DOT/FAA/AM-00/29

Office of Aviation Medicine
Washington, D.C. 20591

Distribution of Butalbital in Biological Fluids and Tissues

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August 2000

Final Report

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U.S. Department of Transportation

Federal Aviation Administration

N O T I C E

Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT/FAA/AM-00/29		

4. Title and Subtitle	5. Report Date
Distribution of Butalbital in Biological Fluids and Tissues	August 2000
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
Lewis, R.J., Southern, T.L., and Canfield, D.V.	

9. Performing Organization Name and Address	10. Work Unit No. (TRAIS)
FAA Civil Aeromedical Institute P.O. Box 25082 Oklahoma City, Oklahoma 73125	11. Contract or Grant No.

12. Sponsoring Agency name and Address	13. Type of Report and Period Covered
Office of Aviation Medicine Federal Aviation Administration 800 Independence Ave., S.W. Washington, D.C. 20591	14. Sponsoring Agency Code

15. Supplemental Notes

This work was accomplished under the approved task AM-B-00-TOX-202.

16. Abstract

During the investigation of fatal aviation accidents, postmortem samples from the pilot/copilot are submitted to the Federal Aviation Administration's (FAA's) Civil Aeromedical Institute for toxicological analysis. Blood specimens are received in approximately 70% of the fatal aviation accidents analyzed by the FAA's Toxicology and Accident Research Laboratory. The lack of blood available is usually due to the severe damage to a pilot's body during an aviation accident and/or to the length of time taken to recover the body following an accident. Therapeutic and toxic levels for most drugs are reported in the scientific literature for blood and plasma only. Therefore, it is imperative for an accident investigator and forensic toxicologist to be able to estimate drug concentrations in a fatal aviation accident victim's blood from the available concentrations in the tissue. This is exemplified by a recent aviation fatality where butalbital was identified in muscle tissue of a pilot, and the investigators wanted to know the approximate butalbital concentration expected in the victim's blood. Butalbital, a short-acting barbiturate found in combination with other drugs such as acetaminophen, aspirin, codeine, and caffeine, is commonly prescribed for the treatment of tension headaches. Certain side effects of butalbital, such as drowsiness, sedation, dizziness, and a feeling of intoxication, could affect pilot performance and become a significant factor in an aviation accident. Thus, our laboratory determined the distribution of butalbital in various postmortem tissues and fluids. The distribution coefficients established for butalbital, expressed as specimen/blood ratios, were found to be as follows: muscle (0.66 ± 0.09), kidney (0.98 ± 0.09), lung (0.87 ± 0.06), spleen (0.75, ± 0.03), brain (0.96 ± 0.07), liver (2.22 ± 0.04), liver fluid (0.89 ± 0.23), heart (0.91 ± 0.17), bile (0.94 ± 0.22), and urine (0.73 ± 0.16). The results demonstrate that muscle, kidney, lung, spleen, brain, liver, and heart can be used reliably to estimate butalbital blood concentrations.

17. Key Words	18. Distribution Statement
Forensic Science, Toxicology, Butalbital, Distribution, Sedative, Postmortem Drug Analysis, Aircraft Accident Investigation	Document is available to the public through the National Technical Information Service Springfield, Virginia 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	7	

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INTRODUCTION

The Federal Aviation Administration's (FAA's) Civil Aeromedical Institute (CAMI) is responsible under the Department of Transportation (DOT) Order 8020.11A, Chap 4, Par 170, to "conduct toxicologic analysis on specimens from … aircraft accident fatalities." Additionally, DOT Order 1100.2C, Chap 53, Par 53-15 requires that CAMI "investigate(s) selected general aviation and air carrier accidents and searches 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 the CAMI's Forensic Toxicology and Accident Research Laboratory where toxicological analysis is conducted on various postmortem fluids and tissues.

Due to the violent collisions, explosions, and fires often associated with aircraft accidents, crew members' bodies may be fragmented, incinerated, disintegrated, or scattered over large areas of rough terrain or bodies of water. In many cases, the search for remains results in only small fragments of tissues being submitted for toxicological analysis. In fact, the FAA's Forensic Toxicology and Accident Research Laboratory receives blood specimens in only 70% of fatal aviation accidents. Therapeutic and toxic levels of a drug are typically reported in the scientific literature only for blood or plasma. Thus, accident investigators and forensic toxicologists are interested in estimating drug concentrations in a fatal aviation accident victim's blood from the available tissue drug concentrations. This is exemplified by a recent aviation fatality where butalbital was found in the muscle of a pilot and the investigators wanted to know the approximate butalbital concentration expected in the victim's blood.

Butalbital, a short-acting barbiturate found in combination with other drugs such as acetaminophen, aspirin, codeine, and caffeine, is commonly prescribed for the treatment of tension headaches (1,2). There are specific side effects, however, that could affect a pilot's performance and become a significant contributory factor in an aviation accident. The most serious of these side effects include drowsiness, sedation, dizziness, and a feeling of intoxication (1,2). Additional side effects of barbiturate exposure include a withdrawal syndrome, characterized by psychosis, personality changes, or seizures, and a rebound syndrome, characterized by chronic and/or daily pain or headaches (3,4).

