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Determination of Etomidate in Human Postmortem Fluids and Tissues

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16. Abstract

Following an aviation accident, biological specimens from the operator of the aircraft are submitted to the Federal Aviation Administration's Civil Aerospace Medical Institute for toxicological analysis. During the course of medical treatment following an aviation accident, pilots who later died as a result of their injuries may have been administered etomidate as an intravenous anesthetic. Our laboratory has developed a sensitive method for the identification and quantitation of etomidate in the biological specimens received from these pilots. Furthermore, we have evaluated the distribution of this compound in various postmortem tissues and fluids from 3 fatal aviation accident cases. When available, 10 specimen types were analyzed for each case, including blood, urine, vitreous humor, liver, kidney, skeletal muscle, lung, spleen, heart muscle, and brain. Specimens were extracted using solid-phase base extraction and analyzed by GC/MS. Deuterated etomidate was not available as an internal standard, so to eliminate any possible matrix effects during extraction all quantitative values in specimens other than blood were determined through standard addition. Blood etomidate concentrations in these three cases ranged from 12 to 41 ng/mL. Distribution coefficients for etomidate were determined for each of the specimen types analyzed. These coefficients are expressed relative to the blood concentration in that case. To our knowledge, this is the first report presenting the distribution of etomidate in humans at therapeutic concentrations.

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INTRODUCTION

Etomidate, [R-(+)-ethyl-1(phenylethyl)-1-H imidazole-5-carboxylate], is an intravenous anesthetic agent first approved for use in the United States in 1983 and marketed under the trade name Amidate®.[1] Prior to the introduction of etomidate, the medical community lacked an ideal agent for the induction of unconsciousness before certain medical procedures. This compound causes rapid hypnosis in patients and may be used repeatedly without tolerance.[1,2]

Etomidate is rapidly absorbed following intravenous administration. Peak plasma concentrations typically occur within 4 minutes of introduction, and the half-life has been reported to range from 2 to 11 hours.[3] Etomidate is extensively metabolized by hydrolysis in both the plasma and in the liver to an inactive carboxylic acid metabolite.[1,3,4] The chemical structure of etomidate can be seen in Figure 1. The internal standard, SERTIS, was used for all etomidate analysis. SERTIS, n-methyl-4-(4-bromophenyl)-1,2,3,4,-tetrahydro-1-napthylamine, is a generic internal standard used by our laboratory when no deuterated analog is available. SERTIS is not sold for human consumption, so there is no risk of this compound being present in human specimens.

Frequently, pilots involved in aviation accidents survive and are treated surgically in an effort to save their lives. However, these pilots do not always survive surgery. If death occurs following medical treatment, postmortem specimens are shipped to our laboratory for analysis. These specimens are subsequently found positive for the compounds used by medical personnel during life-saving procedures. Etomidate is one such compound. There are relatively few published reports describing the analysis of etomidate, and only 1 of these publications describes the analysis by GC/MS.[5-9] Furthermore, scientific information concerning the distribution of etomidate in human specimens is not available. This manuscript presents the quantitation and distribution of etomidate in postmortem human specimens, including urine, vitreous humor, skeletal muscle, liver, kidney, lung, spleen, brain, and heart muscle.

MATERIALS AND METHODS

Chemicals and Reagents

All aqueous solutions were prepared using double deionized water (DDW), which was obtained using a Milli-QT_{plus} Ultra-Pure Reagent Water System (Millipore®, Continental Water Systems, El Paso, TX). All chemicals described below were purchased in the highest possible purity and used without any further purification. Etomidate was purchased from Bedford Labs (Bedford Laboratories, Bedford, OH) and received as a 2 mg/mL standard in 35% v:v propylene glycol. The internal standard, SERTIS, was obtained from Pfizer (Pfizer Inc., New York, NY). Methanol, acetonitrile, ammonium hydroxide, acetic acid, ethyl acetate, sodium fluoride, and potassium phosphate monobasic were purchased from Fisher Scientific (Fisher Scientific Co., Pittsburgh, PA). The pH of all

