CASE REPORT

Kenneth E. Ferslew,[1] *Ph.D.; Andrea N. Hagardorn,*[1] *B.S.; and William F. McCormick,*[2] *M.D.*

Postmortem Determination of the Biological Distribution of Sufentanil and Midazolam after an Acute Intoxication

REFERENCE: Ferslew, K. E., Hagardorn, A. N., and McCormick, W. F., **"Postmortem Determination of the Biological Distribution of Sufentanil and Midazolam after an Acute Intoxication,"** *Journal of Forensic Sciences*, JFSCA, Vol. 34, No. 1, Jan. 1989, pp. 249-257.

ABSTRACT: A case is presented of a death caused by self-injection of sufentanil and midazolam. Biological fluids and tissues were analyzed for midazolam by high performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS) and for sufentanil by GC/MS. Midazolam was extracted from basified fluids or tissues homogenated with *n*-butyl chloride and analyzed by HPLC by using a phosphate buffer : acetonitrile (60 : 40) mobile phase on a μ-Bondapak C18 column at 240 nm. Sufentanil was extracted from basified fluids and tissue homogenates with hexane : ethanol (19 : 1). GC/MS methodology for both compounds consisted of chromatographic separation on a 15-m by 0.25-mm inside diameter (ID) DB-5 (1.0-μm-thick film) bonded phase fused silica capillary column with helium carrier (29 cm/s) splitless injection at 260°C; column 200°C (0.8 min) 10°C/min to 270°C; and electron ionization and multiple ion detection for midazolam (*m/z* 310), methaqualone (IS, *m/z* 235), sufentanil (*m/z* 289), and fentanyl (IS, *m/z* 245). Sufentanil concentrations were: blood 1.1 ng/mL, urine 1.3 ng/mL, vitreous humor 1.2 ng/mL, liver 1.75 ng/g, and kidney 5.5 ng/g. Midazolam concentrations were: blood 50 ng/mL, urine 300 ng/mL, liver 930 ng/g, and kidney 290 ng/g. Cause of death was attributed to an acute sufentanil/midazolam intoxication and manner of death a suicide.

KEYWORDS: toxicology, sufentanil, midazolam, overdose, drug abuse

Midazolam (Versed®) is a water-soluble, short-acting benzodiazepine central nervous system depressant. It is administered either intramuscularly or intravenously for preoperative sedation, conscious sedation for short diagnostic procedures, and for induction of general anesthesia either alone or before administration of other anesthetic agents [*1*]. Midazolam is also an amnestic agent when administered intravenously producing partial or complete loss

Presented at the 40th Annual Meeting of the American Academy of Forensic Sciences, Philadelphia, PA, 15-20 Feb. 1988. Received for publication 11 March 1988; revised manuscript received 13 April 1988; accepted for publication 18 April 1988.

[1]Toxicologist and research specialist, respectively, Section of Toxicology, Department of Pharmacology, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, TN.

[2]Deputy chief medical examiner of the State of Tennessee, Section of Forensic Pathology, Department of Pathology, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, TN.

of recall for up to several hours [2,3]. Induction of anesthesia occurs within 2 to 2.5 min of intravenous administration of midazolam [1]. Elimination half-life for midazolam ranges from 1.2 to 12 h with a volume of distribution of 0.95 to 6.6 L/kg [4-6]. Clinical effects of midazolam do not directly correlate with its blood concentrations in that effects are shorter than would be indicated by its elimination half-life. Intravenous administration of midazolam at any dose can produce pronounced cardiovascular and respiratory depression. Intravenous administration should be titrated to effect and not administered as a bolus. Midazolam has an abuse potential comparable to valium [7].

Sufentanil (Sufenta®) is a potent opioid analgesic reported to be as much as ten times more potent than fentanyl [8,9]. It is administered intravenously as an analgesic adjunct in the maintenance of balanced general anesthesia or as a primary anesthetic agent for induction and maintenance of anesthesia for major surgical procedures. Sufenta has an immediate onset of action with a distribution time of 1.4 min, a redistribution time of 17.1 min, and an elimination half-life of 164 min [10,11]. Sufenta, being a very potent opioid, can produce adverse reactions of severe respiratory depression and skeletal muscle rigidity. Drug dependence similar to morphine can occur, and therefore, Sufenta has the potential for abuse [12].

