

## Article

# Tissue Distribution of Suvorexant in Three Forensic Autopsy Cases

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## Abstract

Suvorexant (Belsomra®) is a relatively new insomnia medication that has been available in USA and Japan since 2014. It is a dual orexin receptor antagonist that promotes sleep by inhibiting the binding of orexin neurons to the OX<sub>1</sub>R and OX<sub>2</sub>R receptors. In this report, we describe the detection and quantitation of suvorexant from the postmortem specimens of three separate autopsy cases handled by our department. Suvorexant was identified by fast gas chromatography/mass spectrometry during routine screening, and quantitated by a fully validated liquid chromatography–tandem mass spectroscopy method. Quantitation was achieved by positive electrospray ionization in the selected reaction monitoring mode. Monitored transitions were  $m/z$  451 > 186 for quantitation and  $m/z$  451 > 104 for qualification. To our knowledge, this is the first instance of suvorexant being quantitated from actual autopsy cases. It is likely that this compound will be encountered more often by the forensic toxicology community going forward.

## Introduction

Suvorexant, also known by its trade name Belsomra®, is a relatively new sedative hypnotic distributed by Merck & Co. for the treatment of insomnia (1). Suvorexant was approved for use by the Federal Drug Administration in 2014 and released in the United States and Japan in August and November of that year, respectively (2, 3). Suvorexant is used to treat insomnia in patients by regulating the sleep–wake cycle. It is similar in usage to other insomnia medication like zolpidem (Ambien®), eszopiclone (Lunesta®) and zaleplon (Sonata®); however, its mechanism of action is quite different. Suvorexant promotes sleep through the binding inhibition of orexin A (hypocretin-1) and orexin B (hypocretin-2), neuropeptides that promote wakefulness (4). It accomplishes this by blocking the ability of these neurons to bind to the orexin-1 (OX<sub>1</sub>R) and orexin-2 (OX<sub>2</sub>R) receptors, effectively inhibiting the activation of arousal or wakefulness (5). The chemical structure, chemical formula, and molecular weight of suvorexant can be seen in Figure 1.

Suvorexant is prescribed in oral doses of between 5 and 20 mg. The onset of sleep typically occurs between 56 and 68 min after oral ingestion. Reported side-effects include somnolence, muscle weakness, abnormal dreams and headache. Its oral bioavailability

is 82% and peak plasma concentrations typically occur between 2 and 3 h after administration. Its mean volume of distribution ( $V_d$ ) has been reported as between 49 and 105.9 L, it is 99% bound to plasma proteins, and its reported half-life ( $t_{1/2}$ ) is 12 h (6). Metabolism is primarily performed by cytochrome P450 (CYP3A4), and over 10 metabolites have been reported, though none appear to be pharmacologically active (7).

To our knowledge, there have been no published reports involving suvorexant detected from human postmortem samples. Several reports regarding the analysis of suvorexant in spiked samples, human clinical studies and animal studies have been published. Analysis of suvorexant has been accomplished by gas chromatography/mass spectrometry (GC–MS), high-performance liquid chromatography–tandem mass spectroscopy (HPLC–MS/MS), ultra-performance liquid chromatography–tandem mass spectroscopy (UPLC–MS/MS), and liquid chromatography–quadrupole/time-of-flight mass spectrometry (LC–Q/TOF–MS) (8–11).

In this study, we describe the identification of suvorexant by GC–MS/MS and LC–MS–MS, the validation of an LC–MS–MS quantitation method for suvorexant, and the tissue distribution in postmortem specimens from three autopsy cases.

## Materials and Methods

### Chemicals and reagents

Suvorexant, as a solid powder in its free form, was purchased from MedChem Express (Monmouth Junction, New Jersey, USA). A methanolic solution (1 mg/mL) of diazepam- $d_5$  was purchased from Cerilliant Corporation (Round Rock, TX, USA) for use as the internal standard (IS). Acetonitrile and methanol, both HPLC-grade, were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Formic acid and *n*-propyl acetate were acquired from Kanto Chemical Co., Inc. (Tokyo, Japan). Solid tissues were homogenized using a  $\mu$ T-12 beads crusher (TAITEC, Saitama, Japan). Captiva Non-Drip (ND) Lipids filtration cartridges (3 mL) were purchased from Agilent (Santa Clara, CA, USA). Water was distilled using an Aquarius Automatic Water Distillation Apparatus (RFD230CA, Advantec, Ehime, Japan). Blank human whole blood was purchased from Biopredic International (Rennes, France). Blank urine was provided by a volunteer and tested prior to use to ensure the absence of interfering compounds.

### Preparation of calibration samples

A stock solution of 1 mg/mL of suvorexant was prepared by weighing out solid powder and dissolving in a solution of acetonitrile:distilled water (1:1). The stock solution was serially diluted in acetonitrile to prepare working stock solutions. Blood and urine calibrators were prepared by spiking 10  $\mu$ L of the appropriate working standard into 200  $\mu$ L of blank sample for final concentrations of 1, 5, 10, 25, 50, 100, 250 and 500 ng/mL. Tissue specimens were quantitated by using the standard addition method, by directly spiking into 0.2 g of homogenized case sample tissue for final concentrations of 0, 25, 50, 100, 250 and 500 ng/mL. A working IS solution was prepared by dilution of the 1 mg/mL stock solution with methanol to a concentration of 2,000 ng/mL.

