Quantification of Daridorexant, Lemborexant, and Suvorexant in Whole Blood using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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INTRODUCTION

Insomnia is a prevalent medical condition that is treated with mainstay pharmacological interventions including the use of benzodiazepines or "Z-drugs". However, these drugs can cause rapid tolerance development, have a high risk of abuse or physical dependence, or lead to rebound insomnia upon discontinuation and other medical complications. Dual orexin receptor antagonists (DORAs) represent a novel, alternative class of sedative hypnotics that selectively target orexin receptors to inhibit wakefulness, and ultimately lead to sustained restful sleep.

Suvorexant, lemborexant, and daridorexant are 3 FDA-approved DORAs classified under Schedule IV of the Controlled Substance Act. Despite their therapeutic benefits, they remain drugs of forensic interest due to their accessibility, long half-lives, and potential risk for next-day residual drowsiness, impaired motor coordination, and decreased alertness. These CNS depressant effects may feature in driving impairment cases or drug-facilitated sexual assault investigations. Although suvorexant has been described in forensic casework, lemborexant and daridorexant remain unstudied for this purpose. Hence, the primary objective of this study was to develop and validate an LC-MS/MS method to quantify suvorexant, lemborexant, and daridorexant in whole blood.

MATERIALS & METHODS

Liquid-Liquid Extraction

- Fortify 500 µL blood
  - 25 µL calibrator or QC mix
  - 25 µL ISTD (Suvorexant-d_6)
- Add 1 mL sodium acetate buffer (0.4 M, pH 3.6)
- Add 2.5 mL of *N*-butyl chloride
- Mix, 5 minutes
- Centrifuge at 3000 rpm, 5 minutes
- Transfer organic layer to clean glass conical tubes
- Evaporate organic layer to dryness under nitrogen (50 °C), ~10 minutes.
- Reconstitute in 30 µL of 50:50 mobile phase A:mobile phase B