The combination of midazolam and sufentanil is a common practice for producing surgical anesthesia. When combined, dosages of each needed to produce anesthesia are decreased. Because intravenous midazolam produces respiratory depression as does the opioid agonist, the combination of these two drugs can produce an additive effect on respiration which may be potentially lethal if untreated [7,12].

Case History

A 30-year-old white male anesthesiologist died either during or after an apparent self-injection of unknown chemical substances into his left basilic vein. The body was found positioned on his knees, bent over, and face down. Three fresh needle puncture marks were present along the medial aspect of the left forearm below the antecubital fossa overlying the basilic vein. The victim was found with three syringes (one containing approximately 2 mL of a clear solution), two needles, two empty vials of midazolam (Versed, 2 mL containing 5 mg/mL), and one empty vial of sufentanil (Sufenta, 2 mL containing 50 μg/mL). Investigation confirmed that the two vials of midazolam and the vial of sufentanil were removed from the narcotic cabinet where the victim worked during his last shift.

Materials and Methods

Specimen Collection

Blood (20 mL) was collected by cardiac puncture of the ventricles and stored in Vacutainer® tubes containing potassium oxalate and sodium fluoride. Vitreous humor was collected from the posterior chambers of both eyes and stored in sterile Vacutainer® tubes. Urine was collected by bladder puncture and stored in a plastic specimen cup. Liver and kidney tissues (approximately 100 g of each) were collected and stored in plastic heat-sealed bags. Plasma was separated by centrifugation at 2000 rpm for 10 min. Biological fluids were stored at 2 to 4°C, while tissues were stored at −70°C until analysis.

Analytical Methods

Biological fluids and tissues were analyzed for midazolam by gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC) and for sufentanil by GC/MS. The instrumentation and specifics for the GC/MS analyses are given in

Table 1. The instrumentation and specifics for the HPLC analyses are given in Table 2. The flow diagrams outline the modified extraction procedures for the midazolam (Fig. 1) [*13*] and sufentanil (Fig. 2) [*14*].

Blood and urine specimens were analyzed for ethanol using gas-liquid chromatography. The biological fluids and the solution within the syringe were screened for the presence of

TABLE 1—*GC/MS analysis.*

Instrument: Finnigan 4000 GC/MS with a Finnigan/INCOS Data System
Injector: splitless, 260°C
Column: 15-m by 0.25-mm ID DB-5 (1.0-μm film) bonded phase fused silica capillary
Column conditions: 200°C (0.8 min) then 10°C/min to 270°C
Transfer line: 270°C
Carrier gas: He, 29 cm/s
Mass spectrometer: electron ionization, ion source 250°C, emission current 0.3 mA, electron multiplier −1525 V, electron energy 70 eV, multiple ion detection, dwell 0.2 s/mass/scan
Ions: midazolam: *m/z* 310, methaqualone: *m/z* 235, sufentanil: *m/z* 289, fentanyl: *m/z* 245

TABLE 2—*HPLC analysis.*

Instrument: Waters M45 Solvent Delivery System, U6K Injector, Z Module and a Lambda Max 480 variable (UV) spectrophotometer with a Hewlett-Packard 3390A recording integrator
Mobile phase: acetonitrile: 0.015*M* phosphate buffer[a] (60:90), pH 3.3 at 2.5 mL/min
Column: 8-mm by 10-cm μBondapak C18 (10μm) Radial PAK cartridge at room temperature
Detector settings: 220 nm, 0.02 AUFS
Recorder setting: 10 mV full scale, 0.5 cm/min

[a]150 mL of 0.1*M* potassium phosphate, monobasic (KH_2PO_4) q.s. 1L, pHed to 3.3 with 0.1*M* phosphoric acid (H_3PO_4).