### Preparation of case samples

Whole blood, urine, and tissue specimens collected at autopsy were kept frozen at  $-30^\circ\text{C}$  until analysis. The preparation method for

postmortem body fluid samples for the routine screening of drugs by fast GC-MS can be found in a previous report (12). For quantitation by LC-MS-MS, 0.2 mL of body fluid or 0.2 g of tissue was accurately weighed into a 5-mL plastic tube. Distilled water (0.2 mL) was added along with 10  $\mu$ L of the IS working solution. Solid tissues were fortified with suvorexant and homogenized in a beads crusher for 60 s at 3,200 rpm. After thorough vortexing and homogenization (tissues only), the addition of 1.2 mL of acetonitrile was added for the extraction of drugs and protein precipitation. After thorough vortexing and centrifugation, the supernatant was passed through a Captiva ND Lipids cartridge. The resulting filtrate was evaporated at  $60^\circ\text{C}$  under a stream of nitrogen until dryness. The residue was reconstituted in 0.1% formic acid in water for analysis on the LC-MS-MS system.

### GC-MS equipment and conditions

The GC-MS system was a GCMS-TQ8030 (Shimadzu, Kyoto, Japan). The column used was a tandem column consisting of a ZB-SemiVolatiles column (2 m  $\times$  0.18 mm i.d.  $\times$  0.5  $\mu$ m; Phenomenex, Torrance, CA, USA) as the pre-column coupled to a BPX5 column (4 m  $\times$  0.15 mm i.d.  $\times$  0.25  $\mu$ m; SGE Analytical Science Pty. Ltd, Melbourne, Australia) as the separation column connected by a SilTite<sup>®</sup>  $\mu$ -Union connector (SGE) (13).

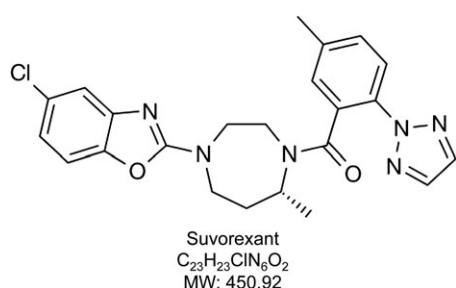
Electron impact (EI) ionization was employed at a voltage of 70 eV. The carrier gas was helium delivered at a constant flow of 2.32 mL/min. The oven temperature program was initially  $70^\circ\text{C}$  for 0.5 min, ramped to  $200^\circ\text{C}$  at  $70^\circ\text{C}/\text{min}$ , increased to  $340^\circ\text{C}$  at  $50^\circ\text{C}/\text{min}$ , and held for 4 min. The inlet temperature was  $270^\circ\text{C}$ , the interface temperature was  $300^\circ\text{C}$ , and the ion source temperature was  $230^\circ\text{C}$ . The injection was made in split mode with a split ratio of 1:22. The injection volume was one microliter and the total run time was 9.16 min.

For routine screening, mass spectral data were collected in the scan mode from  $m/z$  40 to 550 at 5,000 amu/s. For targeting by MS/MS, detection was made in the multiple reaction monitoring mode (MRM). MRM conditions for the analysis of suvorexant can be found in Table I.

### LC-MS-MS equipment and conditions

The LC system was a Prominence liquid chromatograph (Shimadzu, Kyoto, Japan) with a binary pump and integrated degasser, autosampler, and heated column compartment. The detection system was a TSQ Quantum Access MAX tandem mass spectrometer (Thermo Scientific, Waltham, MA, USA). The column utilized was a Hypersil GOLD PFP column (50 mm  $\times$  2.1 mm i.d., particle diameter of 0.5  $\mu$ m; Thermo Scientific, Waltham, MA, USA).

The column temperature was set at  $40^\circ\text{C}$  and the injection volume was 10  $\mu$ L. The mobile phase consisted of a gradient between 0.1% formic acid in water (mobile phase A) and 0.2% formic acid in acetonitrile (mobile phase B). The gradient was 5% B for 5.5 min,



**Figure 1.** Chemical structure of suvorexant.

**Table I.** Chromatographic separation and mass spectrometry conditions for suvorexant

GC-EI-MS (common method)		GC-EI-MS (fast method)		GC-EI-MS-MS (fast)				LC-ESI-MS/MS			
Rt (min)	Prominent ions ( $m/z$ )	Rt (min)	Prominent ions ( $m/z$ )	Rt (min)	Precursor ion ( $m/z$ )	Product ions ( $m/z$ )	Collision energy (eV)	Rt (min)	Precursor ion ( $m/z$ )	Product ions ( $m/z$ )	Collision energy (eV)
23.7	186, 104, 221, 450	5.25	186, 104, 221, 450	5.25	186	104 77	18 24	5.75	451	186 104	26 54

linear ramp to 100% B at 8.5 min, and held at 100% B for 4 min. The flow was set at 0.2 mL/min and the eluate was delivered to the MS/MS by electrospray ionization in the positive mode. The total run time was 15 min.

