N. B. Wu Chen,[1] *Ph.D.; M. I. Schaffer,*[1] *Ph.D.; R.-L. Lin,*[1] *Ph.D.; J. P. Hadac,*[1] *M.A.; and R. J. Stein,*[1] *M.D.*

Analysis of Blood and Tissue for Amoxapine and Trimipramine

REFERENCE: Wu Chen, N. B., Schaffer, M. I., Lin, R.-L., Hadac, J. P., and Stein, R. J., **"Analysis of Blood and Tissue for Amoxapine and Trimipramine,"** *Journal of Forensic Sciences,* JFSCA, Vol. 28, No. 1, Jan. 1983, pp. 116-121.

ABSTRACT: A method for the identification and quantitation of two tricyclic antidepressants, amoxapine (Asendin®) and trimipramine (Surmontil®) is presented here. Samples were extracted with hexane at pH 10, back-extracted with 1.0*N* sulfuric acid. The acidic layer was adjusted to pH 10 and re-extracted with hexane. Electron impact mass spectra were obtained. The base peak and molecular ion for amoxapine were at *m/z* 245 and 313, respectively. The base peak and molecular ion for trimipramine were at *m/z* 58 and 294, respectively. There were three forensic toxicology cases involving amoxapine in Cook County, IL, in 1980 and 1981. The concentrations of amoxapine in blood for these three cases were 1.66 mg/L, 7.16 mg/L, and 2.95 mg/L, respectively.

KEYWORDS: toxicology, amoxapine, trimipramine, Asendin®, Surmontil®, tricyclic antidepressants, chromatographic analysis, gas chromatographic/mass spectrometric analysis

Amoxapine (Asendin®) is a tricyclic antidepressant of the dibenzoxazepine class. It is an antidepressant apparently similar in effectiveness to other tricyclic antidepressants, such as amitriptyline and imipramine [*1-3*]. Trimipramine (Surmontil®) is a tricyclic antidepressant of the dibenzazepine class. Its effectiveness is comparable to amitriptyline and imipramine [*4-6*].

In Cook County, IL, specimens are analyzed for organic bases by a gas chromatographic (GC) procedure [*7*]. The presence of amoxapine, trimipramine, and other tricyclic antidepressants in postmortem specimens were detected by this method. An OV-101 column was used. Positive results were presumptively confirmed on an OV-225 column by GC. Confirmation was then performed by gas chromatography/mass spectrometry (GC/MS) on either an OV-225 or an OV-101 column. Quantitation was performed with an internal standard method by GC on either an OV-101 or an OV-225 column.

Equipment

A computerized Hewlett-Packard 5840A gas chromatograph, equipped with both a flame ionization detector (FID) and a nitrogen-phosphorus detector were used for the GC analy-

Presented at the 34th Annual Meeting of the American Academy of Forensic Sciences, Orlando, FL, 8-11 Feb. 1982. Received for publication 13 March 1982; revised manuscript received 29 April 1982; accepted for publication 2 June 1982.

[1]Toxicologist and GC/MS section chief, chief toxicologist, assistant chief toxicologist, toxicologist, and chief medical examiner, respectively, Cook County Institute of Forensic Medicine, Office of the Medical Examiner, Chicago, IL.

ses. A Finnigan 3200 gas chromatograph/mass spectrometer with an Incos 2300 data system was used for the GC/MS analyses. An Eberbach horizontal shaker was used for the solvent extraction. A Brinkmann concentrator was used for the evaporation of solvent.

Methods

The pH 10 carbonate/bicarbonate buffer used in the following solvent extraction procedure was prepared by adjusting 1.0*M* sodium carbonate solution to pH 10 with 1.0*M* sodium bicarbonate solution.