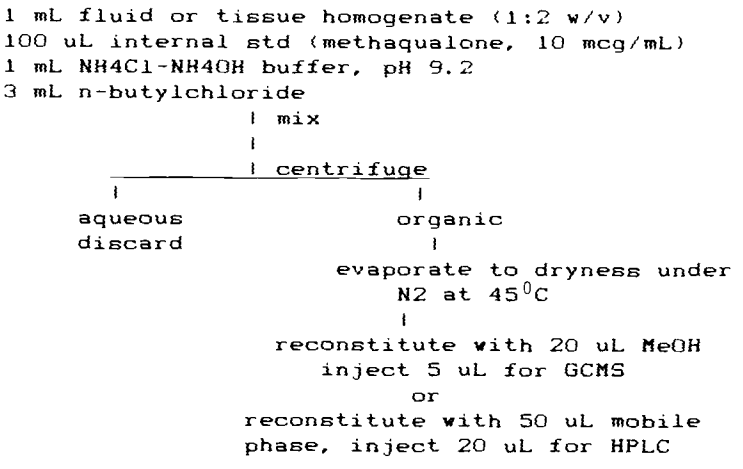

FIG. 1—*Midazolam extraction.*

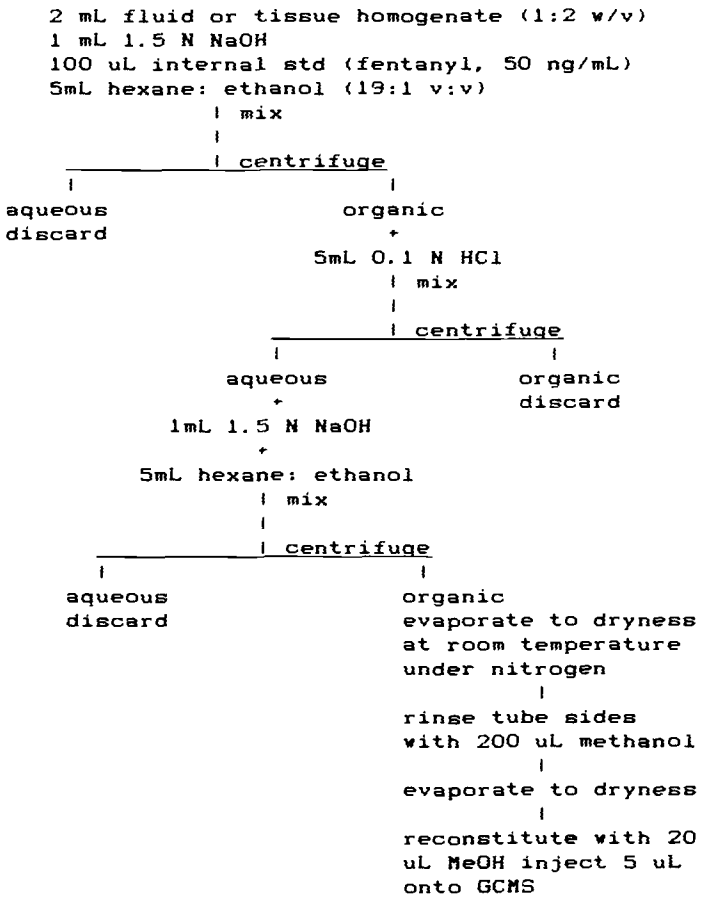

FIG. 2—*Sufentanil extraction.*

numerous acidic, basic, and neutral drugs and metabolites including narcotics and other analgesics, barbiturates and other sedative hypnotics, benzodiazepines, cannabinoids, cocaine, phencyclidine, phenothiazines, sympathomimetic amines, and tricyclic antidepressants by a combination of thin-layer chromatography, gas chromatography, GC/MS, enzyme immunoassays (that is, EMIT®) and specific colorimetric procedures.

Results

The mass spectrum and selected ion chromatograms for the midazolam analysis are illustrated in Figs. 3 and 4 while the HPLC chromatogram is illustrated in Fig. 5. The mass spectra and selected ion chromatograms for the sufentanil analysis are illustrated in Figs. 6 through 8. The concentrations of sufentanil and midazolam were detected from the analyses of the biological fluids and tissues. They are given in Table 3. GC/MS analysis of the clear solution found in the syringe with the victim revealed it to be a 1% solution of lidocaine. Analysis of the blood of the victim revealed no measurable concentration of lidocaine.

