Comparison of Physicochemical Properties of Suvorexant with Quantitative Results using Authentic Samples

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INTRODUCTION

Suvorexant (Belsomra®) is a novel dual orexin receptor antagonist (DORA) that is used for the treatment of insomnia. It became commercially available in 2015 and is currently under Schedule IV of the Controlled Substances Act. Peak plasma concentrations are reached within two hours of administration and are typically <200 ng/mL following a 10 mg oral dose. The volume of distribution is 0.5-0.9 L/kg and it is highly protein bound (99.5%) to α-1-acid glycoprotein and serum albumin. Bioavailability (oral) is ~82%. Suvorexant is a sedative hypnotic with a long half-life (~12 hours) which makes it a drug of forensic interest due to the prominence of sedative hypnotics in driving impairment and drug-facilitated sexual assault (DFSA) investigations. However, the prevalence of suvorexant in forensic toxicology casework is unknown and there have been relatively few published case reports. Moreover, its physicochemical properties have not yet been fully studied. Properties, such as lipophilicity and partition coefficients, can be important from the standpoint of analysis (extraction, chromatographic separation), pharmacology (absorption, distribution) and interpretation (*i.e.,* potential for postmortem redistribution or PMR). No studies have been published to date describing the analysis of suvorexant metabolites in forensic specimens. The purpose of this study was to investigate the molecular lipophilicity of suvorexant, compare experimental and theoretically derived log P values, and identify the drug and its primary oxidative metabolite (M9) (**Figure 1**) in biological specimens from thirteen forensic toxicology investigations.

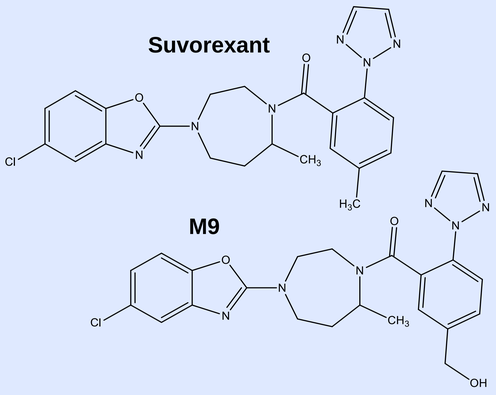

Figure 1. Chemical structures of suvorexant and M9 metabolite.

RESULTS AND DISCUSSION

Suvorexant (octanol/water) partition coefficients (Log P) were evaluated and the experimentally determined value was in close agreement with theoretical Log P values (**Table 1**). Computational approaches that utilized electrotopological characteristics (ALogPS) and additive-constitutive algorithms (ACD Labs/LogP) provided the best estimates. Log P values were independent of pH and ionic strength, consistent with its behavior as a neutral drug (**Figure 2, Table 2**). Suvorexant was identified in antemortem and postmortem blood at concentrations ranging 3-42 ng/mL, which are well within the therapeutic range (**Table 3**). Vitreous concentration could not be determined likely due to the low concentration of the drug in blood (3.5 ng/mL) and its extensive lipophilicity. Blood/plasma ratios could not be estimated due to hemolysis of specimens and unknown sample collection times. Central/peripheral (C/P) ratios for the two paired cases were 2.0 and 2.2, which is consistent with the only published report for suvorexant in three forensic cases (0.9-1.4)[3]. Although neutral and highly lipophilic, suvorexant has a low to moderate V_d and may not exhibit marked postmortem redistribution. PMR is influenced by many factors, including lipophilicity, protein binding and pKa. The drug is reported to undergo metabolism to pharmacologically inactive hydroxylated and glucuronidated species, but there are no commercially available metabolites for suvorexant at this time, precluding their quantitative analysis. In this study, phase I suvorexant metabolites were generated *in vitro* using recombinant cytochrome P450 enzymes (rCYPs). The M9 metabolite was qualitatively identified in all blood and serum/plasma specimens (n=17). Mass accuracies (within ±5 ppm), retention time and MS^2 spectra were used for identification purposes.

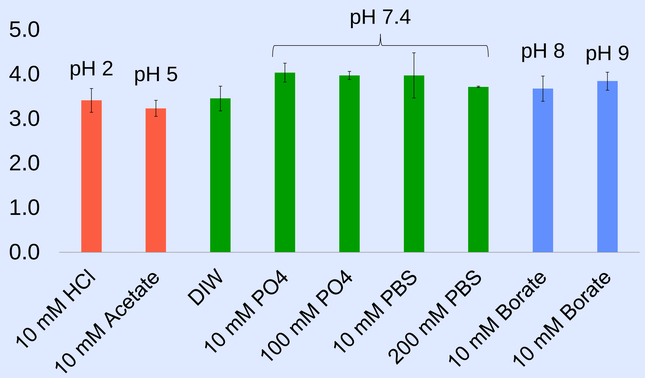

Figure 2. Partition coefficient (Log P) values for suvorexant in various aqueous systems (n=3).