To determine the appropriate transitions to monitor for detection in the selected reaction monitoring (SRM) mode, a shooting standard of suvorexant at a concentration of 1 µg/mL in mobile phase was infused into the MS/MS for determination of the optimal precursor and product ions and their respective collision energies. The optimum transitions and collision energies for suvorexant can be found in Table I. The SRM transitions determined by this method were consistent with the MS/MS conditions reported by publications referenced in this article (9–11).

### Method validation

The LC–MS–MS quantitation method used in this report was validated with recommended guidelines (14). Bias and precision were assessed by the analysis of quality control (QC) samples in blank whole blood and blank urine prepared at concentrations of 3, 100 and 400 ng/mL measured in triplicate over five different runs. Acceptable results were bias and precision (coefficient of variation, CV) within  $\pm 20\%$ . The calibration model was evaluated by the least-squares linear regression method over five different runs. Carryover was assessed by analyzing blank matrix extracts following the injection of the highest calibration level (500 ng/mL). Endogenous matrix interference was evaluated by extracting 10 different whole blood and urine sources that were negative for suvorexant and the IS. To evaluate interferences from other commonly encountered analytes, blank whole blood and urine samples were spiked with 20 commonly encountered drugs (7-aminoflunitrazepam, acetaminophen, caffeine, carbamazepine, codeine, dextromethorphan, diazepam, fluoxetine, methamphetamine, methadone, nordiazepam, oxazepam, phenobarbital, pseudoephedrine, salicylic acid, temazepam, tramadol, trazodone, valproic acid and zolpidem) at 1,000 ng/mL. Positive controls containing 100 ng/mL of suvorexant and IS, and negative controls containing no suvorexant or IS, were evaluated against the spiked samples to determine interferences. Ionization suppression/enhancement was assessed by a post-extraction addition approach by comparing 10 different blood and urine samples extracted in duplicate and spiked with suvorexant and IS post evaporation at 20 and 200 ng/mL. Ten samples of blank mobile phase were spiked at the same concentrations. Matrix effect (ME) was assessed by comparing the peak area ratios (analyte/IS) for the extracted whole blood/urine and the spiked mobile phase. Acceptable results were ME and CV values of less than 25% and 15%, respectively. Extraction recovery was measured by comparing the peak areas of suvorexant from samples extracted from spiked whole blood/urine to non-extracted samples at 100 ng/mL. Limit of detection (LOD) and limit of quantitation (LOQ) were determined by analyzing suvorexant spiked into three blank whole blood and urine samples from different sources at decreasing concentrations processed in three separate analyses. The LOD was determined to be the lowest concentration that produced a peak with a signal to noise ratio of greater than 3:1 at the expected retention time. The LOQ was determined to be the lowest concentration that produced a peak with a signal to noise ratio of at least 10:1 at the expected retention time and was within  $\pm 20\%$  of the spiked concentration. Stability was measured by reanalyzing the QC samples after storage at 4°C at 6, 12, 18 and 24 h.

### Application to Actual Autopsy Cases

Between October of 2016 and August of 2017, the Department of Forensic Medicine at Fukuoka University encountered three autopsy cases involving suvorexant.

#### Case 1

A female in her early forties was found washed up on a beach. She had a history of depression and was prescribed several medications, including Belsomra®. An autopsy was conducted soon after her body was discovered. Autopsy findings revealed that water and white foam were present in the airway and the lungs displayed oedema aquosum. There were no other remarkable wounds found on the body. Diatoms found in the lungs, liver and kidneys were consistent with diatoms from the water near where she was found. Toxicology results revealed the presence of several prescription medications in the blood. The medications, excluding suvorexant, and their concentrations in cardiac blood can be found in Table II. Olanzapine, quetiapine, 7-aminoflunitrazepam and flunitrazepam were detected. Ethanol in the blood was negative. The cause of death was determined to be drowning.

#### Case 2

A male in his mid-50s was found dead lying prone in his bedroom. He had many pharmaceutical prescriptions, including Belsomra®. The decedent was autopsied soon after his death was discovered. Autopsy findings revealed no remarkable wounds, however, the oral cavity, esophagus, tracheobronchi and stomach all contained a bluish-green substance. Rohypnol®, a benzodiazepine hypnotic used to treat insomnia and infamous for its intense blue-green color once dissolved, was among the medications prescribed to the deceased. Ethanol was not detected, but toxicology testing revealed many drugs present in the blood and urine. The 15 other drugs, excluding suvorexant, and their

**Table II.** Toxicology results from the three cases in this report, excluding suvorexant

Compound name	Case 1 Cardiac blood ng/mL	Case 2 Cardiac blood ng/mL	Case 3 Cardiac blood ng/mL
7-Aminoflunitrazepam	27	39	
Biperiden		8	
Bisoprolol			21
Chlorpromazine		159	
N-Desalkylflurazepam			327
Diazepam		58	
Etizolam		6	
Flunitrazepam	<5	<1	
Ketoprofen		<1	
Nateglinide		68	
Nifedipine		285	
Olanzapine	776		
Oxazepam		3	
Promethazine		999	
Quetiapine	150		
Risperidone		55	
Spironolactone		22	
Triazolam			10
Valsartan		1,070	
Zotepine		106	

concentrations in cardiac blood can be found in Table II. Toxic levels of promethazine and elevated levels of nifedipine, chlorpromazine and zotepine were detected in the blood and urine. Due to the number of medications, some of which were at toxic or elevated levels, and a lack of other relevant autopsy findings, the cause of death was determined to be combined drug intoxication.