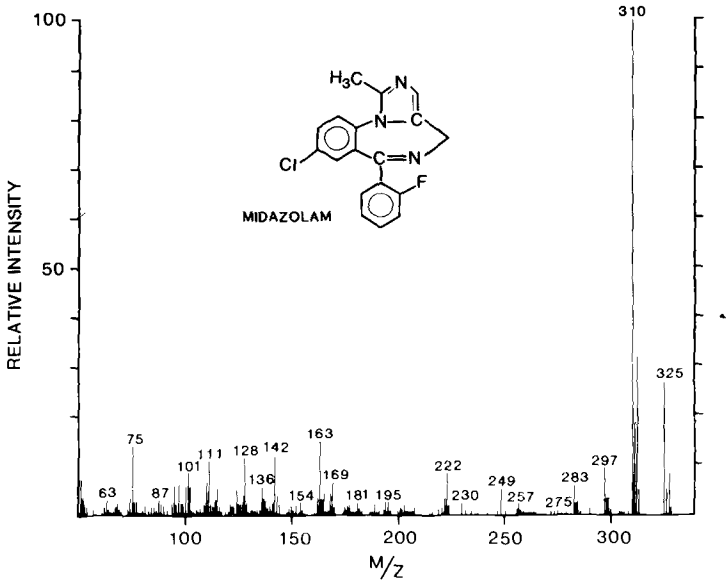

FIG. 3—*Mass spectrum of midazolam.*

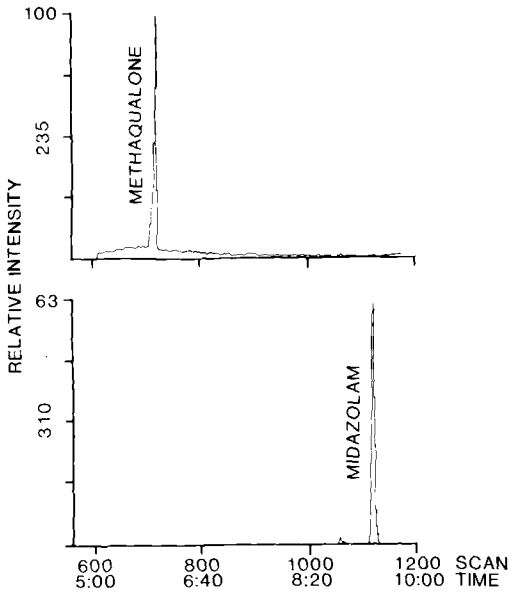

FIG. 4—*Selected ion chromatograms of midazolam extracted from liver.*

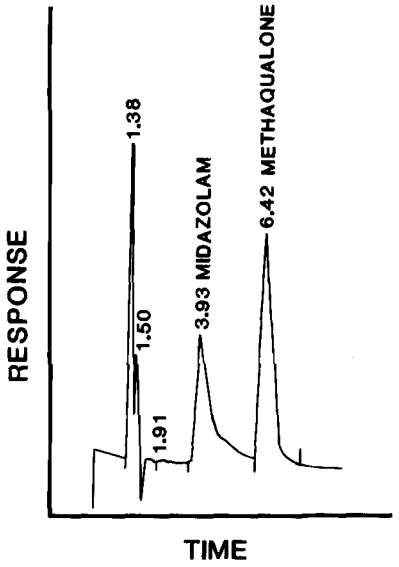

FIG. 5—*HPLC chromatogram of midazolam.*

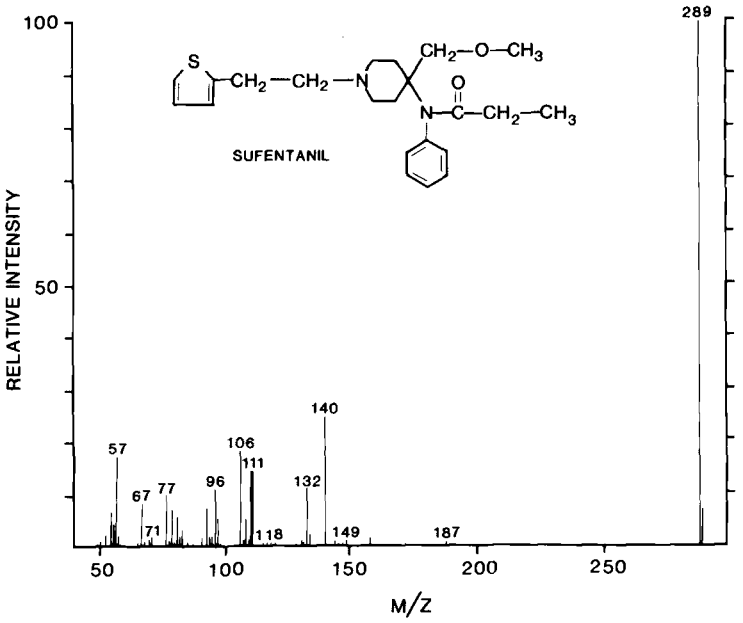

FIG. 6—*Mass spectrum of sufentanil.*

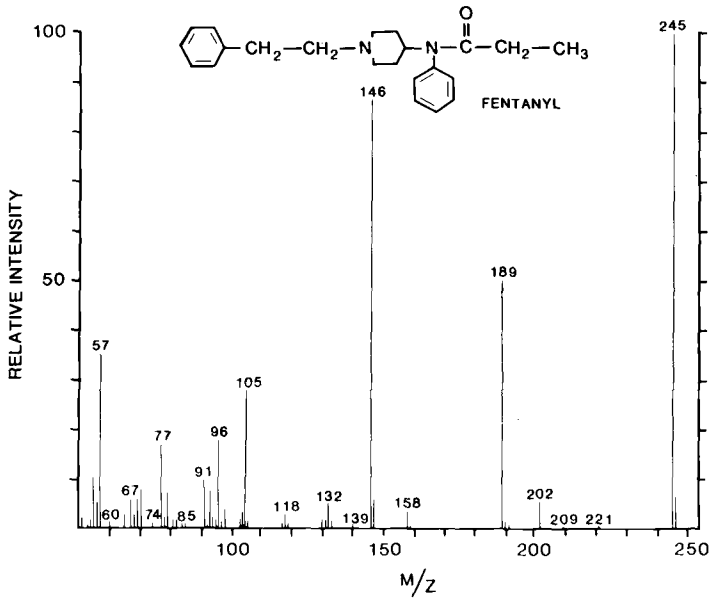

FIG. 7—*Mass spectrum of fentanyl.*

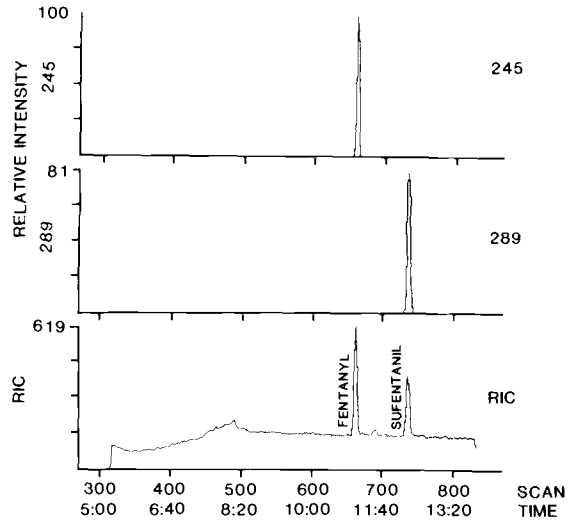

FIG. 8—*Selected ion chromatograms of sufentanil extracted from liver.*

Pathological Findings

Complete autopsy revealed only the three fresh needle puncture marks present along the medial aspect of the left forearm below the antecubital fossa overlying the basilic vein. No other evidence of recent trauma was found. Histopathological examination showed diffuse severe visceral congestion with mild pulmonary edema as well as cerebral edema and early anoxic neuronal ischemia (necrosis). These findings are consistent with an acute asphyxial death.

