Tissue Distribution of Isoflurane After Lethal Intoxication

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Abstract

Introduction: Isoflurane, 1-chloro-2, 2, 2,-trifluoroethyl difluoromethyl ether, is a volatile anesthetic used for general anesthesia in animals under veterinary surgical care. The adverse effects of inhaling isoflurane include respiratory depression, hypotension, cardiac arrthymias, nausea, and vomiting.
Case Histories: Case #1: A 53 year old professor, was found decomposed in his home. The decedent's body was wrapped in plastic with multiple bottles of isoflurane on the scene, along with containers of alcohol.
Case #2: A 44 year old anesthesiologist was found expired in his home along with several empty bottles of isoflurane and a sock with a chemical smell. He had a history of substance abuse and depression.
Objectives: 1. To validate a method for isoflurane analysis using routine headspace gas chromatography with flame ionization detection (HS/GC/FID), that will allow separation, detection, and quantification of isoflurane in different biological matrices. 2. To characterize the postmortem distribution of isoflurane in an isoflurane intoxicated individual.
Materials and Methods: Isoflurane was obtained from JD Medical. Specimens and internal standard were aliquoted into crimp-sealed headspace vials using a Hamilton pipettor-diluter. Separation of isoflurane and other volatiles was achieved with Restek, Rtx®-BAC1 and a Rtx®-BAC2, capillary columns using an Agilent 6890 Plus GC equipped with dual FID and a HP-7694 Headspace Auto-sampler. Confirmation was performed by headspace gas chromatography mass spectrometric detection using an Agilent 6890 GC/5975 MS-EI in full scan mode using a Rtx®-BAC1 column.
Results: Case #1: A 53 year old white male, who was a university professor. The body was a normally developed, middle aged, white male with cutaneous and visceral changes of decomposition, fluid accumulation in the thoracic cavity, and microscopic evidence of hepatic steatosis.
The concentrations of isoflurane in tissues for Case #1 are shown in the table below.

Specimen Concentration	Thoracic Cavity Fluid	Femoral Blood	Brain	Gastric Contents	Bile	Muscle	Lung	Liver
(µg/mL)	5	34	54.5	33µg/125mL	7	9	9	102

Other blood and tissue results for Case #1 were as follows:

Specimen Concentration (µg/mL)	Thoracic Cavity Fluid	Femoral Blood	Brain	Gastric Contents	Bile	Muscle	Lung	Liver
Ethanol (g/dL)	0.160	0.087	0.044	0.070	0.046	0.121	0.093	0.077
Acetaldehyde	Pos	-	-	Pos	Pos	-	-	-
Acetone	-	-	-	Trace	-	-	-	-
Ethyl Acetate	Trace	-	-	Trace	-	-	Trace	Trace
1-Propanol	Trace	-	Trace	Trace	-	Trace	Pos	Trace
1-Butanol	-	-	-	-	-	Pos	-	-

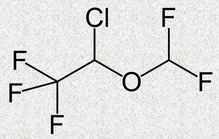
Case #2: An autopsy with toxicology testing was performed at the Office of the Chief Medical Examiner, New York, New York. Other than slight generalized visceral congestion, no findings of note were observed. There were no stigmata of intravenous drug abuse. The concentration of isoflurane was analyzed only in the cardiac blood by NMS Labs; < 3 µg/mL of isoflurane was detected. No other volatiles were found. Comprehensive toxicology screens were performed on cardiac blood, femoral blood, and urine; no other drugs were detected.
Conclusion: Case #1: Isoflurane was distributed throughout the tissues with values ranging from 5.0-102 µg/mL. This finding supported the ruling of an acute isoflurane and ethanol intoxication. The death was ruled an accident.
Case #2: The death was ruled an accidental acute isoflurane intoxication.

Keywords: Isoflurane, Volatiles, Anesthetic

Objectives

- Using headspace gas chromatography with flame ionization detection, to validate a method for isoflurane analysis that will separate, detect and quantify isoflurane in different biological matrices.
- To characterize the postmortem distribution of isoflurane in an individual who died from isoflurane exposure.

Introduction



- Isoflurane is a volatile compound used for general anesthesia[1] in dogs and horses under veterinarian care.
- Isoflurane is a clear, colorless liquid having a slight odor and is easily miscible in organic liquids[2].
- Toxicity includes respiratory depression, hypotension, cardiac arrhythmia, nausea and vomiting[1], reduction of pharyngeal reflexes and relaxation of skeletal muscles[3].
- Isoflurane should be administered with an intravenous anesthetic, because induction of anesthesia with isoflurane alone can lead to coughing and apneic periods[4].
- Isoflurane is abused for the pleasurable "high" sensation inhalation causes, which has been described as similar to that of sniffing glue.

Case #1:

- A 53 year old white male, who was a university professor.
- The decedent had a history of alcohol abuse.
- The decedent was found in a state of moderate decomposition in his home on a welfare check. He was completely wrapped in a large plastic bag with the top of his scalp exposed. Also inside the bag was a bucket containing several shoes as well as a bottle of isoflurane.
- In the house there were numerous bottles of isoflurane along with a handkerchief on a table, and a large amount of empty beer cans.

Introduction

Case #2:

- A 44 year old white male, who was employed as an anesthesiologist.
- The decedent had a history of substance abuse and depression.
- The decedent was found expired in his home and had several bottles of isoflurane in the residence. There was also a sock with a chemical smell recovered from the scene.