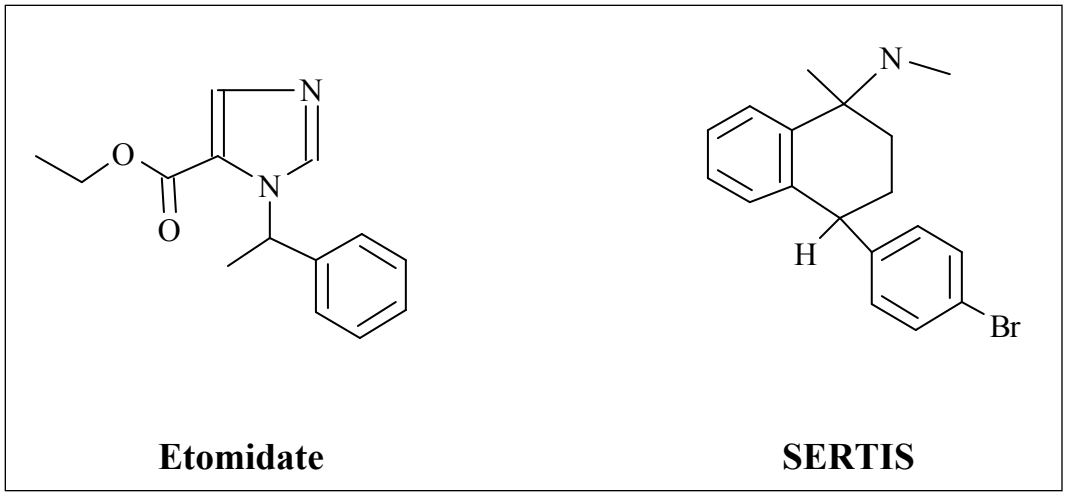

Figure 1. Chemical structures of etomidate and SERTIS.

solutions was measured using a Corning model 430 pH meter (Corning Life Sciences, Acton, MA) connected to a Corning 3-in-1 model pH electrode.

Gas Chromatographic/Mass Spectroscopic Conditions

All analyses were performed using a bench-top gas chromatograph/mass spectrometer (GC/MS), which consisted of a Hewlett Packard (HP) 6890 series GC, interfaced with a HP 5973 quadrupole MS (Agilent Technologies Inc., Santa Clara, CA). The MS was tuned on a daily basis using perfluorotributylamine. The electron multiplier voltage was set at 106 eV above the tune value. Chromatographic separation was achieved using a Varian FactorFour® crosslinked 100% methyl siloxane capillary column 12 m x 0.2 mm i.d., 0.33 µm film thickness (Varian Co., Harbor City, CA.). Helium was employed as the carrier gas and used at a flow rate of 1.0 mL/min. The GC/MS transfer line and source temperatures were set to 280°C and 250°C, respectively. An HP 6890 autosampler was used to inject 1 µL of extract into the GC/MS. The GC was equipped with a split/splitless injection port operated at 250°C in the splitless mode with the purge time of 0.5 min. The oven temperature profile was established as follows: 70°C – 290°C at 30°C/min and a final hold time of 2.67 min, resulting in a total run time of 10 min. Initially, neat standards of each compound (1 µL of a 100 ng/µL solution) were injected individually and analyzed using the full scan mode of the GC/MS, which scanned from 50 to 600 AMU. The full scan mass spectra from these 2 compounds can be seen in Figures 2 and 3. Quantitation and qualifier ions for each analyte were then selected based on both abundance and mass-to-charge ratio (*m/z*). To increase reproducibility and reduce interference, high-mass ions were selected when possible. The ions chosen for each respective analyte can be seen in Table 1. Upon selection of unique ions, the MS was run in selected ion monitoring (SIM) mode with a dwell time of 30 msec for each recorded ion.

Sample Selection and Storage

A search of the CAMI database identified 3 etomidate-positive fatalities from separate civil aviation accidents from the previous 3 years that had a majority of the desired biological tissues and fluids (blood, urine, vitreous humor, liver, kidney, muscle, lung, spleen, heart, and brain) available for analysis. In all cases, blood was stored at -20°C in tubes containing 1.00% (w/v) sodium fluoride/potassium oxalate until analysis. All other specimens were stored at -20°C, with no added preservative, until analysis.

