanol (100 mg/L) and utilized for the preparation of positive controls at 0.04 and 0.25 mg/L. Citalopram concentrations in the controls and specimens were calculated from linear regression of the calibrator responses based on the peak area ratio (peak area citalopram to that of the internal standard).

Instrumentation

A Hewlett-Packard (HP) (Palo Alto, California) 6890 gas chromatograph (GC) with HP GC ChemStation with Windows REV. A.04.02, system software was used for initial screening and quantitation. Separation was achieved utilizing an RTx-50, 50% phenyl-50% methyl polysiloxane capillary column (30 m by 0.32 mm i.d., 0.25 µm film thickness) (Restek Corporation, Bellefonte, Pennsylvania). The initial oven temperature was 130°C held for 3 min, followed by increasing the temperature at a rate of 10°C/min to 270°C, held for 10 min, then 10°C/min to 295°C and held for 5 min. The injection port and nitrogen phosphorus detector temperatures were 250 and 300°C, respectively. Helium was utilized as a carrier gas at a flow rate of 3 mL/min. The presence of citalopram was confirmed utilizing an HP 5973 mass selective detector (MSD) interfaced with an HP 6890Plus GC and an HP ALS 7673. The MSD was operated in the electron impact ionization mode with an electron energy of 70 eV and a full scan mass-to-charge (m/z) ratio range of 35 to 450 amu. A DB-5 (J & W Scientific, Folsom, California) column (30 m by 0.255 mm i.d., and 0.25 µm film thickness) was utilized. The oven temperature was programmed from 50°C (held for 2 min) to 200°C at 50°C/min, and then from 200°C to 290°C at 12°C/min, with helium as the carrier gas with a flow of 0.7 mL/min. The injection port and transfer lines were maintained at 200 and 280°C, respectively. The ion source temperature was set at 280°C.

Procedure

All reference solutions and case specimens were refrigerated at 5°C until analysis. To 5 mL aliquots of calibrator (in duplicate), positive and negative (drug free blood) controls or case specimens were added 2 mL saturated sodium chloride and 100 uL of 6 N sodium hydroxide. Then 1 mL of internal standard (5 mg/L solution of promazine) was added followed by 2 mL of an ammonium chloride/ammonium hydroxide buffer at pH 9.2. After the addition of 10 mL ethyl acetate, the samples were rotated for 20 min and centrifuged for 5 min at 3000 rpm. The upper organic layer was removed and 2 mL 0.5 N sulfuric acid added. After rotation for 20 min and centrifugation for 5 min at 3000 rpm, the lower aqueous layer was removed and 4 mL TRIS buffer at pH 9.2 was added. The samples were vortexed for 10 s and 5 mL hexane/isopropanol (9:1) added. After further rotation and centrifugation, the upper organic layer was removed and transferred to a clean tube and one drop of 0.1% hydrochloric acid in methanol added. All tubes were then evaporated to dryness at 40°C under nitrogen using a TurboVap™ LV Evaporator (Zymark Corp., Hopkinton, Massachusetts). Extracts were reconstituted with 100 µL methanol, briefly vortexed, transferred to GC autosampler vials and 1 µL injected into the instrument.

Results and Discussion

Citalopram chromatographed well on the RTx-50 column with a relative retention time of 1.02 min, eluting after the internal standard, near sertraline (Fig. 2). A typical calibration curve produced a correlation coefficient > 0.997 (e.g., regression equation: $y = 0.001x + 0.0057$, $r^2 = 0.9996$). A peak eluting approximately 0.6 min after the parent drug was tentatively identified as the desmethyl metabolite (Fig. 2*). The assay was linear between the concentration range 0.01 to 2.00 mg/L with a limit of detection of 0.01 mg/L and a limit of quantitation of 0.02 mg/L. Accuracy at 250 ng/mL was $> 92\%$ and between day precision determined as %CV was 4.55% ($n = 4$). By GC/MS (Fig. 3), citalopram has a base peak of m/z 58, with a molecular ion at m/z 324 and other prominent ions at m/z 208 and 238. The postulated desmethyl metabolite has a base peak at m/z 44, with prominent ions at 310 and 238 (Fig. 4).

