Journal of Chromatography B 1091 (2018) 87–95

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Quantification of suvorexant in blood using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry

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ARTICLE INFO

Keywords:
Suvorexant
LC-Q/TOF-MS
Blood
Forensic toxicology

ABSTRACT

Suvorexant is a novel drug for the treatment of insomnia that is marketed under the trade name Belsomra®. Unlike other hypnotics, suvorexant is a dual orexin receptor antagonist that is believed to have a lower abuse potential compared to other therapeutics. Although sedative hypnotics feature prominently in forensic toxicology investigations, there have been limited reports that describe the analysis of suvorexant in biological samples. Following a 10-mg oral dose, peak concentrations are typically < 200 ng/mL. A highly sensitive assay is required because forensic toxicology laboratories are often required to identify a drug several hours after a single dose. A new analytical procedure for the quantification of suvorexant in whole blood was developed that will aid in the identification of this new drug in forensic toxicology casework. A simple acidic/neutral liquid-liquid extraction (LLE) was used to isolate suvorexant from whole blood followed by liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry analysis using positive electrospray ionization (ESI). The extraction efficiencies of various solvents in blood were evaluated in addition to limit of detection, limit of quantitation, precision, accuracy and bias, calibration model, matrix effects, interferences, and carryover. The recovery of suvorexant was evaluated using four different extraction solvents (*N*-butyl chloride, ether/toluene (1:1), hexane/ethyl acetate (9:1), and methyl *tert*-butyl ether (MTBE). Although no significant differences in analytical recovery were observed, *N*-butyl chloride demonstrated improved reproducibility, efficiency and convenience. A weighted (1/x) quadratic calibration model was selected over a range of 2–200 ng/mL (R^2 = 0.995). Using only 0.5 mL whole blood, limits of detection and quantification were 0.5 ng/mL. Intra-assay (n = 5) and inter-assay (n = 15) precision (% CV) were ≤ 13% and bias ranged from −5 to 2% at concentrations of 5, 50, and 160 ng/mL. Matrix effects were 16% (9% CV) and 15% (8% CV) for 20 ng/mL and 100 ng/mL (n = 20), respectively. No qualitative interferences or carryover were observed; however, a quantitative interference with the internal standard (estazolam-D5) could be attributed to sertraline when present at a 10-fold higher concentration. In the absence of a commercially available deuterated internal standard, the potential for quantitative interferences using LC-based methods are discussed.

1. Introduction

Insomnia is a prevalent medical condition that affects approximately one-third of the adult population in America and can be described as difficulty falling asleep, staying asleep, or experiencing non-restorative sleep [1,2]. Insomnia has been associated with depression, anxiety disorders, irritability, inability to concentrate, and a general diminished quality of life [2]. Most commonly, pharmacological interventions include the use of benzodiazepines and other drugs that modulate the gamma-amino butyric acid (GABA) receptor. However, these receptor agonist hypnotics can stimulate GABA and may pose consequences such as rebound insomnia, next-morning sedation, amnesia, potential for abuse, and physical dependence [3].

Suvorexant, also known as MK-4305, is a novel drug that is used for the treatment of insomnia [4]. Suvorexant is marketed under the trade name Belsomra® and is manufactured by Merck & Co. as a dual orexin receptor antagonist (DORA). In August 2014, the Food and Drug Administration approved suvorexant and in February of 2015 it became commercially available. Currently, suvorexant is listed under Schedule IV of the Controlled Substances Act [5]. Its mechanism of action is unique from other clinically approved hypnotic drugs, because it affects the activity of orexin neurons in the lateral hypothalamus, which are thought to play a key role in the regulation of wakefulness [6]. As a dual orexin receptor agonist, suvorexant blocks both OX_1R and OX_2R receptors which promote sleep by inhibiting orexin A and B. Thus, suvorexant aids in the transition from wakefulness to sleep, and has no

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https://doi.org/10.1016/j.jchromb.2018.05.006
Received 27 March 2018; Received in revised form 29 April 2018; Accepted 7 May 2018
Available online 25 May 2018