### Case 3

A male in his 80s was discovered by his son who came home and found him incompletely suspended by his neck from an extension cord in a workroom. The deceased had become blind from diabetes ~10 years ago. He had a history of acute myocardial infarction 25 years ago. At the time of death, he had been visiting the hospital regularly for diabetes and anxiety. Belsomra®, triazolam and other medications were prescribed. An autopsy was performed less than 24 h of the discovery of the body. Autopsy revealed a deep, distinct furrow encircling the neck with subcutaneous bleeding and a

fracture of the upper left corner of the thyroid cartilage. Ethanol in the blood was 0.02 mg/mL. The concentrations in cardiac blood of the other drugs found in his system are shown in Table II. The manner and cause of death was suicide by hanging.

## Results and Discussion

### Results of GC-MS(/MS) screening

The chromatogram and spectra from the fast GC-MS screening of suvorexant from the right heart blood of Case 1 can be seen in Figures 2 and 3, respectively. The low concentration of suvorexant in Case 2 made screening in the scan mode difficult, so an MRM mode method was developed to improve the sensitivity (Figure 4). In the fast GC-MS analysis utilized by our department, the retention time for suvorexant was 5.25 min. Suvorexant has a high boiling point (669°C) and is highly retained using gas chromatography (11). To demonstrate the high degree of retention, a shooting standard of

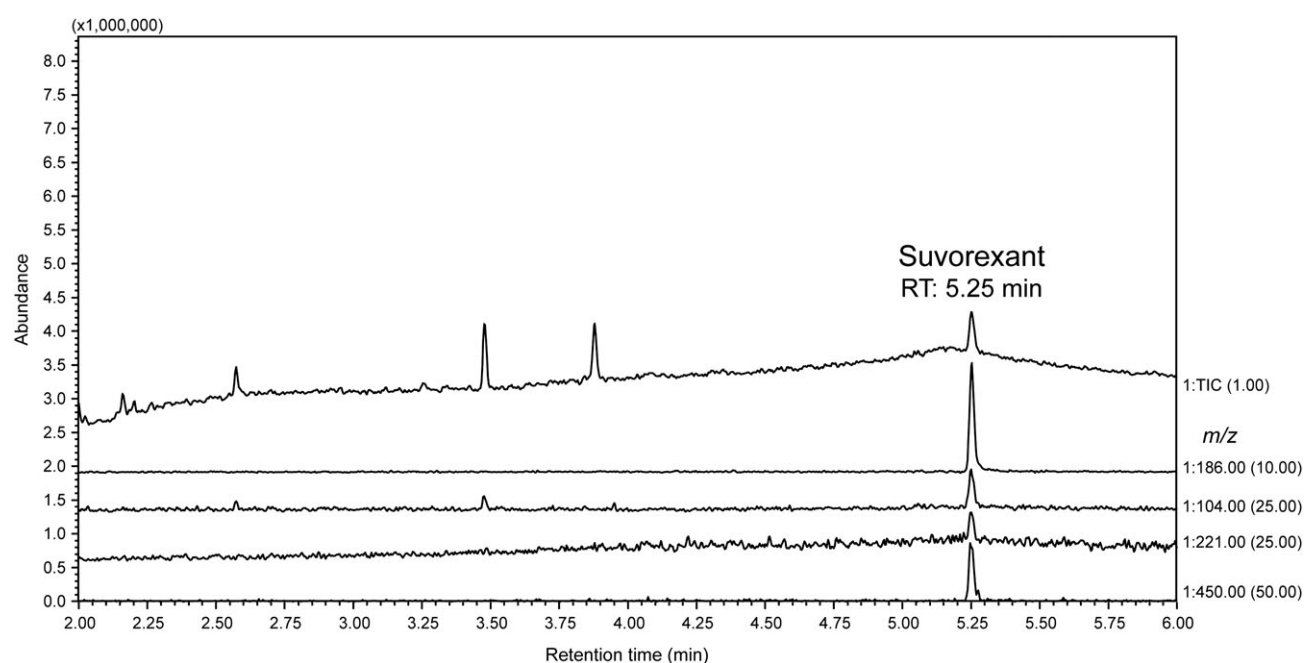


Figure 2. Fast GC-EI-MS extracted ion chromatogram of suvorexant in the right heart blood of Case 1.