Since scientific information concerning the distribution of butalbital at therapeutic and toxic levels is not available, our laboratory determined its distribution in various postmortem tissues and fluids. A search of the Forensic Toxicology and Accident Research Laboratory database identified four fatal aviation cases reported positive for butalbital where a full complement of biological fluids and tissues, including blood, muscle, liver, kidney, bile, lung, spleen, brain, urine, and heart was available. This report details the quantitation and distribution of butalbital in these postmortem specimens.

MATERIALS AND METHODS

Chemicals and Reagents

Butalbital and butalbital-d_5 standards were purchased from Radian (Austin, TX) at 1.00 mg/mL concentrations in methanol. The derivitization reagent, MethElute, was obtained from Pierce Chemical Co.(Rockford, IL). All other necessary chemicals and reagents were obtained from commercial sources in high purity and were used with no further purification.

Gas Chromatographic-Mass Spectroscopic (GC-MS) Conditions

All analyses were performed using a benchtop gas chromatograph/mass spectrometer (GC/MS), which consisted of a Hewlett Packard (HP) 6890 series GC connected to a HP 5973 quadrupole MS operating with a transfer line temperature of 280 °C and a source temperature of 250 °C. The MS was autotuned on a daily basis using perfluorotributylamine. The electron multiplier voltage was set at the autotune voltage with no offset. All chromatographic separations

were achieved using an HP-ULTRA-1 crosslinked 100% methyl siloxane capillary column, 12 m X 0.2 mm i.d., 0.33 μm film thickness. Helium was the carrier gas with a flow of 1.0 mL/min. An HP 6890 autosampler was used to inject 1 μL of sample extract into the GC. The GC was equipped with a split/splitless injection port operated in the splitless mode with a purge time of 0.5 min and a temperature of 250 °C. The oven temperature profile was 70 °C to 170 °C at 30 °C/min with an initial hold of 0.5 min, then ramped at 40 °C/min to a final temperature of 290 °C, which was held for 1 min, yielding a total run time of 7.83 min. Butalbital had a retention time of 3.5 min. Standard solutions of butalbital and butalbital-d_5 were separately analyzed using the full scan mode from 50 to 500 amu in order to select unique quantitation and qualifier ions. The ions chosen were 196, 138, 181 for butalbital, and 201, 200, and 184 for butalbital-d_5. Using these ions, the mass spectrometer was operated in selected ion monitoring mode (SIM) with a Dwell time of 40 msec.

Butalbital concentrations were determined using an internal standard calibration procedure. Calibration curves were prepared by plotting the linear regression of the analyte/internal standard response factor versus the analyte concentration. Response factors were determined for each specimen, and the various analyte concentrations were then obtained from the respective calibration curves.

Preparation of Standards

Calibration curves were prepared by diluting a butalbital standard in whole blood yielding concentrations ranging from 25 to 800 ng/mL. Controls were prepared in whole blood at 80, 160, and 320 ng/mL using separate drug standards than were used for the calibration curve. The internal standard solution was prepared by diluting a standard of butalbital-d_5 with H_2O to yield a final concentration of 400 ng/mL.

Sample Preparation and Extraction Procedure

Tissues were homogenized with a Brinkmann Tissue Homogenizer (Brinkmann Instruments, Westbury, NY) following a 1:1 dilution with water. Three mL aliquots of specimen fluids, calibrators, and controls, and 2 g samples of tissue homogenates were transferred to individual 15 mL screwtop vials. Smaller sample sizes were used if the extracted specimen concentrations were expected to be above the calibration curve. To each specimen, calibrator, and control, 1 mL of the internal standard solution (400 ng/mL) was added. The samples were vortexed and allowed to stand for 10 min. The cellular debris and proteins were precipitated and removed from the samples by adding 9 mL of cold acetonitrile, mixing on a rotory extractor for 15 min, and finally centrifuging at 820 × g for 5 min. The supernatant was transferred to 15 mL vials and evaporated in a water bath at 40 °C under a stream of dry nitrogen to a volume less than 1 mL. To this 3 mL of 0.1 M sodium acetate buffer, pH 7.0 was added. The extracts were transferred to solid-phase extraction (SPE) columns that were pre-conditioned with 3 mL methanol followed by 2 mL sodium acetate buffer, pH 7.0. The SPE columns were Bond Elute Certify II columns procured from Varian (Harbor City, CA). Care was taken not to dry the column prior to sample addition. A column flow rate of 1-2 mL/min was maintained in each step using a Varian 24 port pressure manifold with a nitrogen pressure of approximately 3 psi. Once the samples had passed through the columns, the columns were washed with 1 mL sodium acetate buffer, pH 7.0, dried with 25 psi nitrogen for 5 min, and then washed with 2 mL hexane:ethyl acetate (95:5). Butalbital was eluted from the columns with 4 mL hexane:ethyl acetate (75:25). Eluents were evaporated to dryness in a water bath at 40 °C under a stream of dry nitrogen. The extracts were reconstituted in 75 μL MethElute and transferred to GC autosampler vials for GC/MS analysis.