RESULTS & DISCUSSION

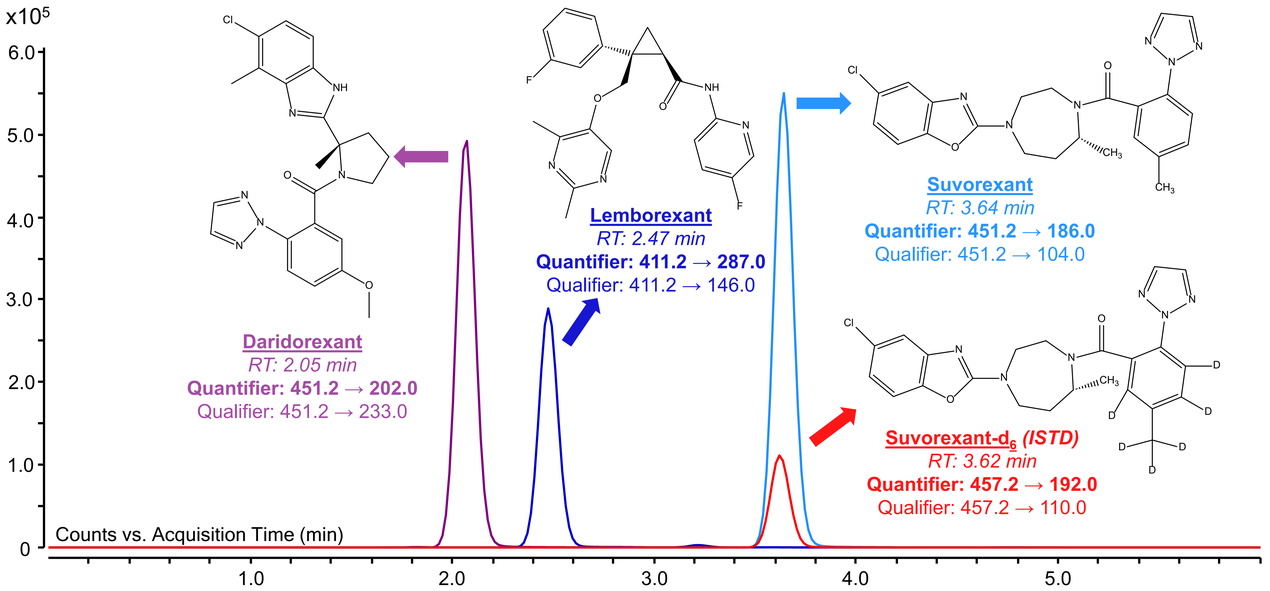

Figure 1. Quantifier transitions of suvorexant, lemborexant, and daridorexant (HQC; 450 ng/mL) with suvorexant-d_6 (ISTD; 100 ng/mL) in extracted human whole blood

Parameters	Suvorexant	Lemborexant	Daridorexant
Calibration range	0.25 – 500 ng/mL		
Calibration Model	1/x linear	1/x quadratic	
LOD/LOQ	0.25 ng/mL		
Bias (%) n= 15 per QC level	-10.9 – 2.3	-7.7 – 4.2	-4.6 – 8.8
Within-run %CV n= 15 per QC level	1.1 – 17.7	1.3 – 10.7	0.9 – 13.9
Between-run %CV n= 15 per QC level	4.9 – 8.9	5.3 – 7.5	6.7 – 9.8
Matrix Effects (%) n= 10 per QC level	LQC: -68.2 HQC: -66.6	LQC: -75.7 HQC: -56.7	LQC: -62.4 HQC: -54.2
Interference Studies	Free from endogenous and exogenous interferences		
2X dilution Integrity (%) n= 15 bias, within-run %CV and between run %CV	-7.6 0.5 – 3.5 5.0	-6.5 1.9 – 7.6 6.1	-3.2 2.0 – 7.1 7.5
Carryover	No carryover at 500 ng/mL		
Processed sample stability	LQC and HQC stable at 4°C for 48 hours		
Recovery Efficiency (%)	91.6 ± 8.4	99.9 ± 11.2	94.2 ± 4.7

Table 1. Results of suvorexant, lemborexant, and daridorexant method validation performed according to ANSI/ASB 036 guidelines. For all analytes, LQC, MQC, HQC, and 2-fold dilution concentrations were 0.75, 100, 450, and 225 ng/mL, respectively.

Case	Average [Suvorexant] (ng/mL) (n=2)	PM Blood Type
1	12.6	Central
	32.5	Peripheral
2	15.5	Central
3	45.9	Peripheral
4	21.1	Peripheral
5	9.8	Central
6	28.7	Peripheral
7	19.7	Peripheral
8	27.3	Peripheral

Table 2. Suvorexant in authentic postmortem forensic specimens (n=8, extracted in duplicate)

MATERIALS & METHODS

LC-MS/MS

Instrumentation:

- Agilent 1290 Infinity II Liquid Chromatograph coupled to an Agilent 6470 Triple Quadrupole MS

Source Parameters	Value
Gas temperature (°C)	300
Gas flow (L/min)	13
Sheath gas temperature (°C)	350
Sheath gas flow (L/min)	12
Capillary voltage (V)	3000
Nebulizer (psi)	45
Nozzle (V)	2000

Column:

- Agilent Infinity Lab Poroshell 120 EC-C18 (2.7 µm, 2.1 x 100 mm) with matching guard

Mobile phase:

- A: 0.1% formic acid in diH_2O
- B: 0.1% formic acid in acetonitrile

Flow rate:

- 0.4 mL/min

Gradient elution profile: (8 min total)

- Start at 35% B, increase to 40% B by 0.50 min, increase from 40% B to 80% B by 3.5 min, hold at 80% B for 1 min, decrease back to 35% B by 6 minutes. Post-equilibration time of 2 minutes was also added.

CONCLUSIONS

A method for the quantification of DORAs in whole blood using LLE and LC-MS/MS was developed and validated (ASB 036). Analysis of authentic postmortem samples demonstrates its applicability to quantify Suvorexant, and novel DORAs (lemborexant and daridorexant) in forensic casework.

DISCLOSURE

The authors declare no conflicts of interest.

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