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Quantification of suvorexant in urine using gas chromatography/mass spectrometry

Mariah Carson, Sarah Kerrigan*

Sam Houston State University, Department of Forensic Science, 1003 Bowers Blvd., Huntsville TX, 77341, United States

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ABSTRACT

Suvorexant (Belsomra®) is a novel sedative hypnotic drug that is prescribed to promote sleep in patients with insomnia. It is the first of a new class of drugs classified as dual orexin receptor antagonists (DORAs). Sedative hypnotics with central nervous system depressant effects feature prominently in forensic toxicology investigations. For this reason, a new analytical method was developed to identify suvorexant in urine using liquid-liquid extraction (LLE) and gas chromatography/mass spectrometry (GC/MS). Due to the absence of a commercially available isotopically labeled internal standard, estazolam-D5 was used due to its azepine, triazole and chlorinated functionality. The limit of detection and limit of quantitation was 10 ng/mL and the linear range of the assay was 10–1000 ng/mL. Accuracy and precision (%CV) were 98–101% and <11% at 30, 250 and 800 ng/mL. Interferences from matrix and fifty common drugs were not present and processed samples were stable for 24 h at room temperature. Suvorexant is a new drug of significant forensic interest due to its hypnotic and central nervous system depressant effects. The absence of commercially available metabolites and its chromatographic properties present some challenges in terms of identification. Nevertheless, a robust, reliable and sensitive assay was developed to identify suvorexant using GC/MS analysis.

1. Introduction

Insomnia is a common sleep disorder reported to affect 30% or more of the adult population [1–3]. Complaints typically consist of difficulty falling asleep, maintaining sleep or experiencing restorative sleep. Pharmacological therapies include the use of gamma-amino butyric acid (GABA) modulators, including benzodiazepines (e.g. estazolam, flurazepam, triazolam) and non-benzodiazepine-type drugs (e.g. zolpidem, zolpiclone, zaleplon), sedating antidepressants (e.g. trazodone) and antihistamines (e.g. diphenhydramine, doxylamine). In February 2015 Merck released suvorexant (Belsomra®), the first of a new category of sleep aids with a novel mechanism of action.

Suvorexant (MK-4305) is a potent and selective dual orexin (hypocretin) receptor (OX1R and OX2R) antagonist. Orexin A and B are neuropeptides that are produced by neurons in the lateral hypothalamus that are known to regulate wakefulness. Just as the loss of orexin neurons results in narcolepsy, suvorexant promotes sleep by inhibiting the wakefulness-promoting orexin neurons of the arousal system [4–6].

Suvorexant was approved by the Food and Drug Administration in August 2014 and was placed in Schedule IV of the Federal Controlled Substances Act shortly thereafter. The manufacturer's recommended dose is 10 mg, 30 min prior to sleep. Studies have shown that the onset of sleep typically occurs within an hour and peak plasma concentrations (250–300 ng/mL) occur within 2–3 h of a single 10 mg oral dose [7]. The drug is highly lipophilic, having a volume of distribution (V_d) of 49 L/kg), is extensively protein bound (99%) and has an oral bioavailability of 82% [3,4,8].

CYP3A4 and to a lesser extent CYP2C19 have been identified as the principal enzymes responsible for the oxidative metabolism of the drug [8]. Proposed metabolic routes involve hydroxylation and carboxylation with subsequent glucuronidation. Although the metabolites are reported to be pharmacologically inactive, none are commercially available as reference standards, so only the parent drug can be targeted in forensic toxicological analyses. Suvorexant is reported to be eliminated predominantly in the feces (66%) rather than the urine (23%) [5]. Half-lives for the drug have been reported between 8 and 19 h and following chronic daily dosing, plasma concentrations are reported to reach steady state within approximately three days [4,9]. Following administration of a 10 mg dose,

* Corresponding author at: Department of Forensic Science, Sam Houston State University, Box 2525, 1003 Bowers Blvd., Huntsville, TX, 77341, United States.
E-mail address: sarah.kerrigan@shsu.edu (S. Kerrigan).

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peak plasma concentrations were >200 ng/mL and the drug was detectable for several days after use [7,9].

To date there have been no published forensic toxicology case reports involving suvorexant. However, given the prevalence of sleep disorders and the extent to which other hypnotics and sedative drugs feature in death and human performance toxicology investigations (notably impaired driving and drug-facilitated sexual assault), suvorexant must be considered. However, the absence of published analytical methods presents a challenge to forensic toxicology laboratories faced with ever increasing demands due to the proliferation of new psychoactive substances in recent years.

According to the Scientific Working Group for Toxicology (SWGTOX), methods used for forensic toxicology purposes should be validated in accordance with established guidelines [10]. To date there has been only one published report describing the analysis of suvorexant in biological fluids. In this report from the drug manufacturer, Breidinger described the analysis of suvorexant in plasma using a 96-well liquid-liquid extraction with liquid chromatography/mass spectrometry (LC/MS/MS) detection. In this report we describe the analysis of suvorexant in urine using gas chromatography/mass spectrometry (GC/MS), which is still the most widely used analytical technique in forensic toxicology laboratories.

Suvorexant was isolated from urine using liquid-liquid extraction and identified using GC/MS and selected ion monitoring (SIM). The method was validated in accordance with the Scientific Working Group for Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology. Deuterated analogs of suvorexant are not yet commercially available. For this reason, estazolam-D5 was selected as the internal standard (IS) because it incorporates some structural features common to suvorexant (a phenyl-1,2,3-triazole), notably the azepine/azepane 7-membered rings, heterocyclic triazole and chlorine moieties) highlighted in Fig. 1.