RESULTS AND DISCUSSION

The mass spectrum of butalbital and butalbital-d_5 provided numerous ions, from which 196 and 201, were selected as quantitation ions, respectively. Ions 138 and 181 were used as qualifier ions for 196, and ions 200 and 184 were used as qualifier ions for 201. A calibration curve (correlation coefficient = 0.998) of six points was constructed from 25 to 800 ng/mL and employed for butalbital quantitation for all specimens. With appropriate dilutions, all results fell within the calibration curve. The extraction procedure provided a clean extract in a relatively short period of time.

Therapeutic levels of butalbital in plasma range from 1 to 10 μg/mL (5). The blood concentrations found in the four cases ranged from 0.221 to 11 μg/mL. With an average blood/plasma ratio of 1.0, the blood concentrations ranged from slightly above therapeutic to slightly

below it (6). The distribution and concentration of butalbital found in the various tissues and fluids are presented in Table 1. The resulting butalbital blood concentrations suggest that the individual victims either took different amounts of drug or took the dose at different times prior to the accidents.

The distribution coefficients for butalbital, expressed as specimen/blood ratios, are summarized in Table 2. The distribution coefficients found for butalbital are: muscle (0.66 ± 0.09), kidney (0.98 ± 0.09), lung (0.87 ± 0.06), spleen (0.75, ± 0.03), brain (0.96 ± 0.07), liver (2.22 ± 0.04), liver fluid

Table 1. Distribution of Butalbital in Postmortem Tissues and Fluids.

Postmortem Tissue and Fluid Concentrations Represented as µg/g or µg/mL				
Specimen	Case 1	Case 2	Case 3	Case 4
Blood	11.000	0.638	0.368	0.221
Muscle	7.078	0.392	0.292	0.130
Kidney	11.162	0.541	0.394	0.214
Lung	9.838	0.528	0.343	0.180
Brain	NA	0.564	0.375	0.216
Spleen	8.485	0.502	0.269	0.160
Heart	8.723	NA	NA	0.227
Liver	24.453	1.415	0.839	0.481
Liver Fluid	8.000	NA	NA	0.234
Bile	8.608	0.699	NA	NA
Urine	NA	0.394	0.308	NA

Table 2. Postmortem Tissue and Fluid Distribution Coefficients for Butalbital.

Distribution Coefficients for Butalbital		
Specimen	Specimen/Blood ± SD	% of blood ± CV
Muscle	0.66 ± 0.09	66 ± 14
Kidney	0.98 ± 0.09	98 ± 9
Lung	0.87 ± 0.06	87 ± 7
Brain	0.96 ± 0.07	96 ± 7
Spleen	0.75 ± 0.03	75 ± 4
Heart	0.91 ± 0.17	91 ± 19
Liver	2.22 ± 0.04	222 ± 2
Liver Fluid	0.89 ± 0.23	89 ± 26
Bile	0.94 ± 0.22	94 ± 23
Urine	0.73 ± 0.16	73 ± 22

(0.89 ± 0.23), heart (0.91 ± 0.17), bile (0.94 ± 0.22), and urine (0.73 ± 0.16). The relatively small standard deviations (SD) associated with these distribution coefficients suggest that butalbital was in the post-absorption phase. Muscle, kidney, lung, spleen, brain, liver, and heart ratios all have a coefficient of variation (CV) of less than 20%. Bile and urine had CVs greater than 20%. This was not unexpected due to the excretory nature of bile and urine. Liver fluid also showed inconsistent ratios, having a CV of 26%. Interestingly, liver fluid contained less then half of the butalbital concentration found in liver tissue. These results suggest that butalbital distribution in select tissues, i.e., muscle, kidney, lung, spleen, brain, liver, and heart, can be used to closely estimate blood values over a broad concentration range and a wide window of time since the last dosage.

CONCLUSION

Ideally, all toxicological analyses would be conducted using blood samples. Due to the nature of forensic toxicology, however, the laboratory must rely upon the specimens that are available. The need is obvious for an ability to estimate drug concentrations in blood from values determined in various other tissues or fluids. In this study, our laboratory established the distribution of butalbital in various postmortem tissues and fluids. The data from this study suggest which postmortem tissues and fluids are reliable for estimating butalbital concentrations in blood. As expected, due to the excretory function of bile and urine, our results indicate that these specimens would not be desirable for estimating butalbital blood levels. Surprisingly, liver fluid had a relatively large CV for its distribution coefficient, and also had substantially lower butalbital concentrations than did liver tissue. This suggests that liver tissue would be a better choice than liver fluid for estimating butalbital blood levels. The results demonstrate that muscle, kidney, lung, spleen, brain, liver and heart can be reliably used to estimate butalbital blood concentrations. The findings from this study clearly emphasize the utility of various postmortem tissues in approximating butalbital blood concentrations.

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