Citalopram was identified in 22 cases submitted to the Cuyahoga County Coroner's Office in Cleveland. Table 1 illustrates the demographic characteristics of each case. Fifteen of the 22 decedents were male and twelve were Caucasian. The decedents ranged in age from 22 to 86 years with a mean age of 52.7 and a median age of 52 years. Table 1 also delineates the circumstances surrounding each death. As may be readily ob-

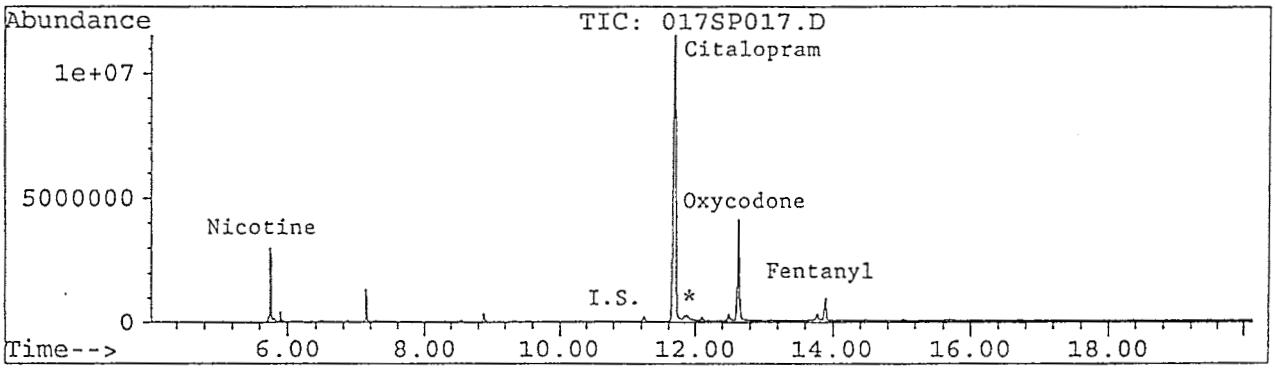

FIG. 2—*Total ion chromatogram of urine basic extraction, Case 6. Retention time of citalopram = 11.699 min. * = postulated desmethyl citalopram metabolite; I.S. = internal standard, retention time = 11.55 min.*