Basic Drug Analysis [7]

In a 15-mL screw cap glass culture tube, 2.0 mL of blood, bile, urine, or tissue homogenate were spiked with 20 μL of diphenhydramine (100 mg/L) internal standard; made basic with 200 μL of concentrated ammonium hydroxide and 1 mL of pH 10 carbonate/bicarbonate buffer (1.0*M*); and extracted with 10 mL of hexane:ethyl acetate (1:1) on a horizontal shaker for 5 min. After centrifugation, the solvent was pipetted into a 15-mL screw cap glass culture tube. Two mL of 1.0*N* sulfuric acid were added and the mixture was shaken on a horizontal shaker for 5 min. After centrifugation, the solvent layer was aspirated and the acid layer was transferred to a 15-mL screw cap glass culture tube. The acid layer was made basic with 300 μL of concentrated ammonium hydroxide and 1 mL of pH 10 carbonate/bicarbonate buffer (1.0*M*), and was extracted with 10 mL of hexane:ethyl acetate (1:1) on a horizontal shaker for 5 min. After centrifugation, the solvent was transferred to a 30-mL Brinkmann concentrator cup and evaporated to dryness at 50°C on a Brinkmann concentrator. The residue was reconstituted with 20 μL of ethyl acetate and 1 μL was injected into a GC. The residue was further diluted with ethyl acetate before injection if necessary for a particular specimen. The GC column for screening was a 1.2-m (4-ft) by 2-mm inside diameter glass column packed with 3% OV-101 on Chromosorb WHP, 80-100 mesh (Hewlett-Packard, Avondale, PA). GC conditions were as follows: column temperature, 190°C isothermal for 2 min, 20°C/min to 240°C, isothermal for 5 min or longer; injector temperature, 275°C; FID temperature, 275°C; and nitrogen carrier gas flow rate, 30 mL/min. The GC column for presumptive confirmation was a 1.2-m (4-ft) by 2-mm inside diameter glass column packed with 5% OV-225 on Chromosorb WHP, 80-100 mesh (Hewlett-Packard, Avondale, PA). FID was used for the analysis.

GC/MS Confirmation

Another aliquot of specimen was extracted for GC/MS. The basic drug extraction procedure described above for GC was used. The residue was reconstituted with 20 μL of ethyl acetate and 1 μL was injected into the GC/MS. Further dilution was made if necessary. The GC column was a 1.2-m (4-ft) by 2-mm inside diameter glass column packed with 5% OV-225 on Chromosorb-WHP, 80-100 mesh (Hewlett-Packard, Avondale, PA). The GC/MS conditions for amoxapine were as follows: column temperature, 240°C, isothermal for 7 min; injector temperature, 275°C; helium carrier gas flow rate, 20 mL/min; glass jet separator temperature, 250°C; transfer line temperature, 250°C. The quadrupole mass spectrometer was operating at electron impact mode; electron energy, 70 eV; emission current, 0.5 mA; analyzer temperature, ambient temperature. The vacuum diverter situated between the GC column and the glass jet separator was not turned off until 20 s after injection and was turned on at the end of each GC/MS analysis. This minimized the accumulation of extraneous material from the column into the analyzer. All GC/MS data were stored and processed with an Incos 2300 Data System containing a computer library of more than 25 000 mass spectra. The GC/MS conditions for trimipramine were the same as described above except the column temperature was at 210°C, isothermal for 2 min.

Amoxapine Quantitation

The procedure was the same as that for basic drug analysis, except hexane was used as the extraction solvent, 50 μL of trifluoperazine (100 mg/L) was used as the internal standard, and the OV-101 column temperature was 240°C, isothermal for 5 min. Quantitation was based on the peak area ratio of amoxapine to the internal standard, trifluoperazine. This peak area ratio and amoxapine concentration were studied and found to be linear between 0.5 and 10 mg/L. All specimens were properly diluted to fall within this range. With each set of biological specimens analyzed daily, an amoxapine standard (2.5 mg/L) was processed accordingly, and its GC response was used for the automatic quantitation for that day. The average of two determinations was reported.

Trimipramine Quantitation

The procedure was the same as that for amoxapine quantitation, except 50 μL of dexbrompheniramine (100 mg/L) was used as the internal standard, and the column temperature was 210°C, isothermal for 3 min. Quantitation was based on the peak area ratio of trimipramine to the internal standard, dexbrompheniramine. This peak area ratio and trimipramine concentration were studied and found to be linear between 0.5 and 10 mg/L. Other quantitation considerations were similar to amoxapine quantitation.