TABLE 3—*Tissue and fluid concentrations.*

SUFENTANIL CONCENTRATIONS	
Blood	1.1 ng/mL
Urine	1.3 ng/mL
Vitreous humor	1.2 ng/mL
Liver	1.75 ng/g
Kidney	5.5 ng/g

MIDAZOLAM CONCENTRATIONS	
Blood	50 ng/mL (GC/MS, HPLC)
Urine	300 ng/mL (HPLC)
Liver	930 ng/g (GC/MS)
Kidney	290 ng/g (GC/MS)

Discussion and Conclusions

The intravenous dosage ranges to produce anesthesia are 0.15 to 0.35 mg/kg for midazolam and 1 to 8 μg/kg for sufentanil [*7,12*]. Midazolam and sufentanil dosages should be decreased when used in combination with narcotics because of their additive effects [*7,12*]. The amounts of drugs found in this case (that is, 20 mg of midazolam and 50 μg of sufentanil) would yield 0.221 mg/kg of midazolam and 0.551 μg/kg of sufentanil based on the victim's estimated body weight of 90.7 kg and are more than sufficient dosages to produce anesthesia in the victim. Subject to reasonable assumptions about the actual time of death after administration of the drugs, the concentrations of sufentanil and midazolam found in the blood are consistent with the dosages administered and the pharmacokinetics of the drugs. The concentrations are congruous to the reported therapeutic blood concentration ranges of these drugs (that is, midazolam 53 to 96 ng/mL and sufentanil 0.05 to 1 ng/mL [*1,4-6,15-17*]) yet adequate to produce lethal respiratory depression if untreated. Note that midazolam can produce pronounced cardiovascular and respiratory depression at any dose and especially in a sensitive individual [*7*].

The concentrations of sufentanil in the tissues and biological fluids are compatible with its pharmacokinetic profile of rapid distribution. Calculation of sufentanil's apparent volume of distribution, based on the blood concentrations detected, results in a value of 0.5 L/kg. The ratios of sufentanil concentrations in blood to those in urine or vitreous humor are very near unity with some accumulation in the liver and the kidney.

Calculation of an apparent volume of distribution for midazolam, based on the blood concentration detected, results in a value of 4.4 L/kg which reflects midazolam's extensive distribution. Larger concentrations in the urine, kidney, and liver also denote this distribution and tissue binding. HPLC and GC/MS methods yielded the same blood concentration of midazolam. The histopathological findings indicate that the victim died of acute asphyxia. Compilation of the pathological findings with the toxicological results along with the understanding of the victim's knowledge of these drugs substantiates that the victim died of acute asphyxiation as a result of suicidal administration of a combination of midazolam and sufentanil. The drugs produced a fatal additive depression of respiration. Lidocaine (1% solution) was evidently used as a local anesthetic at the site of the intravenous injection. This is the first case reported, to the knowledge of the authors, of a death attributable to an acute sufentanil/midazolam intoxication.

Acknowledgment

The authors wish to thank Investigators Brewer and Morton of the Carter County Sheriff's Department for their assistance in this case and Ms. Donna Corbitt for her preparation of

the manuscript. We also wish to acknowledge Roche Laboratories, Nutley, New Jersey, and Janssen Pharmaceutica, Piscataway, New Jersey, for their assistance in supplying analytical standards of midazolam and sufentanil.

References

[1] Gemperle, M. and Kapp, W., "Midazolam and Anaesthesia," *British Journal of Clinical Pharmacology*, Vol. 16, Suppl. 1, 1983, pp. 1875-1905.

[2] Dundee, J. W. and Wilson, D. B., "Amnestic Action of Midazolam," *Anaesthesia*, Vol. 35, No. 5, 1980, pp. 459-461.

[3] Kortila, K., "Clinical Effectiveness and Residual Effects of New Agents and Techniques," *Journal of Dental Research*, Vol. 62, 1983, p. 166.

