Rapid and Sensitive Quantification of Psilocin in Human Whole Blood using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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INTRODUCTION

Psilocybin is the principal component found in the *Psilocybe* mushrooms. There have been emerging clinical breakthroughs in the use of psilocybin as an alternative therapeutic candidate to treat neurological and neurodegenerative diseases. However, psilocybin is also of forensic concern as it is a Schedule I Controlled Substance under the 1970 U.S Controlled Substance Act, with its pharmacodynamic/pharmacokinetic profile and long-term side effects still poorly understood.

Given the rapid dephosphorylation and instability of psilocybin in human biological matrices, current quantitative analytical methods prioritize the quantification of the comparatively more stable psilocin and/or other psilocybin metabolites. Considering that whole blood is the first type of matrix collected in controlled clinical drug trials, and more importantly, the most common type of matrix tested in forensic toxicology casework for human performance investigations, it remains critical to develop a validated method that could be applicable in both clinical and forensic laboratories.

Thus, this research aims to: (1) to optimize a solid-phase extraction procedure in human whole blood, and (2) to develop and validate a sensitive LC-MS/MS method to quantify psilocin according to ANSI/ASB 036 guidelines.

MATERIALS AND METHODS

Sample Preparation

- 500 µL human whole blood
- 50 µL calibrator/QC mix
- 20 µL ISTD
- 10 µL 0.1M ascorbic acid
- 1 mL 100 mM phosphate buffer (pH 6)
- Centrifuge at 4000 *rpm* for 10 min

Solid-Phase Extraction

- Load supernatant onto Polychrom® Clin II SPE columns
- Wash with (1) 2 mL diH_2O, (2) 2 mL methanol, (3) 2 mL ethyl acetate
- Dry under vacuum for 5 min
- Elute with 2 mL ammonium hydroxide (2%) in ethyl acetate (v/v)
- Evaporate under nitrogen
- Reconstitute in 100 µL of 90A:10B MP

RESULTS & DISCUSSION

Table 1. Method validation results. LQC, MQC and HQC were 2 ng/mL, 20 ng/mL and 160 ng/mL respectively.

Parameters	Validation Procedure	Results
Linearity	Least square regression model with 8 non-zero calibrators over 5 days	0.78, 1.56, 3.12, 12.5, 50, 100, 150, 200 ng/mL (R^2 > 0.995)
Calibration Model	Residual plot of calibrators over 5 days	1/x
LOD/LOQ	Lowest calibrator extracted in triplicate using 3 unique sources over 3 runs (n=9)	0.78 ng/mL
Bias	Extracted in triplicate over 5 days (n=5)	-9 to 16% (*LQC*),-7 to 5.4% (*MQC*), -8.3 to 14.3% (*HQC*)
Within-run %CV		3.2 to 10.6% (*LQC*),2.9 to 15.4% (*MQC*), 2.5 to 15.5% (*HQC*)
Between-run %CV	Combined replicate pools of triplicate extracted QCs over 5 days (n=15)	10.2% (*LQC*), 9.1% (*MQC*),11.5% (*HQC*)
Matrix Effects	Post-extraction addition with 10 unique blood sources (n=20)	LQC: 10% (*Psilocin*) and 16% (*Psilocin-d$_{10}$*) HQC: 4% (*Psilocin*) and 12% (*Psilocin-d$_{10}$*)
Interference Studies	(1) Monitor Psilocin's signal in negative control (2) Fortify LQCs (n=3) with mix of 176 different drugs (>8000 ng/mL), (3) Fortify LQCs (n=9) with bufotenine (psilocin isomer) at 10x QC concentration	(1) No interference, (2) -1.9% (*Bias*), (3) Bufotenine is chromatographically separated from psilocin
Dilution Integrity	1:1 dilution of HQC in triplicate over 5 days (80 ng/mL)	2.9 to 16.9% (*Bias*) 3.5 to 17.9% (*Within-run precision*) 12.2% (*Between-run precision*)
Carryover	Re-injecting extracted blank following highest calibrator (200 ng/mL) over 5 runs	No carryover observed
Processed sample stability	Assessed peak area ratio (Psilocin/Psilocin-d_{10}) after 48 hours in the autosampler (4°C)	LQC and HQC stable beyond 48 hours
Recovery Efficiency	Evaluated at 167 ng/mL over 3 runs (n=9)	89.9-95.2%