Calibrator and Control Preparation

A calibration curve for etomidate was prepared by serial dilution, utilizing bovine whole blood as the diluent. Controls were also prepared using bovine whole blood as the diluent. Calibrators were prepared from 1 stock standard solution, while controls were prepared from a second separate stock solution with a different manufacturer's lot number. A calibration curve was prepared at concentrations ranging from 0.39 – 800 ng/mL. A minimum of 7 calibrators were used to construct the calibration curve. Controls were prepared at concentrations of 80 and 320 ng/mL and extracted, with each batch of unknowns to verify the accuracy of the calibration curve. The internal standard solution was prepared at a concentration of 400 ng/mL in DDW by dilution from the stock standard of this compound.

Quantitation of blood specimens was achieved via an internal standard calibration procedure. Response ratios for etomidate were determined for every blood sample analyzed. The response ratio was calculated by dividing the area of the analyte peak by the area of the internal standard peak. Calibration curves were derived by plotting a linear regression of the analyte/internal standard response ratio versus the analyte concentration for each respective calibrator. Standard addition was used to determine the concentration of etomidate in every specimen type other than blood. This was necessary due to the possible matrix effects associated with the extraction of an internal standard that is not a deuterated analog of the compound of interest. The standard addition procedure used in this work has been described extensively elsewhere.[10] Briefly, a specimen was divided in half, 1 half was spiked with a precisely known amount of etomidate, the other half was not altered. Internal standard was added to each tube and the 2 halves were analyzed. After analysis, the ratio of the etomidate concentration in the unspiked sample compared to the spiked sample allowed us to derive the concentration in the original specimen.

Sample Preparation and Extraction Procedure

Postmortem specimens, calibrators, and controls were extracted in the following manner. Tissue specimens were homogenized using an Omni post-mounted homogenizer (Omni Int., Marietta, GA). The generator used with this homogenizer had a diameter of 30 mm and rotated at 22,000 rpm. Tissues were homogenized following a 1:2 dilution with 1.00% NaF in DDW. Three mL aliquots of postmortem fluids, calibrators, controls, and 3.00 g aliquots of each tissue homogenate (1.0 g tissue) and each standard addition sample were transferred to individual 16 x 150 mm screw-top tubes. To each specimen, calibrator, and control, 1.00 mL of the internal standard (400 ng) was added. Samples were vortexed briefly and allowed to stand at room temperature for 10 min. Nine mL ice-cold acetonitrile was added to each sample. The mixture was then placed on a rotary mixing wheel and

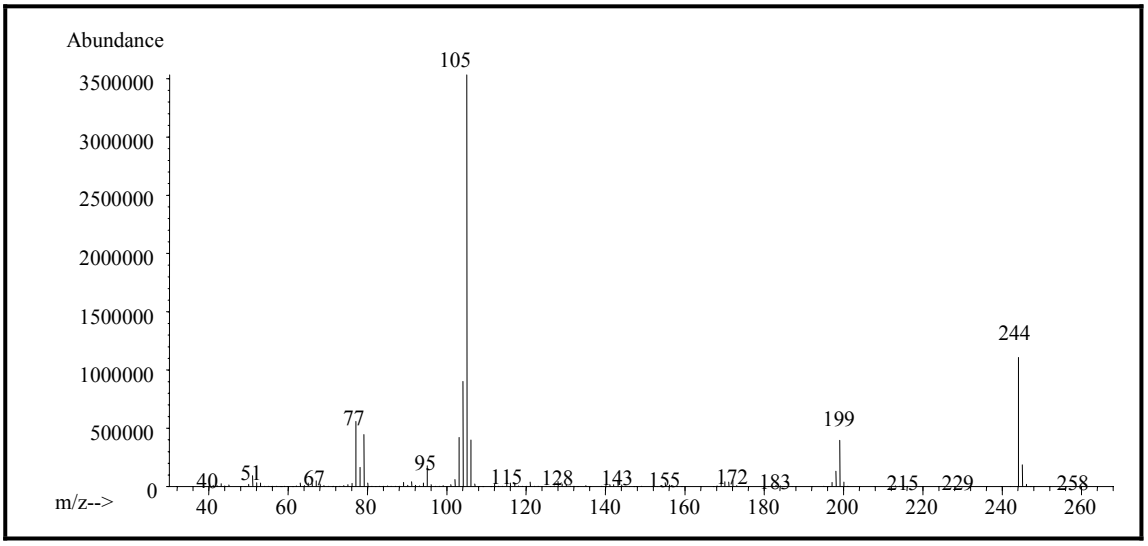

Figure 2. Full scan mass spectra of etomidate.