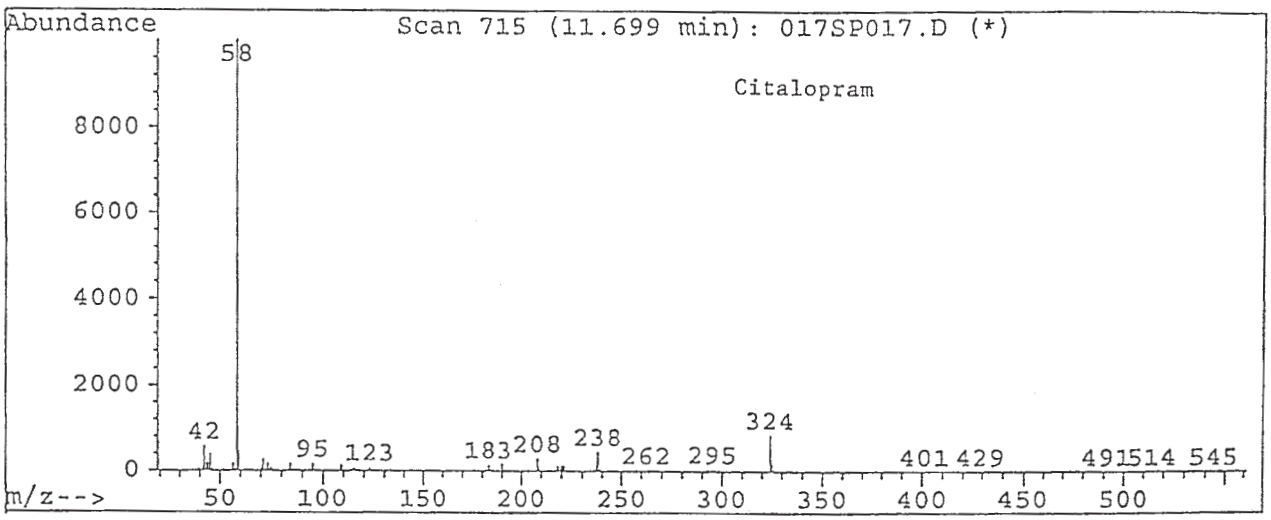

FIG. 3—*Mass spectrum of citalopram from urine, Case 6.*

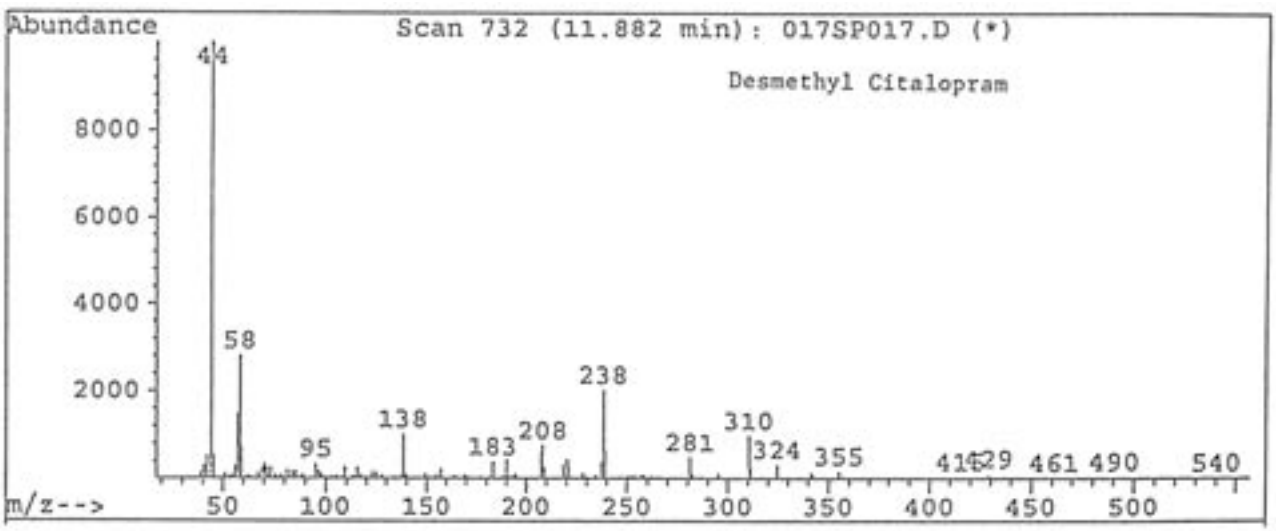

FIG. 4—*Mass spectrum of desmethyl metabolite from urine, Case 6.*

TABLE 1— *Demographic characteristics of postmortem cases.*

No.	Gender	Age	Race	Circumstances
1	Male	42	Caucasian	Hanged himself at place of business. Found by a friend.
2	Male	51	Black	Conveyed from nursing home to hospital.
3	Male	43	Caucasian	Lost control of motorcycle traveling at high speed and hit a guardrail.
4	Female	68	Caucasian	Conveyed from health and rehab center to hospital and expired in E.R.
5	Male	47	Caucasian	Found by father at home on couch.
6	Male	48	Caucasian	Found unresponsive on a couch at home by wife.
7	Female	30	Caucasian	Found unresponsive in full cardiopulmonary arrest at mother's home.
8	Male	39	Black	Found unresponsive on floor at home.
9	Female	70	Black	Found expired in bed at nursing home.
10	Male	64	Caucasian	Found expired in bed at nursing home.
11	Male	86	Caucasian	Failed to respond after surgery.
12	Female	29	Black	Collapsed at home in full cardiac arrest.
13	Male	38	Caucasian	Found in home in bed by mother.
14	Male	59	Caucasian	Wife found husband unresponsive on garage floor with car engine running.
15	Male	45	Black	Became ill and collapsed on street.
16	Male	53	Black	Conveyed from nursing home to hospital in full arrest.
17	Female	55	Black	Conveyed from nursing home to hospital in full arrest.
18	Female	63	Caucasian	Found at home in bed by police.
19	Female	68	Black	Conveyed from home to hospital in full arrest.
20	Male	22	Caucasian	Found at home hanging from ceiling in garage.
21	Male	73	Black	Found by wife expired in bed at home.
22	Male	67	Black	Conveyed from nursing home to hospital in full arrest.

served, the majority of decedents were found unresponsive at home or at a nursing home. Table 2 lists the citalopram and other drug concentrations measured in each of the cases. Citalopram was detected in heart blood in each case in a concentration range from 0.09 to 1.64 mg/L ($N = 22$, mean $\pm$ SD $= 0.51 \pm 0.43$, median $= 0.34$). Citalopram was detected in the femoral blood in each case for which it was submitted (range 0.09 to 0.76 mg/L, mean $\pm$ SD $= 0.34 \pm 0.23$, median $= 0.28$, $N = 14$). Generally, the femoral blood citalopram concentration was lower than the corresponding heart blood level with an average heart blood/femoral ratio of 1.26 (range 0.75 to 1.98, $n = 14$). Citalopram was also detected in every urine and bile sample submitted in these cases. The concentrations were generally several times greater than the corresponding blood concentrations. Therefore, these fluids would be suitable screening specimens. Citalopram concentrations in urine ranged from 0.05 to 276 mg/L. Liver was obtained in three cases and citalopram was detected in Cases 15, 19, and 20 in a concentration range of 2.22 to 8.08 mg/kg.