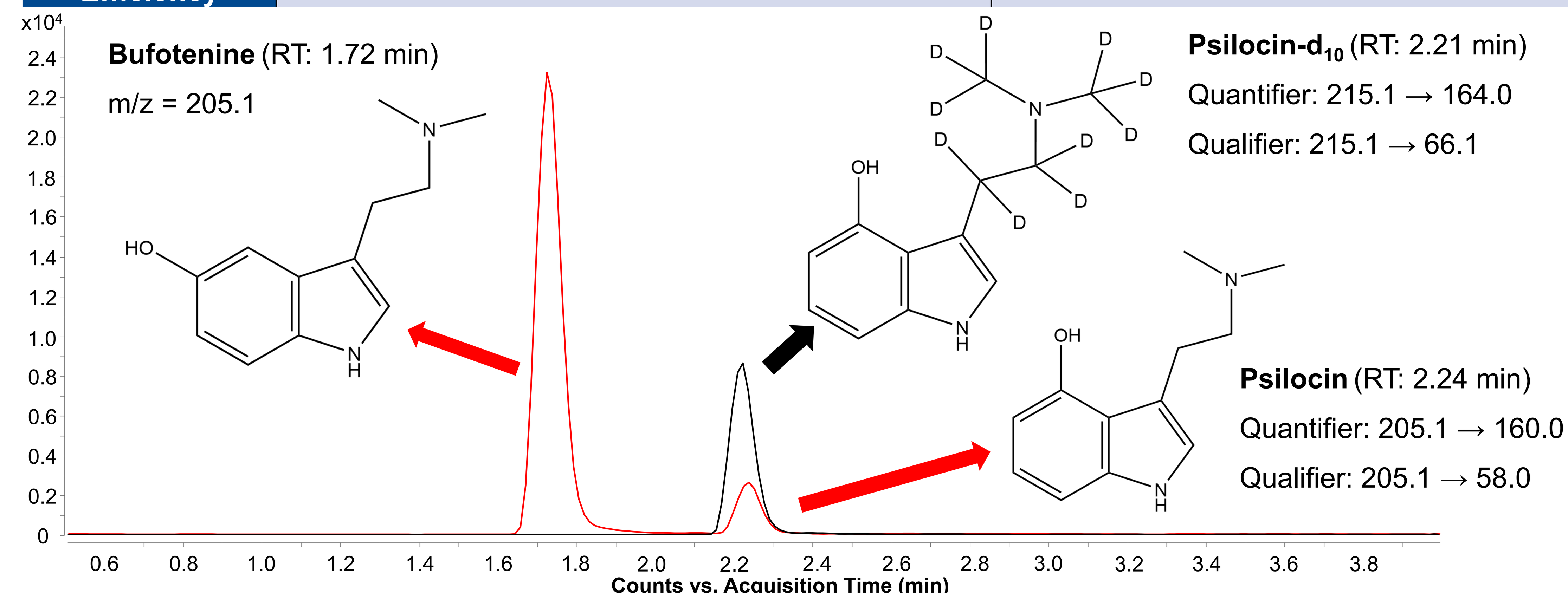

Figure 1. Chromatographic separation of Bufotenine (20 ng/mL) and Psilocin (2 ng/mL) with Psilocin-d_{10} (ISTD; 3 ng/mL) in extracted human whole blood

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MATERIALS AND 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)	5
Sheath gas temperature (°C)	350
Sheath gas flow (L/min)	11
Capillary voltage (V)	3500
Nebulizer (psi)	45
Nozzle (V)	0

Column:

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

Mobile phase

- A: 5 mM ammonium formate + 0.01% formic acid in di$H_2$0
- B: 0.1% formic acid in acetonitrile

Flow rate:

- 0.5 mL/min

Gradient elution profile: (6 min total)

- 0.5 min hold at 4% B, 0.5-4 min: 4% B → 30% B, 4-4.10 min 30% B → 90% B, 4.10-4.60 min hold at 90% B, 4.60 min-4.70 min 90% B → 4% B. Re-equilibrate for 1.3 min.

CONCLUSION

An efficient SPE and LC-MS/MS method for the quantification of psilocin in whole blood was developed and met all acceptable ASB 036 validation criteria. A proof-of-concept study will need to be conducted to further support the method's applicability to authentic forensic/clinical samples.

DISCLOSURE

The authors declare no conflicts of interest.

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