2. Experimental

2.1. Chemicals and reagents

Methanol was purchased from J.T. Baker (Center Valley, PA). Sodium acetate, glacial acetic acid, ether and toluene were purchased from Mallinckrodt (Phillipsburg, NJ). Sodium fluoride was purchased from (Anachemia, Rouses Point, NY). All solvents were LC-grade and all chemicals were AR grade or higher. Suvorexant ([(7*R*)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl][5-methyl-2-(2H-1,2,3-triazol-2-yl)phenyl]methanone) was purchased in powdered form from Adooq Bioscience (Irvine, CA). Estazolam-D5 and the following drugs that were utilized in the interference study were purchased from Cerilliant Corp. (Round Rock, TX): 7-aminoclonazepam, 7-aminoflunitrazepam, acetaminophen, alprazolam, amitriptyline, amobarbital, amphetamine, buproprion, butalbital, caffeine, carbamazepine, carisoprodol, clonazepam, cocaine, codeine, cyclobenzaprine, dextromethorphan, diazepam, fluoxetine, flurazepam, gabapentin, hydrocodone, hydromorphone, ketamine, methylenedioxymethamphetamine (MDMA), meperidine, meprobamate, methadone, methaqualone, morphine, nordiazepam, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenobarbital, phenytoin, propoxyphene, pseudoephedrine, salicylic acid, secobarbital, sertraline, temazepam, delta-9-tetrahydrocannabinol (THC), carboxy-THC, tramadol, trazodone, valproic acid and zolpidem. Drug standards were purchased as methanolic solutions and diluted to appropriate concentrations in solvent. Working standards of suvorexant (0.01 mg/mL and 0.001 mg/mL) and estazolam-D5 (0.01 mg/mL) were routinely used for the preparation of calibrators and controls. Liquid-liquid extraction (LLE) was achieved using a mixture of ether/toluene (1:1) and sodium acetate buffer (pH 3.6, 0.4 M). Certified drug-free urine was purchased from Utak Laboratories (Valencia, CA). Additional urine samples required for interference studies were collected from healthy drug-free volunteers. Urine samples were refrigerated and preserved with 1% (w/v) sodium fluoride.

2.2. Extraction

Suvorexant was isolated from urine using a simple acidic/neutral liquid-liquid extraction. Internal standard solution was added to urine (2 mL) to achieve a final concentration of 250 ng/mL. Following the addition of 2 mL sodium acetate buffer (pH 3.6, 0.4 M), 5 mL of ether/toluene 1:1 was added. The samples were gently mixed on a rotary mixer for 10 min and centrifuged at 3000 rpm for 5 min. The organic layer was transferred to a clean glass conical vial and evaporated to dryness under nitrogen at 50 °C. Extracts were reconstituted in methanol (20 μL) and transferred to autosampler vials for analysis.

2.3. Instrumentation

An Agilent 7890A Gas Chromatograph equipped with an Agilent 5975C Mass Selective Detector (Santa Clara, CA) was used for instrumental analysis. A 30 m × 0.25 mm DB5-MS column with a 0.1 μm film thickness was used. A total of 2 μL of sample was injected onto the GC with a 10:1 split ratio. The inlet temperature was 280 °C and the temperature program was as follows: Initial temperature 260 °C (0.1 min), increased to 290 °C at a rate of 30 °C/min and a final hold time of 16 min. The total run time was 17.1 min. Twelve pre- and post-injection washes (in methanol) were performed between injections. Data was acquired using SIM analysis following a 2.4 min solvent delay. Estazolam-D5 (*m*/*z* 210.1 (73%); 264.1 (103%); **299.1** (100%)) were acquired using a dwell time of 50 ms and suvorexant (*m*/*z* 104.1 (17%); **186.1** (100%); 450.2 (13%)) were acquired using a dwell time of 75 ms. Quantitation ions are shown in bold and ion ratios are shown in parentheses. Ion ratio acceptance was considered to be ±20% (relative) for all ions except lower intensity ions (*m*/*z* 450 and 104), which utilized an ion ratio acceptance of ±5% (absolute).

2.4. Assay validation

Extraction efficiency was determined by comparing the relative peak area (suvorexant/IS) of extracted and non-extracted samples in replicate. Urine fortified with internal standard (250 ng/mL) was supplemented with suvorexant (100 ng/mL) pre- (N = 6) and post (N = 6) extraction. Limits of detection (LOD) and quantitation (LOQ) were established empirically by fortifying drug-free urine with suvorexant at successively lower concentrations. Three independent sources of urine were used, each analyzed in duplicate over three runs (N = 18) at each concentration tested. The LOD was defined as the lowest concentration of drug to produce a signal-to-noise (S/N) ratio of 3:1 or more, a relative retention time within 2% of the expected value and ion ratios within acceptable limits. The LOQ was defined as the lowest concentration of drug to produce a signal-to-noise (S/N) ratio of 10:1 or more, a relative retention time within 2% of expected, ion ratios within acceptable limits and a calculated concentration within 20% of the true value.

The calibration model was established using urine fortified with 10, 50, 100, 250, 500, 750 and 1000 ng/mL suvorexant over five independent runs. Accuracy and precision were evaluated at low, medium and high concentrations (30, 250 and 800 ng/mL, respectively) using three urine samples from independent sources, each extracted in duplicate over five days. Processed sample stability