Citalopram was detected in all vitreous humor samples assayed. The concentrations were generally lower than those detected in the blood samples with one exception, Case 21. Cerebrospinal fluid was assayed in two cases. Citalopram was detected at a concentration of 0.22 (Case 15) and 0.05 (Case 21) mg/L. Citalopram was detected in all gastric content samples submitted, in a concentration range of 0.07 (Case 4) to 1.75 (Case 10) mg/L ($N = 15$). Table 2 also lists other drugs measured in each case and also the cause and manner of death as determined by the Coroner. Citalopram was not considered to have played a role in any of the deaths in the cases presented. Therefore, these data may be used as a reference source of the levels of this new drug, which may be observed as an incidental finding.

Levine et al. (10) and Mozayani et al. (11) also reported the distribution of citalopram in postmortem specimens. In the Levine study, citalopram concentrations determined from 13 cases were summarized. These investigators also reported higher levels in urine than blood and detected a higher concentration of drug in liver than blood (liver/blood 3.1 to 13, $n = 6$). In the second study, data from two cases were reported. Blood citalopram levels of 0.29 and 0.82 mg/L were considered to represent therapeutic levels in the two cases. In both these cases the bile citalopram concentration was several times higher than the blood concentration, consistent with the findings of the current study. The concentrations of citalopram measured in the liver (7.0 and 4.76 mg/kg) were comparable to the levels measured in the three cases in this study. Also the Mozayani study (11) reported levels of citalopram in the vitreous humor, which were similar to levels detected in blood in one case and 25% of the levels detected in blood in Case 2. Dragsholt et al. (12) reported citalopram data from 86 cases in Denmark from 1989 to 1997. After assessing the data and circumstances surrounding each case the authors suggested therapeutic, toxic, and lethal concentration ranges of citalopram. They suggested lethal concentrations were in the range of 2 to 6 mg/kg. In the current study, concentrations of approximately 2 to 8 mg/kg were measured in the liver of three cases in which citalopram was an incidental finding. Therefore, these data appear to disagree with the Danish study assuming the concentrations reported reflect tissue levels in the work of Dragsholt et al. (12).

In conclusion, this report summarizes data from 22 postmortem cases in which a new antidepressant, citalopram, was measured in multiple biological specimens. Since the presence of the drug was determined to be an incidental finding in these deaths, these data may serve as the basis of a database of biological fluid concentrations in postmortem specimens following therapeutic doses.

TABLE 2— *Citalopram and other drug concentrations in biological specimens.*

No.	Citalopram (mg/L)					Other Drugs	Cause of Death	Manner
	BLOOD					Heart Blood		
	HT	FEM	URINE	BILE	VH	(mg/L)		
1	0.17	0.09	1.73	0.79	0.02	Fluoxetine/ Norfluoxetine 1.15	Hanging	Suicide
2	1.14	NS	NS	NS	QNS	Diazepam 0.15	Pulmonary Thromboembolism incident to peritoneal-shunt pump	Therapeutic Complication
3	1.64	NS	NS	4.90	0.76	Ethanol 0.10g%	Blunt impacts to head, trunk and extremities with skeletal, visceral and soft tissue injuries	Accidental
4	0.21	NS	0.07	0.33	0.04		Chronic obstructive pulmonary disease	Natural
5	0.41	0.30	276	2.77	UNS	Carbamazepine 7.70 Morphine 0.17	Acute intoxication by heroin	Accidental
6	0.27	0.26	112	2.50	0.32		Multifocal severe stenosing coronary atherosclerosis	Natural
7	0.84	0.76	NS	4.15	0.79		Undetermined cause of death following complete anatomic and toxicological examinations	Natural
8	0.17	NS	NS	NS	NS		Atherosclerotic heart disease	Natural
9	0.21	NS	NS	NS	NS		Generalized atherosclerosis with atherosclerotic heart disease	Natural
10	1.05	0.53	NS	1.41	0.43	Olanzapine 0.17	Hypertrophic Cardiomyopathy, probably hypertensive and athero-sclerotic cardiovascular disease	Natural
11	0.51	0.43	0.57	1.79	0.50	Lidocaine 7.22	Cardiorespiratory arrest & anoxic encephalopathy incident to acute gastric ulcer with gastrointestinal hemorrhage	Natural
12	0.1	0.12	NS	0.70	0.45	Dextromethorphan 0.47 Buproprion metabolites: morphol 0.16; threo 2.25 erythro 0.65 Bupropion 0.07	Chronic bronchial asthma with acute asthmatic bronchitis	Natural
13	0.09	0.12	1.14	0.83	QNS	Ethanol 0.15 g% Desmethyldiazepam 0.26 Morphine 0.06	Severe atherosclerotic coronary artery disease. Recent intravenous heroin abuse	Accidental
14	0.24	0.20	0.05	NS	0.20	Ethanol 0.18 g% Carbon Monoxide 68%	Asphyxia by carbon monoxide (inhalation of auto exhaust)	Suicide
15	0.85	NS	12.25	16.10	0.39	Diphenhydramine 0.45 Verapamil 0.69	Acute pancreatitis with peritonitis and serosanguineous effusions. Due to Chronic ethanol abuse	Natural
16	0.09	0.11	0.21	0.74	NS	Amitriptyline 0.06 Lidocaine 0.38 Nortriptyline 0.21	Intracerebral hemorrhage. Due to Hypertensive atherosclerotic cardiovascular disease	Natural
17	1.13	0.71	15.83	NS	NS	Verapamil 1.77	Hypertensive atherosclerotic heart disease with cardiomegaly and congestive heart failure	Natural
18	0.23	0.20	NS	1.79	NS		Intraoral gunshot wound with perforations of vertebral column and spinal cord	Suicide
19	0.54	0.62	12.66	NS	0.53		Hypertensive, hypertrophic and congestive cardiomyopathy	Natural
20	0.71	0.38	2.93	NS	0.13	Olanzapine 0.10	Hanging	Suicide
21	0.11	NS	3.10	3.02	0.20	Phenytoin 26.9	Blunt impacts to head with brain injuries	Accident
22	0.56	NS	NS	NS	NS		Atherosclerotic heart disease	Natural