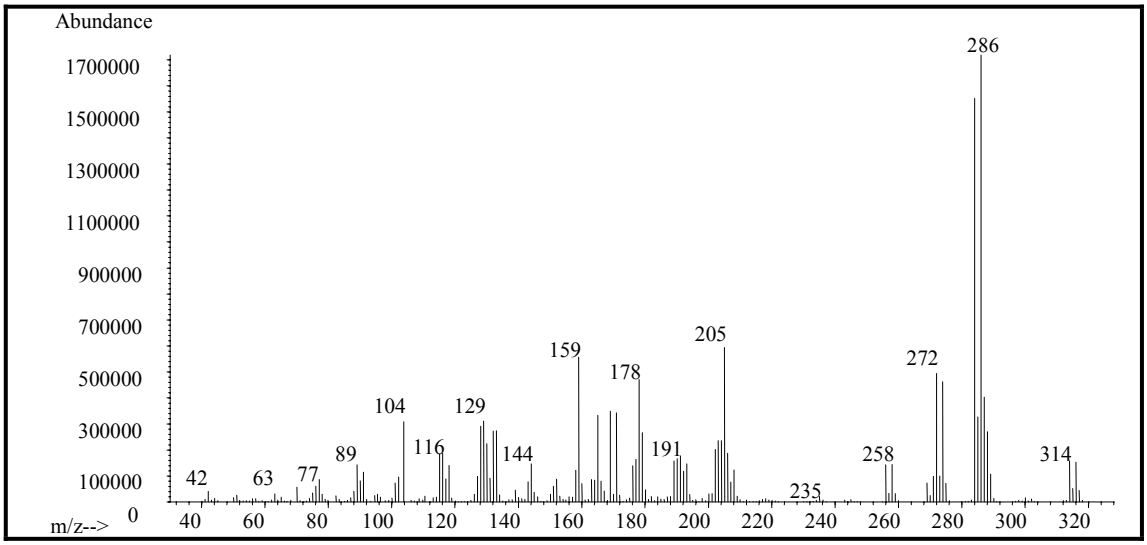

Figure 3. Full scan mass spectra of SERTIS.

Table 1. Ions utilized for the quantitation of fluoxetine etomidate.

Compound	Ions utilized for quantitation (*m/z*)*
Etomidate	**105**, 244, 199
SERTIS	**286**, 284, 314

* Ions in bold used for quantitation, other ions used as qualifiers.

mixed at 15 rpm for 15 min. Centrifugation at 820×*g* for 5 min removed cellular debris and proteins. Following centrifugation, the supernatant was transferred to clean 16 x 125 mm culture tubes and evaporated in a TurboVap® Concentration Workstation at 40°C (Caliper Life Sciences, Hopkinton, MA) under a stream of dry nitrogen to a volume of approximately 1 mL. Following acetonitrile evaporation, 4.00 mL 0.10 M phosphate buffer, pH 6.00, was added to each sample. The extracts were transferred to Bond Elute Certify® solid-phase extraction (SPE) columns obtained from Varian (Varian Co., Harbor City, CA.), which had been pre-conditioned with 2.00 mL methanol, followed by 2.00 mL 0.10 M phosphate buffer, pH 6.00. Care was taken not to dry the column prior to adding the extract. Column flow rates of 1-2 mL/min were maintained in each SPE step using a Varian 24 port Cerex™ SPE processor (Varian Co., Harbor City, CA.) with a nitrogen pressure of 3 psi. Once each sample had passed through its respective column, the columns were washed with 1.00 mL of 1.00 M acetic acid and then dried completely with 25 psi nitrogen for 5 min. The columns were again washed with 6.00 mL. Following the methanol wash, the columns were again dried completely with 25 psi nitrogen for 5 min. The analytes were eluted off the columns with 3.00 mL of 2% ammonium hydroxide in ethyl acetate, which was prepared fresh daily. Eluents were evaporated to dryness in a TurboVap® set at 40°C under a stream of dry nitrogen. Once dry, the contents of each tube were reconstituted in 50 µL of ethyl acetate and transferred to GC/MS vials for analysis.