Results and Discussion

In the gas chromatogram obtained in our routine basic drug GC screening as described above, the retention times for diphenhydramine, dexbrompheniramine, trimipramine, amoxapine, and trifluoperazine were 1.11 min, 2.42 min, 3.22 min, 5.15 min, and 5.47 min, respectively.

The base peak and molecular ion for trimipramine were at *m/z* 58 and 294, respectively, as shown in its electron impact mass spectrum (Fig. 1). The retention times for dexbrompheniramine and trimipramine were 1.21 and 1.72 min, respectively, as obtained in our trimipramine quantitation procedure. No trimipramine-related deaths were found in Cook County, IL, in 1980 and 1981, but three amoxapine-related deaths were found during those two years. As shown in the case histories (Table 1) of these three cases, either amoxapine (Asendin) bottles were found at the death scenes (Case A and Case C), or the decedent admitted the intake of amoxapine before his death (Case B). Amoxapine was found to be present in the postmortem specimens of all these three cases. The retention time and mass spectrum of a peak in the specimen were consistent with those of amoxapine standard. The base peak and molecular ion for amoxapine were at *m/z* 245 and 313, respectively, as shown in its electron impact mass spectrum (Fig. 2). The retention times for amoxapine and trifluoperazine were 2.01 and 2.33 min, respectively, as obtained in our amoxapine quantitation procedure.

Tissue distribution of amoxapine in these three cases is presented in Table 2. Amoxapine concentrations in blood for Cases A, B, and C were 1.66, 7.16, and 2.95 mg/L, respectively. Aside from gastric contents, amoxapine concentration was the highest in bile, with 75.2 mg/L for Case A and 823 mg/L for Case B. Amoxapine concentration in liver ranked the second highest, with 36.0 mg/kg for Case B and 23.2 mg/kg for Case C. Amoxapine concentration in the heart (left ventricle) was 14.9 mg/kg for Case B.

After a systematic search [*7*], drugs other than amoxapine were ruled out or found in the postmortem specimens of these three cases. These results as well as the cause and manner of death were tabulated in Table 1. The immediate cause of death of Case C was "amoxapine toxicity and asphyxia in a plastic bag." Case A was a multiple drug overdose death (amitriptyline, nortriptyline, and amoxapine). Case B was an acute amoxapine intoxication death with blood amoxapine concentration at 7.16 mg/L. The amoxapine concentration for Case B and the multiple tricyclic antidepressants concentrations for Case A were comparable with

TABLE 1—*Amoxapine-related deaths in Cook County, IL, in 1980 and 1981.*

Case	Concentration in Blood, mg/L	Cause of Death	Manner of Death	Other Drugs Found, Concentration in Tissue	Case History
A	1.66	combined drug overdose (amitriptyline, nortriptyline, amoxapine)	undetermined	amitriptyline (2.86 mg/L in blood); nortriptyline (1.02 mg/L in blood); ethanol (44 mg/dL in blood); tripelennamine (positive in urine)	female/white, age 20; found unresponsive with empty bottles (Asendin, amitriptyline, Valium®, and perphenazine).
B	7.16	acute amoxapine intoxication	suicide	ethanol (28 mg/dL in blood)	male/white, age 46; took 20 Asendin (100 mg) tablets and two beers. Died in emergency room. Previous suicide attempt.
C	2.95	amoxapine toxicity and asphyxia in a plastic bag	suicide	no other drugs found	female/white, age 61; found unresponsive with plastic bag over head and empty asendin bottle. Previous suicide attempt.