HT= Heart FEM= Femoral

QNS=Quantity not sufficient NS=Specimen not submitted UNS=Unsuitable

References

1. Physicians' desk reference, 54th ed. Montvale, NJ: Medical Economics Company, Inc., 2000.
2. Baumann P. Pharmacology and pharmacokinetics of citalopram and other SSRIs. Int Clin Psychopharmacol 1996;Suppl 1(11):5–11.
3. Celexa for the treatment of depression. In: Medical Sciences Bulletin 251 (August 1998). http://pharminfo.com/pubs/msb/celexa251.html.
4. Rasmussen BB, Brosen K. Is therapeutic drug monitoring a case for optimizing clinical outcome and avoiding interactions of selective serotonin reuptake inhibitors? Ther Drug Monit 2000;22:143–54.
5. Leinonen E, Lepola U, Koponen H. Substituting carbamazepine with oxcarbazepine increases citalopram levels. A report on two cases. Pharmacopsychiatry 1996;29:156–8.
6. Akerman KK, Jolkkonen J, Huttunen H, Penttila I. High performance liquid chromatography method for analyzing citalopram and desmethylcitalopram from human serum. Ther Drug Monit 1998;20:25–9.
7. Matsui E, Hoshino M, Matsui A, Okihara A. Simultaneous determination of citalopram and its metabolites by high performance liquid chromatography with column switching and fluorescence detection by direct plasma injection. J Chromatogr 1995;668:299–307.
8. Reymond P, Amey M, Souche A, Lambert S, Konrat H et al. Determination of plasma levels of citalopram and its demethylated and deaminated metabolites by gas chromatography and gas chromatography-mass spectrometry. J Chromatogr 1993;616:221–8.
9. New drug. In: Toxi News Anays Diagnostics, Inc. Lake Forest, CA. 2000;19(2):1–2.
10. Levine B, Zhang X, Smialek JE, Kunsman GW, Frontz ME. Citalopram distribution in postmortem cases. Society of Forensic Toxicologists Annual Meeting; 2000 Oct. 2–6, Milwaukee, WI, (Abstract p. 42).
11. Mozayani A, Nix R, Jachimczyk JA. Distribution of citalopram in postmortem cases. Society of Forensic Toxicologists Annual Meeting; 2000 Oct. 2–6, Milwaukee, WI. (Abstract p. 43).
12. Dragsholt C, Worm K, Simonsen KW, Kringsholm B. Therapeutic, toxic and lethal concentrations of citalopram. Proceedings of the Joint Congress of the Society of Forensic Toxicologists and The International Association of Forensic Toxicologists; 1998 Oct. 8; Albuquerque, NM, (Abstract 101).

Additional information and reprint requests:
Dr. Amanda J. Jenkins
The Office of the Cuyahoga County Coroner
11001 Cedar Avenue
Cleveland, OH 44106