Extraction Efficiency

The method used for the determination of analyte recovery has previously been reported.[11] Briefly described, 2 groups of controls, X and Y, prepared using negative whole blood, were extracted in the same manner as described above. Group X was spiked with a precisely known concentration of etomidate prior to SPE extraction, while group Y was spiked with the same precisely known concentration of etomidate following SPE 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 (100 * (X/Y) = % recovery) for etomidate.

RESULTS AND DISCUSSION

Method Validation

The procedure described herein, which utilizes SPE and GC/MS for the detection of etomidate, is rapid, reproducible, and sensitive. Analyte peaks were completely resolved, and each provided quantitation ions with unique *m/z*, so no interference was observed. Retention times for etomidate and SERTIS were typically 4.75 and 6.10 min, respectively. No analyte suffered interference from endogenous/exogenous matrix components. Standard addition was used for specimen types other than blood while using a blood calibration curve, thereby eliminating any matrix effect concerns.

The full-scan mass spectra of etomidate and SERTIS provided numerous high mass ions; these spectra can be seen in Figures 2-3. Quantitation and qualifier ions for each compound are shown in Table 1. Acceptability criteria employed for analyte identification and quantitation were as follows: 1) ion ratios for a given analyte, measured as the peak area of a qualifier ion divided by the peak area of the quantitation ion, were required to be within ± 20% of the average of the ion ratios for calibrators used to construct the calibration curve; 2) each ion monitored was required to have a minimum signal-to-noise ratio (S/N) of 10; and 3) the analyte was required to have a retention time within ± 2.00% of the average retention time for calibrators used to construct the calibration curve. Analytes not meeting these criteria were reported as either negative or inconclusive.

The linear dynamic range (LDR), limit of detection (LOD), and limit of quantitation (LOQ) for etomidate were determined using whole blood as the matrix. The LDR was determined to be 0.78 – 400 ng/mL. The correlation coefficient for the calibration curve used to evaluate the LDR was 0.998 when employing a weighting factor of 1/X. The injection of an ethyl acetate blank following the 400 ng/mL calibrator showed no carryover contamination. Subsequently, ethyl acetate blanks were utilized between each postmortem specimen throughout the sample sequence to verify that no sample-to-sample contamination had occurred. The LOD was defined as the lowest etomidate concentration detectable that met the above-discussed identification criteria. The LOQ was defined as the lowest etomidate concentration detectable that not only met all identification criteria discussed above but also had an experimentally determined concentration within ± 20% of its prepared value. The LOD for etomidate was determined to be 0.39 ng/mL, while the LOQ was determined to be 0.78 ng/mL.

The extraction efficiency for etomidate was determined at two different concentrations. The recovery of etomidate at 50 ng/mL was determined to be 79%, while the recovery at 400 ng/mL was 91%. Recovery values above 75% are exceptional when considering the simplicity of the extraction and the use of whole blood as the matrix for these experiments.

Intra-day and inter-day accuracy and precision were examined for this extraction procedure. Accuracy was measured as the relative error between the experimentally

determined and prepared concentrations of a whole-blood control. Precision was measured as the relative standard deviation (RSD) of the experimentally determined concentrations of a group of whole blood controls. Pools of controls were created at 80 ng/mL and 320 ng/mL in volumes large enough to be used for the entire accuracy and precision investigation. These controls were stored at 4°C for the duration of this study. For the intra-day accuracy and precision experiment, a calibration curve was extracted, along with 5 replicates of each control concentration. As shown in Table 2, etomidate at both concentrations yielded relative errors within 5% of the target concentration. Furthermore, the RSD associated with this experiment was found to be 2% at each concentration. These results demonstrate the exceptional accuracy and precision of this method.