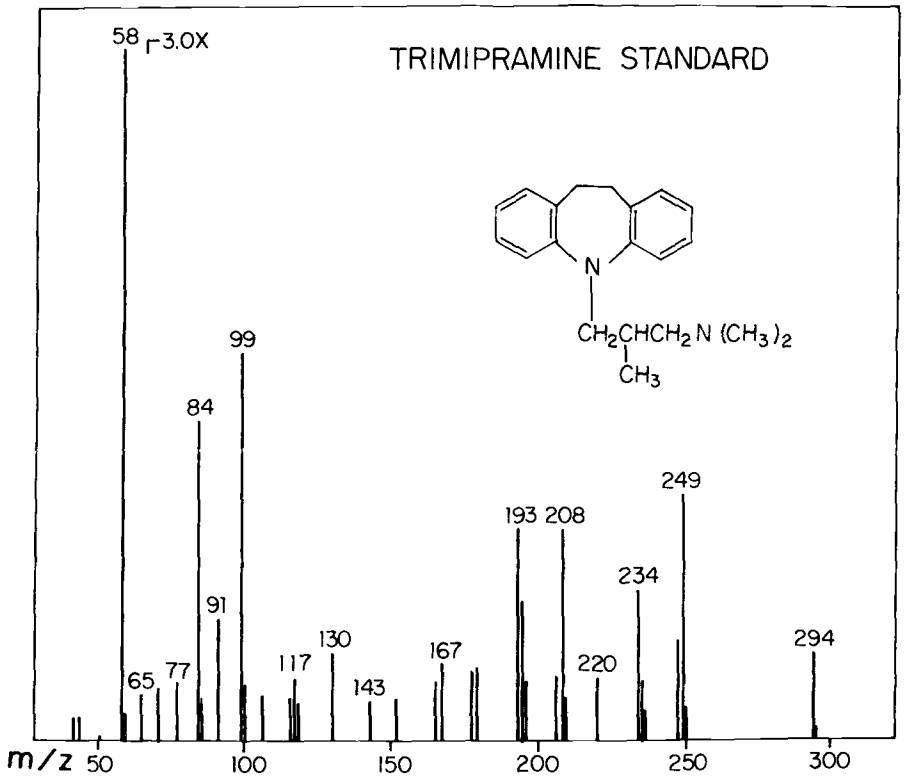

FIG. 1—*Electron impact mass spectrum of trimipramine standard.*

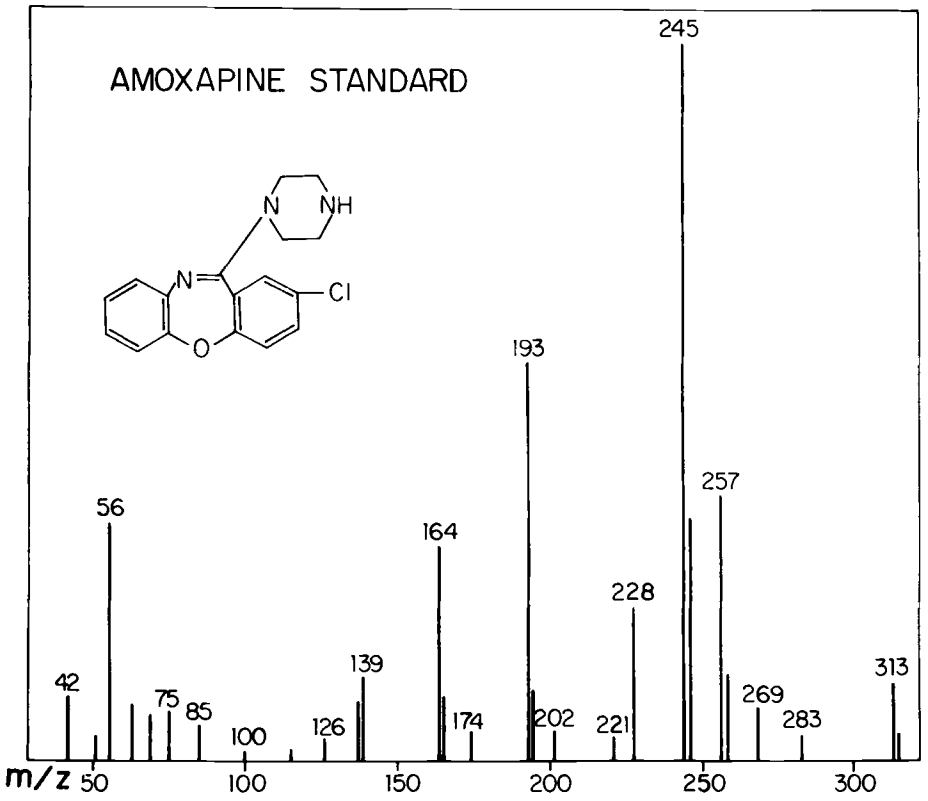

FIG. 2—*Electron impact mass spectrum of amoxapine standard.*

TABLE 2—*Tissue distribution of amoxapine in three cases.*

Case	Blood, mg/L	Bile, mg/L	Urine, mg/L	Liver, mg/kg	Heart, mg/kg	Gastric content, mg Total
Case A	1.66	75.2	22.0	...	...	56
Case B	7.16	823	...	36.0	14.9	9.8
Case C	2.95	...	...	23.2	...	...