Aqueous System	pH	Mean ± SD	%CV
Deionized water	-	3.45 ± 0.28	8.0%
HCl (10 mM)	2	3.41 ± 0.27	7.8%
Acetate buffer (10 mM)	5	3.23 ± 0.18	5.6%
Phosphate buffer (10 mM)	7.4	4.04 ± 0.21	5.3%
Phosphate buffer (100 mM)	7.4	3.97 ± 0.09	2.3%
PBS (10 mM)	7.4	3.97 ± 0.51	12.8%
PBS (200 mM)	7.4	3.72 ± 0.01	0.4%
Borate buffer (10 mM)	8	3.68 ± 0.28	7.7%
Borate buffer (10 mM)	9	3.84 ± 0.20	5.3%

Table 2. Mean partition coefficients (n=3) for suvorexant using various aqueous systems across a range of pH and ionic strengths (mM).

Method	Log P
Experimental (octanol/water)	3.45
ALogPS	3.86
ACD Labs/LogP	3.62
KowWin	4.65

Table 1. Experimental and theoretical Log P values for suvorexant.

Sample #	Type	Matrix	Result (ng/mL)	Qualitative M9 ID
1	AM	Blood	3.2	Yes
2	PM	Blood (P)	3.5	Yes
		Blood (C)	7	Yes
		Vitreous humor	ND[1]	No
3	PM	Blood (P)	16.8	Yes
		Blood (C)	36.5	Yes
4	AM	Blood	21	Yes
		Serum/Plasma	3.6	Yes
5	PM	Blood (P)	36.8	Yes
6	PM	Blood (C)	13.2	Yes
7	PM	Blood (P)	40.5	Yes
8	PM	Blood (P)	27.7	Yes
9	PM	Blood (P)	15.6	Yes
10	PM	Blood (P)	26.5	Yes
11	AM	Blood	29.6	Yes
		Serum/Plasma	12.9	Yes
12	PM	Blood (P)	17.2	Yes
13	PM	Blood (P)	41.5	Yes

Table 3. Concentrations of suvorexant in forensic investigations.
[1]Not determined (below LOD).

MATERIALS AND METHODS

Instrumentation

An Agilent 1290 Infinity Binary LC system coupled to 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 guard (2.1 x 5mm, 2.7 µm) that were maintained at 35°C. Mobile phase A and B consisted of 0.1% formic acid in deionized water and in acetonitrile, respectively. Separation was achieved at 0.4 mL/min using the following gradient: 40% B (0 min), 40-80% B (0-3 minutes), hold 80% B (1 min), 80-40% B (1 min), post-equilibration (2 min). Targeted MS/MS data acquisition was used for suvorexant quantitation in authentic specimens and Log P determination. Auto MS/MS was used for qualitative metabolite identification using the following gradient for separation: Hold 40% B (3 min), 40-80% (1 min), hold 80% B (2 min), 80-40% (1 min), post-equilibration (2 min).

Quantitation of Suvorexant

Calibrators and controls were prepared in whole bovine blood using a previously validated acidic/neutral liquid-liquid extraction method[1]. Adjudicated case samples were extracted using this method (LOD & LOQ of 0.5 ng/mL) and estazolam-D5 (100 ng/mL) was used as the internal standard.

Partition Coefficients

Log P values were determined for suvorexant using predictive software programs ALogP.S 2.1, ACD Labs/LogP, and Log KowWin 1.67. Experimental Log P values were determined using the shake-flask method with 1-octanol and various aqueous phases (**Table 1**).

Metabolite Identification

In the absence of commercially available standards for suvorexant metabolites, major metabolites reported in the literature were generated using recombinant cytochrome P450 enzymes (CYP 3A4, 2C19, 2D6, 2C9, 2C8, 2C18, 2B6, and 1A2)[2]. Metabolite formation was monitored for 0-240 min and 120 min rCYP incubations were used as positive controls. Metabolite identification was performed qualitatively for authentic specimens using mass accuracy (± 5 ppm), MS^2 spectra, and retention time matching.

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CONCLUSION

Interpretation of results in forensic toxicology investigations can be complicated by postmortem distribution and PMR, and a drug's physicochemical properties can greatly affect its behavior before and after death. Factors that are essential for successful interpretation are the volume of distribution, lipophilicity, basicity, and metabolism. Drug concentrations may also be influenced by sampling site which demonstrates the importance of analyzing substances in a variety of specimen types. This study aimed to expand the knowledge of suvorexant distribution in forensic case specimens and identify the potential for using the primary plasma metabolite (M9) in future forensic casework.

REFERENCES

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2. Ciu, D. et al. (2016) *Xenobiotica*. 46, 1-14.
3. Waters, B. et al. (2018) *Journal of Analytical Toxicology*. 42, 276-283.