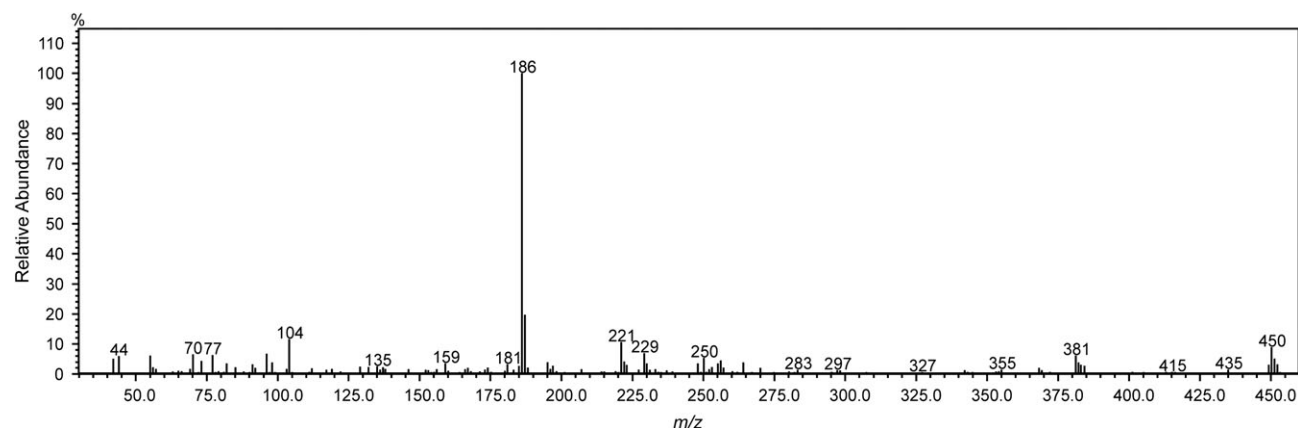
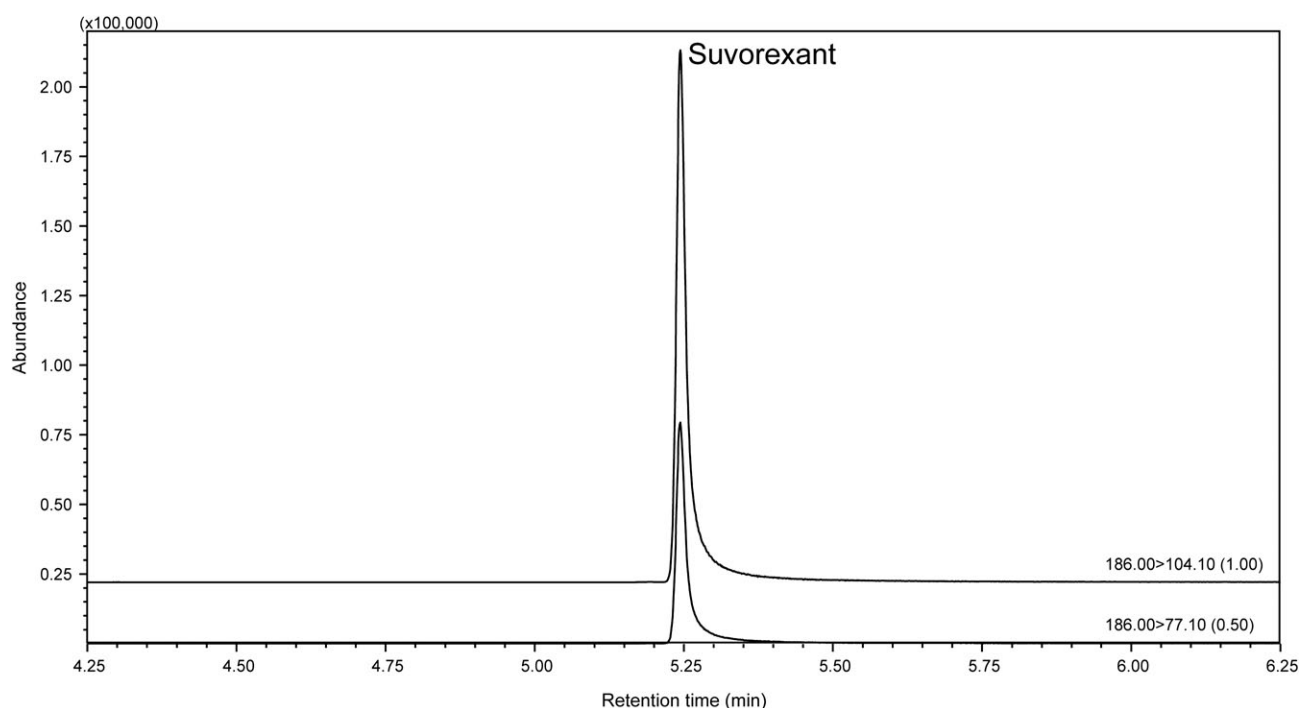
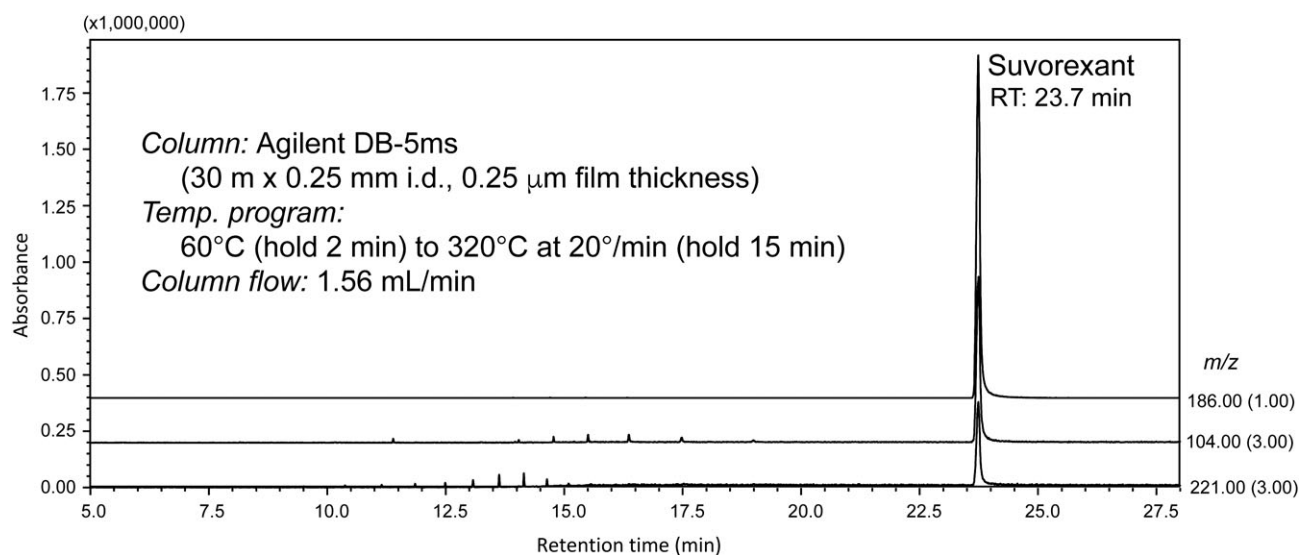


