Detection and Validation of PCP, Ketamine, Analogs and Metabolites in Blood Using LC-MS/MS

Andrea Bernal, BS; Britni Skillman, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

Sam Houston State University

INTRODUCTION

Recently, the production and distribution of novel psychoactive substances (NPS) has persisted worldwide. With constantly evolving drug trends, detecting NPS in forensic casework can be difficult. Among these trends, the CFSRE has identified NPS stimulants and hallucinogens as a major category of concern in U.S. casework. Specifically, several analogs of phencyclidine and ketamine have increased in prevalence to include analytes such as 3-methyl-PCP, 3-methoxy-PCP, and 2-fluoro-2-oxo-PCE. This project aims to provide a robust analytical method to detect and characterize these substances in forensic toxicology.

MATERIAL & METHODS

Calibrators and controls were prepared by fortifying whole, preserved bovine blood with analyte and internal standard. A liquid-liquid extraction (LLE) procedure was developed using 0.5 mL sample (Figure 1). The method was validated according to ANSI/ASB Standard 036.

LLE

1. Fortify 0.5 mL blood:
   25 µL calibrator or QC mix
   25 µL ISTD
2. Add 1.5 mL 10 mM borate buffer (pH 9)
3. Add 3 mL *N*-butyl chloride
4. Rotate 5 minutes
5. Centrifuge at 4200 RPM, 10 min
6. Transfer organic layer to glass conical tubes
7. Dry down under nitrogen (40°C), ~10 min
8. Reconstitute with 50 µL starting mobile phase

Figure 1. *LLE Protocol*

DISCLOSURES

The authors do not have any conflicts of interest to disclose.

RESULTS & DISCUSSION

Peak	Analyte	Retention Time (min)	Internal Standard	Ion Transitions (m/z)
1	Deschloroketamine	1.403	Norketamine-d4	204.1 → 91.0, 173.1
2	Norketamine	1.639	Norketamine-d4	224.1 → 125.0, 207.0
3	Deschloro-N-ethyl ketamine	1.776	Ketamine-d4	218.1 → 91.0, 173.1
4	2-Fluoro-2-oxo PCE	1.819	Ketamine-d4	236.1 → 109.0, 163.1
5	Ketamine	1.892	Ketamine-d4	238.1 → 125.0, 207.0
6	Fluorexetamine	2.034	Norketamine-d4	236.1 → 109.0, 95.0, 83.0
7	Methoxetamine	2.593	Ketamine-d4	248.2 → 203.1, 121.0
8	PCP	4.045	PCP-d5	244.2 → 86.1, 159.1
9	3-Methoxy PCP	4.165	PCP-d5	274.2 → 86.1, 121.1
10	3-Methyl PCP	4.334	PCP-d5	258.2 → 86.1, 105.0