Table 2. Intra-day accuracy and precision for repeated determinations over 7 days.*

	Etomidate	
	Day 1	
Target Conc.	80	320
Mean SD	79 2	333 8
Relative Error	-1%	+4%
R.S.D.	2%	2%
	Day 2	
Target Conc.	80	320
Mean SD	73 2	315 7
Relative Error	-9%	-2%
R.S.D.	3%	2%
	Day 4	
Target Conc.	80	320
Mean SD	80 1	334 4
Relative Error	0%	+4%
R.S.D.	1%	1%
	Day 7	
Target Conc.	80	320
Mean SD	82 2	335 7
Relative Error	+3%	+5%
R.S.D.	2%	2%

*n=5 at each concentration for each day; controls were run on days 1, 2, 4, and 7.

Inter-day accuracy and precision were evaluated by extracting 5 replicates of each of the 2 control concentrations on Days 2, 4, and 7. The quantitative values determined on each of these days were derived from the calibration curves originally prepared on Day 1. The results obtained after storage of each control lot at 4°C for 2, 4, and 7 days can be seen in Table 2. Etomidate concentrations determined over this 7-day period showed no significant difference from those obtained on Day 1. The RSDs for etomidate were less than 5% on Days 2, 4, and 7. Even though no apparent decrease in concentration was observed over the 7-day storage period at 4°C, as a good laboratory practice, we recommend prompt toxicological analysis once a postmortem specimen has been thawed and preparing a new calibration curve at the beginning of each new analysis.

Postmortem Concentrations of Etomidate

Blood concentrations found in these 3 cases ranged from 12 to 41 ng/mL. Therapeutic levels of etomidate in plasma range from 220 to 410 ng/mL.[3] The published plasma/whole blood ratio is 0.62. Therefore, therapeutic blood concentrations for etomidate in whole blood range from 354 to 661 ng/mL. Blood concentrations in these cases were all found to be below therapeutic levels. The concentration of etomidate in each postmortem specimen from these 3 cases can be seen in Table 3. With a volume of distribution of 2-7 L/kg,[1] etomidate was expected to be found at higher concentrations in the tissue specimens analyzed. As can be seen in Table 3, this was the case. It appears that etomidate is rapidly distributed following administration. It has also been found that this compound is extremely lipid soluble.[12] Therefore, brain concentrations are expected to be relatively high. The following mean concentrations of etomidate were found: blood 25 ng/mL, urine 9 ng/mL, vitreous humor 3 ng/mL, liver 128 ng/g, lung 40 ng/g, kidney 150 ng/g, spleen 119 ng/g, muscle 61 ng/g, brain 128 ng/g, and heart 119 ng/g. The individual concentrations from each case can be seen in Table 3. The distribution coefficients for etomidate, expressed as specimen/blood ratios, are summarized in Table 4. The distribution coefficients for etomidate were determined to be: urine 0.4 ± 0.1, vitreous humor 0.17 ± 0.08, liver 4 ± 1, lung 1.6 ± 0.5, kidney 5 ± 1, spleen 4 ± 1, muscle 3 ± 1, brain 5 ± 1, and heart 6 ± 2. As can be seen in Table 3, etomidate distribution coefficients for urine, bile, vitreous humor, muscle, kidney, lung, spleen, brain, liver, and heart had coefficient of variation (CV) values between 20 and 47%. It is widely accepted that basic drugs with large volumes of distribution can undergo postmortem redistribution. This redistribution may account for some of the larger CV values observed.

Table 3. Etomidate concentrations obtained from 3 pilot fatalities.*

Case	Blood	Urine	VH	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart
1	12	6	3	29	12	48	32	33	49	83
2	21	—	2	92	46	152	79	87	132	167
3	41	11	—	262	63	251	246	63	202	108

* All concentrations shown in units of ng/mL or ng/g
—Specimen type not available for analysis

Table 4. Postmortem etomidate-positive specimen distribution coefficients.