Materials and Methods

Materials:

- The chemicals that were used were isoflurane which was obtained from JD Medical, and n-propanol (as an internal standard) which was obtained from Burdick and Jackson.
- The Hamilton Micro-Lab-600 series pipettor-diluter was used to sample the specimens and add internal standard into headspace vials.
- An Agilent Gas Chromatograph, 6890 Plus series GC system equipped with dual FID and HP-7694 Headspace Autosampler was used to detect and quantify isoflurane. The chromatographic columns were a Rtx®-BAC1, 30 m x 0.32 mm I.D., 1.8 µm, cat. #18003, and a Rtx®-BAC2 Plus, 30 m x 0.32 mm I.D., 1.2 µm cat. #18002, (Restek Corp., Bellefonte, PA, 16823).
- An Agilent 6890 GC/5975 MS-EI GC/MS with electron impact ionization in full scan mode was used to confirm isoflurane. Confirmation was made using a Restek Rtx®-BAC1 capillary column, 30 m x 0.32 mm I.D., 1.8 µm film thickness, cat. #18003, used with helium as the carrier gas.

Methods:

- Isoflurane was immiscible in water and therefore stock solutions for the calibrators and quality controls were made in methanol. The stock solutions were then spiked into water to make the calibrators and quality controls.
- Isoflurane was analyzed using a modified version of an in-house HS/GC/FID analysis (described below). The resolved components were detected using an FID, identified by their characteristic retention time (RT) and quantified by comparing the peak area ratio for components in the samples to similar ratios generated from replicate analysis of a 6 point curve.
- The HS/GC/FID operating parameters were as follows: the injection port temperature was 250˚C; the initial oven temperature was 40˚C with an initial hold time of 1.25 minutes, a ramp rate of 12˚C per minute, a final temperature of 55˚C with a final hold time of 2.5 minutes. The total run time was 5.0 minutes. The headspace transfer line temperature was 200˚C. Sample volume 1.0 mL and 1,250 µL internal standard.

Materials and Methods

- Isoflurane was confirmed using an Agilent 6890 GC/5975 MS-EI with electron impact ionization in full scan mode. The analytes were identified by their characteristic RT and spectra. The ion source and quadruple were set at 230˚C and 150˚C, respectively, with a mass range of m/z 20-250 amu.
- The GC operating parameters were as follows: the injection port temperature was 165˚C; the initial oven temperature was 37˚C with an initial hold time of 3.00 minutes, a ramp rate of 12˚C per minute, a final temperature of 55˚C with a final hold time of 8.00 minutes. The total run time was 12.5 minutes. The chromatographic column was a Rtx®-BAC1, 30 m x 0.32 mm I.D., 1.8 µm, cat. #18003.
- The injector was operated in the split mode with a split ratio of 10:1 at a temperature of 200˚C and the injection port temperature was 165˚C. Sample volume was 600 µL with 1,250 µL internal standard. ½ cc of Headspace was manually injected using a 12.7 mm/30G BD insulin syringe.
- Isoflurane: Linearity: 5.0 - 100 µg/mL; LOQ = 5 µg/mL; correlation coefficients >0.998% and >0.988% for Rtx®-BAC1 and -BAC2 Plus respectively; %CV for intra and inter-run precision was 3.7% and 4.1% for the low QC and 4.0% and 3.1% for the high QC, respectively.
- There is partial co-elution with isoflurane and isopropanol in both Rtx®-BAC1 and -BAC2 Plus.

Results

- The distribution of isoflurane in tissues and fluids is shown in the abstract table.
- Isoflurane reportedly has a brain/blood coefficient of 1.6[4] which is consistent with the data reported here: the brain (54.5 µg/mL) was higher than in the femoral blood (34 µg/mL), ratio = 1.6.
- A comprehensive screen for other drugs revealed only ibuprofen and caffeine and a femoral blood ethanol of 0.160 g%. The presence of ethanol would have been additive with the CNS depressant effects of isoflurane.
- There have been five deaths reported from self-administration of isoflurane. Isoflurane levels from previously reported[1] lethal intoxications, and the present report, are listed in table 1. The results of this study are consistent with previous findings.

Table 1—Isoflurane Distribution in Fatality Cases (µg/mL)

Case	Blood	Brain	Lung	Liver	Kidney
#1	34	54.5	9	102	
#2	< 3				
#3[5]	9.9	107	17	31	22
#4[5]	46		35	97	27
#5[5]	48	307		1000	53
#6[6]	1.8	29		110	
#7[7]	26	46	26		

Results

- The inter-laboratory results from CCMEO and NMS Labs for the thoracic cavity fluid and the femoral blood were within 32% and 36% respectively.

Autopsy Findings:

- **Case #1:** A complete autopsy was performed at the CCMEO, Cleveland, Ohio. It consisted of an external and internal examination of the body, a microscopic examination, a toxicological examination, and a police investigation. Autopsy findings included cutaneous and visceral changes of decomposition with decomposition fluid accumulation in the thoracic cavities and microscopic examination revealed hepatic steatosis.
- **Case #2:** A complete autopsy with external and internal examination and toxicology testing was performed. There was a slight generalized visceral congestion, and no other findings were observed. There were no stigmata of intravenous drug abuse.

Conclusions

Case #1:

- Isoflurane was found throughout tissues and fluids with values ranging from 5.0 to 102 µg/mL.
- These findings were consistent with the suspicion that this was an acute isoflurane exposure. The cause of death was ruled an accidental acute intoxication by the combined effects of ethanol and isoflurane.

Case #2:

- Isoflurane was only analyzed in the cardiac blood.
- The cause of death was ruled accidental acute isoflurane intoxication.

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