Figure 3. Full scan EI mass spectrum for suvorexant.



**Figure 4.** Fast GC-EI-MS/MS MRM chromatogram of suvorexant from the right heart blood of Case 2.



**Figure 5.** GC-EI-MS chromatogram of suvorexant using common screening conditions.

suvorexant (10  $\mu\text{g/mL}$ ) was analyzed using conditions that are commonly used for GC-MS screening. The resulting chromatogram can be seen in Figure 5. With a retention time of 23.7 min, this compound could easily be missed if data acquisition were not long enough or if the analyst did not know to search for it.

### Results of LC-MS-MS method validation

The LC-MS-MS product ion spectrum and chromatogram for suvorexant can be seen in Figures 6 and 7, respectively. A summary of the bias and precision of the quantitative method can be seen in Table III.

The within-run CVs for blood and urine were 3–16% and 2–15%, respectively. The between-run CVs for blood and urine were 3–12% and 3–15%, respectively. Linear calibration curves were constructed using spiked blank whole blood or urine, or standard addition by spiking directly into the tissue homogenates. The average coefficient of determination ( $R^2$ ) for the blood and urine curves were 0.995 and 0.997, respectively. The  $R^2$  for the standard addition curves were >0.992 (0.992–0.996). Carryover was not observed following the highest calibrator containing 500 ng/mL of suvorexant. Post-extraction addition for whole blood and urine was used to determine ME. For whole blood, the mean ME was –13 and –12% at 20 and 200 ng/mL,

