Quantification of Suvorexant in Blood Using LC-Q/TOF-MS

Britni Skillman, BS*, Kelsie Bryand, MS and Sarah Kerrigan, PhD
Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

ABSTRACT

A new analytical procedure for the quantification of suvorexant in whole blood was developed that will aid in the identification of this novel hypnotic in forensic toxicology casework. A simple acidic/neutral liquid-liquid extraction (LLE) was used to isolate suvorexant from blood followed by quadrupole time-of-flight liquid chromatography/mass spectrometry (LC-Q/TOF-MS) analysis. 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)). The new method was validated in accordance with the Scientific Working Group for Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology. A weighted (1/X) quadratic calibration model was selected over a range of 0-200 ng/mL (R^2=0.995). Using 0.5 mL whole blood, limits of detection and quantification were 0.5 and 1 ng/mL, respectively. 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. No carryover or qualitative interferences were identified from the matrix or other common drugs.

INTRODUCTION

Suvorexant, also known as MK-4305, is a novel drug that is used for the treatment of insomnia. 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 placed under Schedule IV of the Controlled Substances Act. The half-life of suvorexant is approximately 12 hours and steady-state plasma concentrations can be reached within three days of daily administration. Peak plasma concentrations occur approximately two hours after administration. The long half-life and extensive lipophilicity of suvorexant may present a number of challenges from a forensic toxicology standpoint. Forensic toxicology laboratories may not yet screen for suvorexant in routine investigations, so very little is understood regarding its prevalence or role in human performance or postmortem toxicology investigations. Moreover, due to its very high boiling point, suvorexant is a late eluting compound using gas chromatography/mass spectrometry (GC/MS), increasing the likelihood that the drug may go undetected.

Sedative hypnotic drugs feature prominently in forensic toxicology investigations, but to date there have been no published reports that describe the analysis of suvorexant in whole blood. The purpose of this study was to develop and validate a method for the detection and quantification of suvorexant in whole blood samples using LC-Q/TOF-MS. We describe a new analytical procedure for the quantification of suvorexant that can be easily adapted to existing acidic/neutral liquid-liquid extraction protocols that are already in widespread use.

RESULTS & DISCUSSION

A new method for the detection of suvorexant in whole blood using LC-Q/TOF-MS was developed and validated. Method performance was evaluated in accordance with SWGTOX recommendations as follows to include calibration model, limit of detection, limit of quantification, extraction efficiency, precision, accuracy, bias, matrix effects and drug interferences. In the absence of an isotopically labeled analog, estazolam-D5 was used as the internal standard (IS) (Table 1). The extraction efficiencies of various solvents in blood were evaluated in addition to limit of detection, limit of quantitation, precision, accuracy and bias. Recovery of suvorexant was compared using four different extraction solvents. All solvent systems evaluated produced high extraction efficiencies and no significant differences were observed (p = 0.12) (Figure 1). MTBE was not quantitatively evaluated due to the lack of cleanliness of the extract. N-butyl chloride was selected for convenience (faster evaporation) and reduced reagent preparation time. The LOD and LOQ were determined to be 0.5 ng/mL and 1 ng/mL, respectively (Figure 2). The optimum calibration model was weighted quadratic (1/X) (R^2 = 0.995) (Figures 3 & 4). No carryover was detected following injection of the highest calibrator (200 ng/mL). Precision and bias were evaluated at concentrations of 5, 50, and 160 ng/mL. Values fell within the 20% acceptance criteria for intra-assay precision, inter-assay precision, and bias (Table 2). No matrix interferences were detected. Matrix effects were evaluated using the post-column infusion method. Although no qualitative interferences were detected in the presence of 53 common drugs, a quantitative interference was attributed to sertraline when it was present at a 100-fold higher concentration than suvorexant. In conclusion, liquid-liquid extraction paired with LC-Q/TOF-MS can be used as a technique for the detection and quantitation of suvorexant in blood samples which may play a key role in toxicological casework.

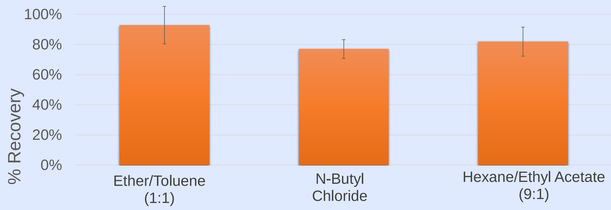

Figure 1. Extraction efficiency using ether/toluene (1:1), N-butyl chloride, and hexane/ethyl acetate (9:1).