those reported in the literature [*8*] for tricyclic antidepressant fatalities. Many clinical studies for amoxapine have been reported and reviewed [*1-3*], but there have been limited reports on amoxapine overdoses [*3,9,10*]. Seizure was reported in amoxapine overdose patients, in one case following the ingestion of 1000 mg of amoxapine [*3*], and in another case following the ingestion of 2100 mg of amoxapine and an undetermined amount of alcohol [*9*]. The above patients survived the amoxapine overdose. Grand mal seizure was reported in the hospital record for the decedent in Case B, after ingestion of 2000 mg of amoxapine and two beers. The decedent told the emergency room nurse about his drug ingestion, suffered a grand mal seizure 30 min later, and died a few hours later. Amoxapine concentrations were determined for blood, bile, liver, heart, and gastric content (Table 2). We feel that Case B was a well-documented case of amoxapine-induced death.

Acknowledgment

We are grateful for the constant support of all our colleagues, especially Joann M. Richmond, M.D., Yuksel Konakci, M.D., and Michelle Robinson.

References

[1] Abramowicz, M., Ed., "Amoxapine (Asendin)—A New Antidepressant," *The Medical Letter on Drugs and Therapeutics*, Vol. 23, No. 8, April 1981, pp. 39-40.

[2] Kaumeier, H. S. and Haase, H. J., "A Double-Blind Comparison between Amoxapine and Amitriptyline in Depressed In-Patients," *International Journal of Clinical Pharmacology, Therapy, and Toxicology*, Vol. 18, No. 4, April 1980, pp. 177-184.

[3] Smith, R. S., Jr., and Ayd, F. J., Jr., "A Critical Appraisal of Amoxapine," *The Journal of Clinical Psychiatry*, Vol. 42, No. 6, June 1981, pp. 238-242.

[4] Abramowicz, M., Ed., "Trimipramine Maleate (Surmontil)—Another Tricyclic Antidepressant," *The Medical Letter on Drugs and Therapeutics*, Vol. 22, No. 3, Feb. 1980, p. 16.

[5] Rifkin, A., Saraf, K., Kane, J., Ross, D., and Klein, D. F., "A Comparison of Trimipramine and Imipramine: A Controlled Study," *The Journal of Clinical Psychiatry*, Vol. 41, No. 4, April 1980, pp. 124-129.

[6] Settle, E. C., Jr., and Ayd, F. J., Jr., "Trimipramine: Twenty Years' Worldwide Clinical Experience," *The Journal of Clinical Psychiatry*, Vol. 41, No. 8, Aug. 1980, pp. 266-274.

[7] Wu Chen, N. B., Schaffer, M. I., Lin, R.-L., Kurland, M. L., Donoghue, E. R., Jr., and Stein, R. J., "The General Toxicology Unknown: I. The Systematic Approach," *Journal of Forensic Sciences*, Vol. 28, No. 2, April 1983.

[8] Baselt, R. C., *Disposition of Toxic Drugs and Chemicals in Man, Volume 1, Centrally-Acting Drugs*, Biomedical Publications, Canton, CT, 1978, pp. 170-188.

[9] Ross, S. R. and Rodgers, S. R., "Physostigmine in Amoxapine Overdose," *American Journal of Hospital Pharmacy*, Vol. 38, No. 8, Aug. 1981, pp. 1119-1122.

[10] Prouty, R. W., "Amoxapine Overdose—A Case Report," *News and Views, A Forum for Forensic Toxicologists*, No. 21, American Academy of Forensic Sciences and The Forensic Sciences Foundation, Colorado Springs, CO, Dec. 1981, p. 6.

Address requests for reprints or additional information to
Nancy B. Wu Chen, Ph.D.
Cook County Institute of Forensic Medicine
Office of the Medical Examiner
1828 W. Polk St.
Chicago, IL 60612