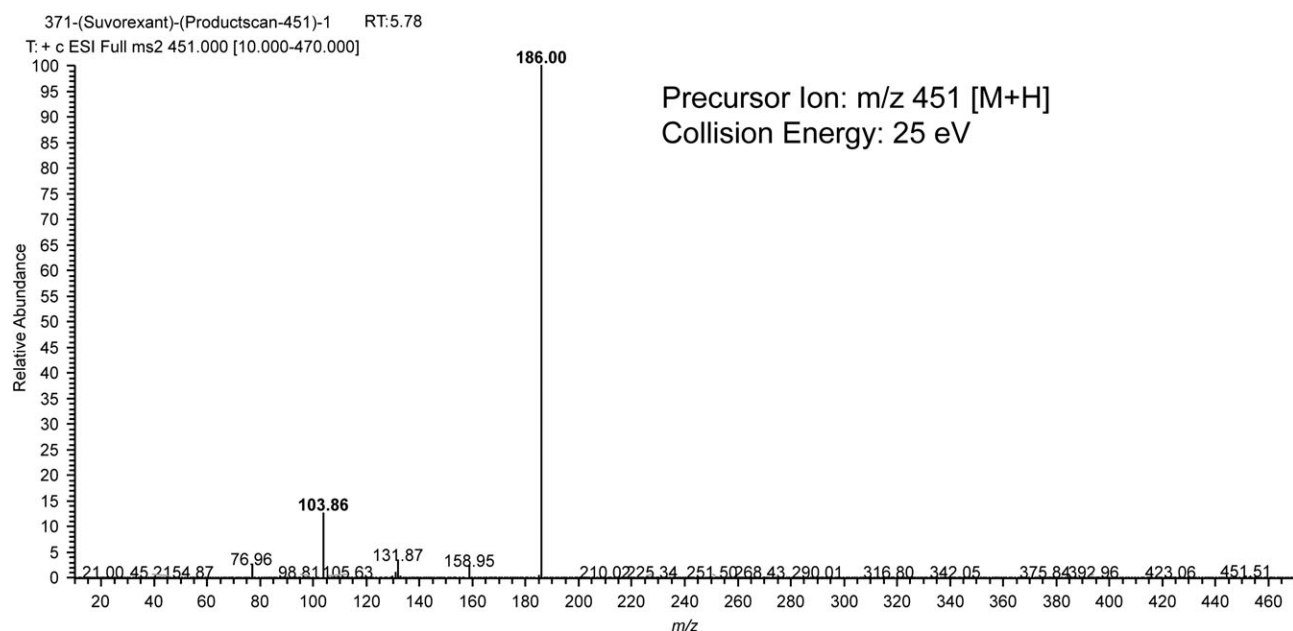


Figure 6. LC-ESI-MS/MS (SRM) product ion spectrum of a suvorexant shooting standard.

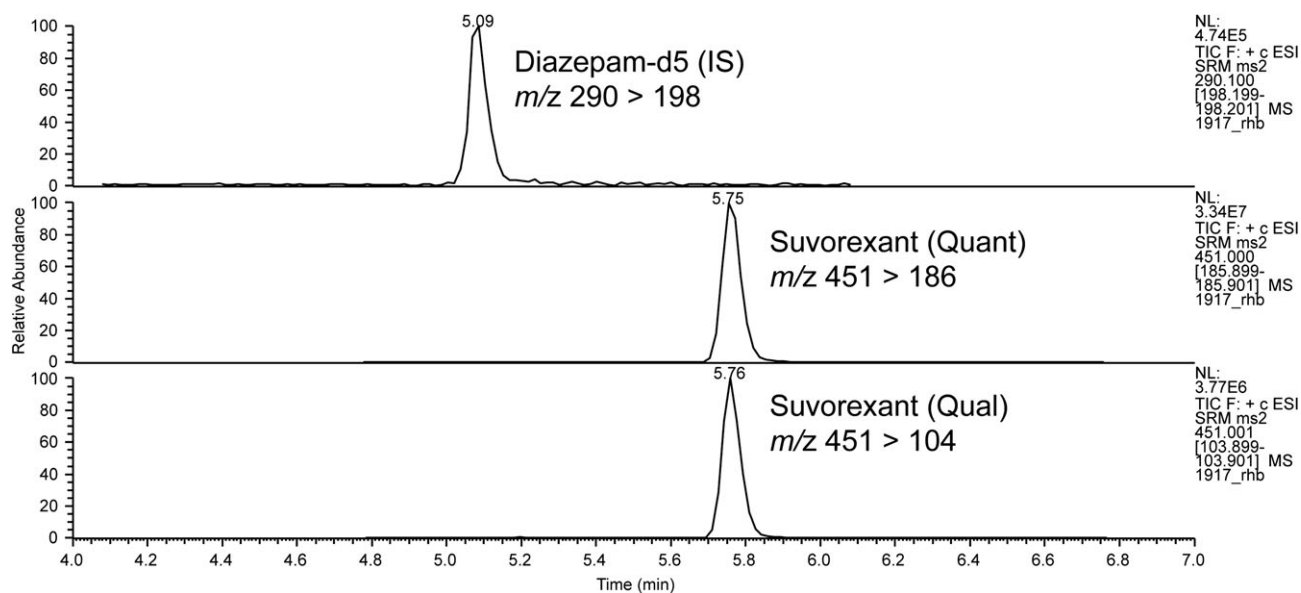


Figure 7. LC-ESI-MS/MS (SRM) chromatogram of suvorexant.

Table III. Bias and precision of the quantitative method

	Nominal concentration (ng/mL)	Calculated concentration (ng/mL, mean $\pm$ SD)	Bias ( $n = 15$ ) (%)	Within-run precision ( $n = 5$ ) (%)	Between-run precision ( $n = 15$ ) (%)
Whole blood	3	2.9 $\pm$ 0.36	-3	6	12
	100	97.7 $\pm$ 10.3	-2	16	11
	400	401.7 $\pm$ 12.0	0.43	3	3
Urine	3	3.2 $\pm$ 0.36	7	2	11
	100	99.5 $\pm$ 14.6	-0.5	15	15
	400	401.3 $\pm$ 12.6	0.33	2	3



respectively. The CV in whole blood was 5 and 3% at 20 and 200 ng/mL, respectively. For urine, the mean ME was -17 and -11% at 20 and 200 ng/mL, respectively. The CV in urine was 13 and 5% at 20 and 200 ng/mL, respectively. The average extraction recovery in whole blood and urine over five separate analyses at 100 ng/mL was  $83 \pm 7\%$  and  $92 \pm 6\%$ , respectively. These are comparable to other recent publications (9–11). There were no interferences observed from either blank matrix or from 20 commonly detected analytes spiked into either matrix. LOD was 0.5 ng/mL in both whole blood and urine. LOQ was 1 ng/mL for both whole blood and urine. Stability at 4°C was confirmed after 6, 12, 18 and 24 h (92–99%).