	Urine/ Blood	VH/ Blood	Liver/ Blood	Lung/ Blood	Kidney/ Blood	Spleen/ Blood	Muscle/ Blood	Brain/ Blood	Heart/ Blood
n	2	2	3	3	3	3	3	3	3
Mean	0.4	0.17	4	1.6	5	4	3	5	6
s.d.	0.1	0.08	1	0.5	1	1	1	1	2
CV	25	47	25	31	20	25	33	20	33

There are no widely accepted criteria for what constitutes a reliable distribution coefficient; however, with certain drugs it may be possible, with caution, to use a tissue or fluid distribution coefficient to crudely estimate a blood concentration in cases where blood is not available, if the distribution coefficient has a CV of $\leq$ 25%. Our laboratory receives blood in only approximately 70% of the cases that we analyze. Therefore, the results obtained from the limited number of cases (n=3) in this study suggest that etomidate concentrations found in liver, kidney, brain, and spleen could be used with caution to estimate blood etomidate concentrations that are slightly below therapeutic levels. We acknowledge that a study involving a greater number of samples from a larger pool of cases needs to be completed to more definitively verify these results. However, based on these findings one could cautiously estimate a range for postmortem etomidate concentrations in blood.

CONCLUSION

In this study, our laboratory developed and validated a novel method for the extraction and analysis of etomidate in human biological specimens. The results obtained from these cases suggest that etomidate is readily absorbed by all tissues and fluids in the body. Although etomidate distribution varied among individuals, there were several specimen types that showed relatively consistent distribution coefficients. Liver, kidney, brain, and spleen each had a CV of less than 25% (n=3). The relatively small CV associated with these distribution coefficients suggests that these specimens may be used with extreme caution to obtain an approximate blood concentration for etomidate when blood is not available for analysis.

REFERENCES

1. Giese, J.L. and Stanley, T.H. Etomidate: A New Intravenous Anesthetic Induction Agent. *Pharmacotherapy,* 3: 251-8 (1983).
2. Doenicke, A. *Etomidate, a New Hypnotic Agent for Intravenous Application.*, 1 ed. (Springer-Verlag, Berlin, 1977).
3. Moffat, A.C., Osselton, M.D., and Widdop, B. eds. *Clarke's Analysis of Drugs and Poisons in Pharmaceuticals, Body Fluids, and Postmortem Materials*, 3 ed. (Pharmaceutical Press, London, UK, 2004).
4. Van Hamme, M.J., Ghoneim, M.M., and Ambre, J.J. Pharmacokinetics of Etomidate, a New Intravenous Anesthetic. *Anesthesiology,* 49: 274-7 (1978).
5. de Boer, A.G., Smeekens, J.B., and Breimer, D.D. Assay of Etomidate in Plasma by Capillary Gas Chromatography with Nitrogen-Selective Detection. *J Chromatogr,* 162: 591-5 (1979).
6. Deng, X. and Simpson, V.J. Gas Chromatographic--Mass Spectrometric Determination of Etomidate in Mouse Brain. *J Pharmacol Toxicol Methods,* 43: 73-7 (2000).
7. Ellis, E.O. and Beck, P.R. Determination of Etomidate in Human Plasma by High-Performance Liquid Chromatography. *J Chromatogr,* 232: 207-11 (1982).
8. Haring, C.M., Dijkhuis, I.C., and van Dijk, B. A. Rapid Method of Determining Serum Levels of Etomidate by Gas Chromatography With the Aid of a Nitrogen Detector. *Acta Anaesthesiol Belg,* 31: 107-12 (1980).
9. Le Moing, J.P. and Levron, J.C. High-Performance Liquid Chromatographic Determination of Etomidate in Plasma. *J Chromatogr,* 529: 217-22 (1990).
10. Siek, T.J. and Dunn, W.A. Documentation of a Doxylamine Overdose Death: Quantitation by Standard Addition and Use of Three Instrumental Techniques. *J Forensic Sci,* 38: 713-20 (1993).
11. Johnson, R.D., Lewis, R.J., Canfield, D.V., et al. Accurate Assignment of Ethanol Origin in Postmortem Urine: Liquid Chromatographic-Mass Spectrometric Determination of Serotonin Metabolites. *J Chromatogr B Analyt Technol Biomed Life Sci,* 805: 223-34 (2004).
12. Heykants, J.J., Meuldermans, W.E., Michiels, L.J., et al. Distribution, Metabolism and Excretion of Etomidate, a Short-Acting Hypnotic Drug, in the Rat. Comparative Study of (R)-(+)-(--)-Etomidate. *Arch Int Pharmacodyn Ther,* 216: 113-29 (1975).