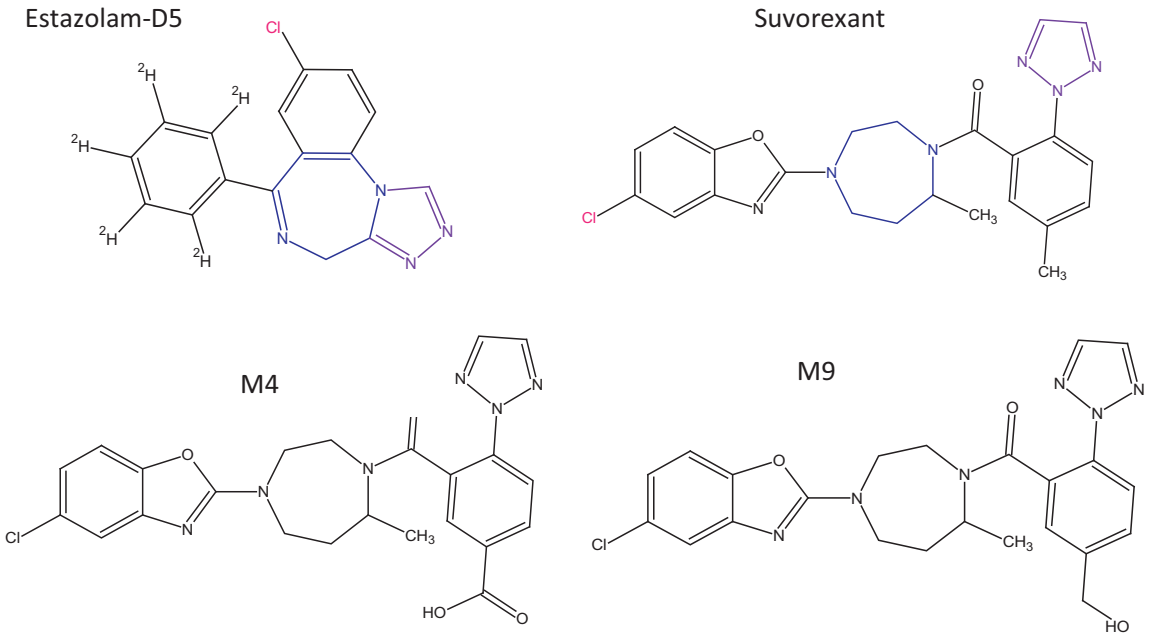

Fig. 1. Chemical structures of suvorexant and its carboxylated and hydroxylated metabolites, M4 and M9, respectively. Estazolam-D5 was selected as the internal standard due to its structural similiarities to suvorexant. These include a 7-membered azepane/azepine ring, a heterocyclic triazole, and a chlorine.

effect on GABA receptors. This drug provides alternative treatment options for insomnia, and is reported to have a lower potential for addiction compared to existing therapeutics [7].

Suvorexant should be administered within 30 min of going to sleep, and not < 7 h of the time of awakening. The recommended oral dose of suvorexant is 10 mg, although doses of 15 and 20 mg are also available [3]. The drug is primarily metabolized by cytochrome P450 CYP3A4 and CYP2C19 enzyme systems. The proposed metabolites of suvorexant are the M4 metabolite produced by carboxylation of the parent drug, and the M9 metabolite produced by hydroxylation (Fig. 1) [8]. Suvorexant metabolites are not yet commercially available, so their analysis is precluded at the present time.

Suvorexant is reported to be eliminated predominantly as inactive metabolite in feces. The drug is extensively protein-bound (99.5%), predominantly to α-1 acid glycoprotein and serum albumin [9]. The half-life of suvorexant is approximately 12 h and steady-state plasma concentrations are reached within three days of daily administration [8]. Peak plasma concentrations occur approximately two hours after administration on an empty stomach, but ingestion of suvorexant following a meal can delay the time to maximum concentration (T_{max}) by an additional 1.5 h [9]. Although race and age do not seem to have an impact on peak plasma concentrations (C_{max}), they are reported to be higher in females by about 9%, and in obese patients by 17%. The oral bioavailability of suvorexant is reported to be approximately 82%, with absorption of the drug having an inversely proportional relationship to the dose administered, resulting in decreased bioavailability as the dose increases [10]. Blood plasma ratios have yet to be reported and very little is known of its distribution in tissues and fluids of toxicological interest [11].

Generally, short half-lives and rapid clearance are preferred for hypnotic medications. The likelihood of residual effects, drowsiness or decreased alertness increases in a dose-dependent fashion. These effects have the potential to interfere with daily activities [7]. The FDA recommends that next-day activities, such as driving, be avoided by patients taking the maximum daily dose of 20 mg [4]. Sedative hypnotics (such as zolpidem) feature prominently in impaired driving and drug-facilitated sexual assault investigations. The long half-life of the drug raises concerns that drivers may be impaired for extended periods following its use. However, most forensic toxicology laboratories do not routinely screen for suvorexant, so very little is understood regarding its prevalence or role in human performance toxicology investigations. Moreover, due to its high boiling point (669 °C, 450.9 g/mol), suvorexant is a very late eluting compound using gas chromatography/mass spectrometry (GC/MS) techniques, which increases the likelihood that the drug might go undetected [12].

There are relatively few published reports that describe the quantitative analysis of suvorexant in biofluids of forensic significance. Merck & Co. published an analytical method for the detection of the drug in plasma using liquid-liquid extraction (LLE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) over a concentration range of 1–1000 ng/mL. This method utilized an isotopically labeled internal standard (suvorexant-$^{13}C^{2}H_3$) that was manufactured in-house by Merck [2]. The method was used to quantify parent drug in plasma samples as part of the clinical study. However, the method was not validated in accordance with generally accepted standards in forensic toxicology [13]. Additionally, this LC-MS/MS procedure utilized only one transition and did not utilize a secondary (qualifying ion), precluding the use of ion ratios for evaluation or acceptance purposes. This approach is not forensically defensible, since it is generally accepted that a minimum of two ions are required [14]. More recently, Iqbal et al. developed a method for the determination of suvorexant in plasma using LLE and LC-MS/MS using rivaroxaban as the internal standard. A linear concentration range of 0.33–200 ng/mL was used with an LOD of 0.1 ng/mL and LOQ of 0.33 ng/mL [15]. Carson et al. reported a quantitative assay in urine using LLE and gas chromatography/mass spectrometry (GC/MS). In the absence of a commercially available deuterated internal standard, estazolam-D5 was used due to its structural similarity to the compound of interest. These include a 7-membered azepine ring, heterocyclic triazole, and a chlorine moiety (Fig. 1). Performance of the assay proposed by Carson et al. was evaluated in accordance with the Scientific Working Group for Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology [13]. The limits of detection and quantitation for the assay in urine were determined to be 10 ng/mL [16].

Similarly, a quadrupole time-of-flight liquid chromatography/mass spectrometry (LC-Q/TOF-MS) method in urine was developed and