### Quantitation results

The concentrations of suvorexant in the body fluids and tissues from all cases can be seen in Table IV. The concentration of suvorexant in the blood sources from Case 1 (421–491 ng/mL) suggested a level higher than the peak plasma levels reported from the recommended 10 or 20 mg oral dose (15–18). Sun *et al.* (16) studied the pharmacokinetics of suvorexant in healthy young men and reported that an oral dose of 50 mg resulted in an average peak plasma concentration of 392 ng/mL (range: 275–559 ng/mL). Based on police records, the decedent in Case 1 was prescribed 20 mg tablets of Belsomra®, with instructions to take one tablet before bedtime in cases of insomnia.

The decedent in Case 2 was also prescribed 20 mg tablets of Belsomra® to be taken once nightly. The suvorexant levels in the blood from Case 2 (11–17 ng/mL) were well below the reported peak plasma levels in any of the clinical studies that have been published. The concentration of suvorexant in the specimens of this case were much lower than in Case 1 (Table IV). A total of 16 drugs were found in the decedent's postmortem blood, some at toxic levels, and thus the death was reported as combined drug intoxication.

The decedent in Case 3 also had a prescription for 20 mg tablets of Belsomra® to be taken once nightly. The concentration of suvorexant in the blood of this case (138–155 ng/mL) were in between those of Cases 1 and 2 (Table IV). The cause of death in this case was determined to be hanging.

### Discussion of tissue distribution

To our knowledge this is the first report of suvorexant being found in postmortem samples. There are many factors that influence the discussion of postmortem toxicology findings in comparison to clinical studies. Interpretation of postmortem drug levels are often subject to postmortem

artifacts, postmortem redistribution (PMR), pharmacogenomics and other factors (19). Thus, it is difficult to speculate on the amount of suvorexant consumed in these cases, and the effect on the deceased. More studies on suvorexant in postmortem samples are needed.

The distribution of suvorexant in the specimens of the three cases can be found in Table IV. Case 1 exhibited the highest levels of suvorexant, with the highest concentrations in the blood samples, followed by the fat, kidney and liver. Case 3 showed the highest level of suvorexant in the fat, followed by the kidney, liver, femoral vein blood and cardiac blood. Case 2 displayed the lowest concentration overall, with the highest level found in the lung, followed by the left heart blood, right heart blood and femoral blood. While it is difficult to identify any significant pattern, it appears the drug readily accumulates in the blood, fat, kidney and liver. Blood/plasma (b/p) ratios have yet to be experimentally examined in humans, but with such high plasma protein binding (99%), it is reasonable to assume the b/p ratio would be around 0.5 or so (20). Plasma is often difficult to extract from postmortem whole blood, which was the case with these samples. Suvorexant does not appear to exhibit marked PMR; central/peripheral (C/P) ratios ranged from 0.89 to 1.36 in these three cases. The  $V_d$  of suvorexant has been reported in several of the sources referenced in this publication, but some of them seem to disagree. Yang *et al.* reports the  $V_d$  as 49 L, while Bennett *et al.* reports it as 105.9 L, and Sullinger *et al.* as 49 L/kg (2, 4, 11). The  $V_d$  is highly associated with the PMR that would be demonstrated in postmortem cases. For example, a  $V_d$  of 49 L/kg would be considered extremely high, and one would expect the result to be a high level of PMR. This is not what we saw in these cases, particularly with Case 1, where the highest levels were in the blood sources, and the C/P levels were close (right heart blood to femoral blood, 1.08). In Case 2, the highest levels were in the fat, kidney and liver, followed by the blood sources (C/P: 1.36). This would suggest a slighter higher level of PMR, but still not to the degree that 49 L/kg would predict. It is our estimation that the true value of  $V_d$  is somewhere in the range of 49–105.9 L, which is also more in agreement with the value reported in Baselt's most recent edition of "Disposition of Toxic Drugs and Chemicals in Man" (11th edition, 2017), which lists the  $V_d$  as a range from 0.5 to 0.9 L/kg (20).

### Conclusion

A relatively new insomnia medication, suvorexant, was found in three recent forensic autopsy cases. Screening and confirmation was achieved by fast GC-MS and fast GC-MS-MS. A reference standard was procured, and quantitation performed on all collected specimens by a validated LC-MS-MS quantitation method. Although numerous metabolites have been identified in previous reports, none were detected in the GC-MS screening of this case. The lack of commercially available metabolites also made targeting of these compounds by LC-MS-MS prohibitive.

Suvorexant is similar to other sleep aids such as zolpidem, and may be found in more cases in Japan in the near future. It is possible that suvorexant may be used in cases of drug-facilitated sexual assault (DFSA), as is the case with zolpidem and other hypnotics. The forensic medicine and toxicology communities should be aware of and on the lookout for this compound in postmortem samples.

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**Table IV.** Distribution of suvorexant in three autopsy cases

Specimen	Case 1 ng/mL (g)	Case 2 ng/mL (g)	Case 3 ng/mL (g)
Right heart blood	455	15	138
Left heart blood	491	17	N/A
Left femoral blood	421	11	155
Urine	N/A	<1	10
Liver	201	6	158
Kidney	280	4	232
Spleen	36	<1	31
Pancreas	55	1	24
Lung	122	40	41
Muscle	93	<1	30
Fat	359	5	278

N/A = not available.

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