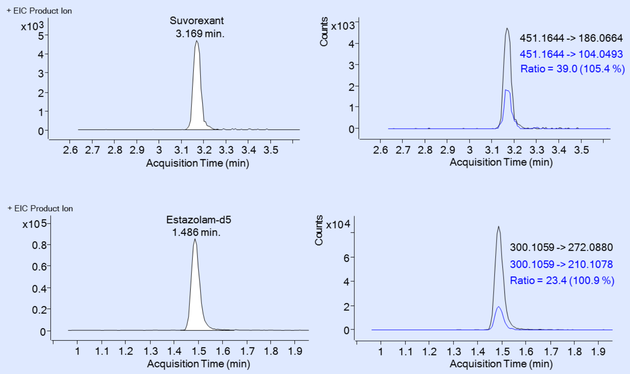

Figure 2. Extracted ion chromatograms for suvorexant at the LOQ (1 ng/mL).

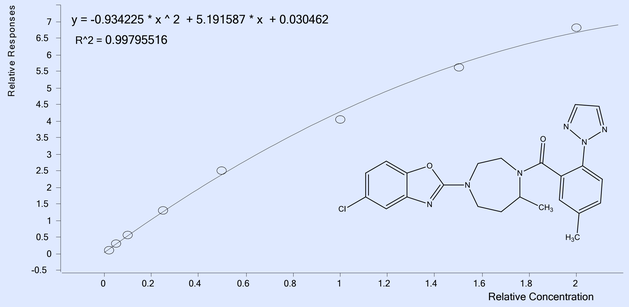

Figure 3. A weighted (1/X) quadratic model was selected.

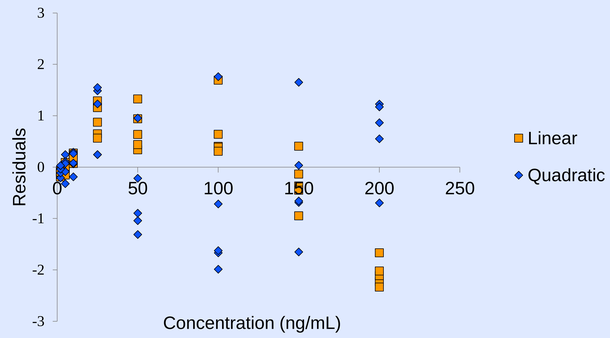

Figure 4. Standardized residuals were compared for linear and quadratic models for a weight of 1/X.

Compound	MS Transition (m/z)	CE (eV)	RT (min)
Suvorexant	451.1644 > 186.0664 451.1644 > 104.0493	50	3.08
Estazolam-D5	300.1059 > 272.0875 300.1059 > 210.1076	30	1.41

Table 1. Ion transitions, collision energy (CE), and retention time (RT) of suvorexant and internal standard using LC-Q/TOF-MS.

Parameters	Results
Calibration Model	2-200 ng/mL (Quadratic, weighted 1/X)
Carryover	No carryover at 200 ng/mL
LOD	0.5 ng/mL
LOQ	1 ng/mL
Precision	4-10% (Intra-assay Precision) n=5
	5-13% (Inter-assay Precision) n=15
Bias	-5 to 2%

Table 2. Summary of validation results using LC-Q/TOF-MS.

MATERIALS AND METHODS

Instrumentation

An Agilent 1290 Infinity Binary LC System and a 6530 Accurate-Mass Q/TOF-MS was operated in electrospray ionization (ESI) positive mode. Gradient elution was achieved using a Poroshell EC-C18 column (2.1 x 100mm, 2.7 µm) and a matching guard column (2.1 x 5mm, 2.7 µm). The column temperature was maintained at 35°C with a flow rate of 0.4 mL/min. Mobile phase A and B consisted of 0.1% formic acid in deionized water and in acetonitrile, respectively. The gradient elution profile consisted of a 40% B to 80% B ramp between 0-3 minutes, a hold of 80% B for 1 minute, and then decrease to 40% B until 5 minutes. Drug-free bovine blood containing sodium fluoride and potassium oxalate was used for the preparation of calibrators and controls.

Extraction

Calibrators and controls were prepared at 0, 2, 5, 10, 25, 50, 100, 150 and 200 ng/mL by fortifying 0.5 mL of blood with suvorexant and estazolam-D5 (100 ng/mL). Extraction was performed by addition of 1 mL sodium acetate buffer (pH 3.6, 0.4M), and 2.5 mL N-butyl chloride followed by mixing and centrifugation. The organic layer was removed and evaporated to dryness under nitrogen (50°C). The extracts were then reconstituted with 30 µL of mobile phase A and B (1:1) and 2 µL was injected into the LC-Q/TOF-MS.

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