[4] Greenblatt, D. J., Abernethy, D. R., Lockniskar, A., Harmatz, J. S., Limjuco, R. A., and Shader, R. I., "Effects of Age, Gender, and Obesity on Midazolam Kinetics," *Anesthesiology*, Vol. 61, No. 1, 1984, pp. 27-35.

[5] Allonen, H., Ziegler, G., and Klotz, U., "Midazolam Kinetics," *Clinical Pharmacology and Therapeutics*, Vol. 30, No. 5, 1981, pp. 653-661.

[6] Heizmann, P., Eckert, M., and Ziegler, W. H., "Pharmacokinetics and Bioavailability of Midazolam in Man," *British Journal of Clinical Pharmacology*, Vol. 16, 1983, pp. 43S-49S.

[7] *United States Pharmacopeia Dispensing Information*, Vol. 1B, 8th ed., United States Pharmacopeial Convention Incorporated, Rockville, MD, 1988, pp. 1487-1490.

[8] Carrier, O., Jr. and Holland W. C., "Supersensitivity in Perfused Isolated Arteries After Reserpine," *Journal of Pharmacology and Experimental Therapeutics*, Vol. 149, 1965, pp. 212-218.

[9] Haeusler, G., Haefelz, W., and Huerlimann, A., "Effect of Surgical and Clinical Adrenergic Denervation on Vascular Responses," in *Physiology and Pharmacology of Vascular Neuroeffector Systems*, J. A. Bevan, R. F. Furchgott, R. A. Maxwell, and A. P. Somlyo, Eds., S. Karger, Basel, 1971, pp. 141-159.

[10] Bovill, J. G., Sebel, P. S., Blackburn, C. L., Oei-Lim, V., and Heykants, J. J., "The Pharmacodynamics of Sufentanil in Surgical Patients," *Anesthesiology*, Vol. 61, 1984, pp. 502-506.

[11] Davis, P. J., Cook, D. R., Stiller, R. L., and Davin-Robinson, K. A., "Pharmacodynamics and Pharmacokinetics of High-Dose Sufentanil in Infants and Children Undergoing Cardiac Surgery," *Anesthesiology and Analgesia*, Vol. 66, 1987, pp. 203-208.

[12] *United States Pharmacopeia Dispensing Information*, Vol. 1A, 8th ed., United States Pharmacopeial Convention Incorporated, Rockville, MD, 1988, pp. 1072-1079.

[13] Suitheimer, C. and Sunshine, I., "Benzodiazepines by Reverse Phase HPLC," in *Methodology for Analytical Toxicology*, Vol. II, I. Sunshine and P. Jatlow, Eds., CRC Press Incorporated, Boca Raton, FL, 1982, pp. 25-30.

[14] Gillespie, T. J., Gandolfi, J., Maiorini, R. M., and Vaughn, R. W., "Gas Chromatographic Determination of Fentanyl and Its Analogues in Human Plasma," *Journal of Analytical Toxicology*, Vol. 5, 1981, pp. 133-137.

[15] Arendt, R. M., Greenblatt, D. J., and Garland, W. A., "Quantitation by Gas Chromatography of the 1- and 4- Hydroxy Metabolites of Midazolam in Human Plasma," *Pharmacology*, Vol. 29, 1984, pp. 158-164.

[16] Smith, M. T., Eadie, M. J., and Brophy, T. O., "The Pharmacokinetics of Midazolam in Man," *European Journal of Clinical Pharmacology*, Vol. 19, 1981, pp. 271-278.

[17] Weldon, S. T., Gillespie, T. J., and Gandolfi, A. J., "Fentanyl and Its Analogs by Gas Chromatography with a Thermionic Specific Detector," in *Methodology for Analytical Toxicology*, Vol. III, I. Sunshine, Ed., CRC Press Incorporated, Boca Raton, FL, 1985, pp. 89-93.

Address requests for reprints or additional information to
Kenneth E. Ferslew, Ph.D.
Quillen Dishner College of Medicine
Section of Toxicology
Box 15160A
Johnson City, TN 37614