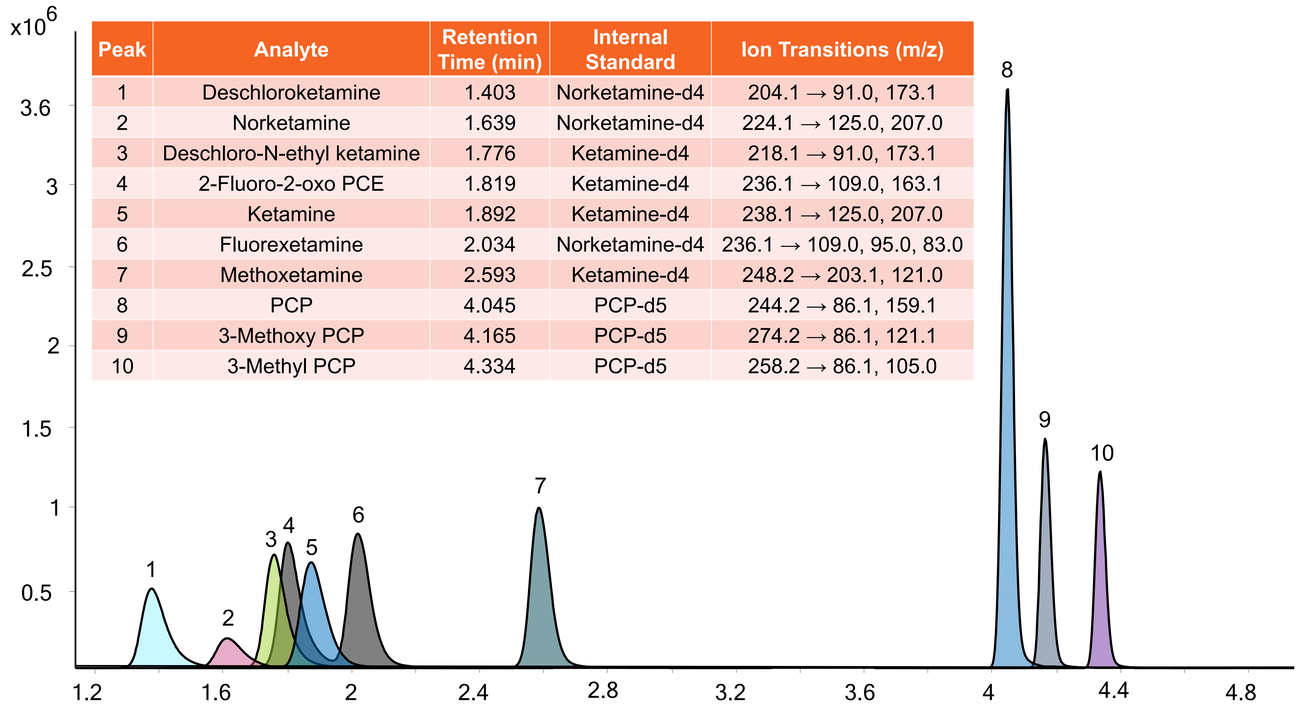

Figure 2. *Example chromatogram for a 100 ng/mL extracted sample (retention time in minutes vs. response)*

Table 2. *Validation Summary Results*

Parameter	Results
Calibration Model	Quadratic 1/x weighted model for all analytes; range of 0.5 – 500 ng/mL; $R^2 > 0.997$
Grand Bias (%)	All values at three concentrations (1.5, 125, and 400 ng/mL) within ± 7.1%
Matrix Effects (%)	Acceptable for all analytes within ± 13.3%
Limit of Detection	0.1 ng/mL
Limit of Quantitation	0.5 ng/mL
Carryover	Acceptable; Average % CO of LOQ < 3.5%
Interferences	No interferences from the matrix, internal standard, commonly encountered drugs of abuse and prescription drugs were detected
Dilution Integrity	Values at 5X, 10X, and 25X dilution were all within ± 11.1% of expected concentration
Processed Sample Stability (72 hours)	All analytes stable

MATERIAL & METHODS

For chromatographic separation and detection, an LC-MS/MS method was optimized on an Agilent 1290 Infinity II LC coupled with an Agilent 6475 LC/TQ. Positive electrospray ionization mode was utilized with optimized source parameters.

Table 1. *Optimized instrumental parameters*

Parameter	Value
Column	Agilent Poroshell 120 EC-C18 (2.1 x 100 mm, 2.7 µm) with guard
Mobile phase A	0.1% formic acid in deionized water
Mobile phase B	0.1% formic acid in acetonitrile
Gradient	0–0.5 min (15% B hold) 0.5–2.5 min (15–22.5% B) 2.5–4.25 min (22.5–65% B) 4.25–5.25 min (65–90% B) 5.25–6.25 min (90% B hold) 6.25–8.50 min (90–15% B)
Column temp	35°C
Flow rate	0.4 mL/min
Source Parameters	200°C drying gas (7 L/min); 400°C sheath gas (12 L/min); 4500 V capillary; 0V nozzle; 50 psi nebulizer

CONCLUSIONS

This study presents a validated LC-MS/MS method for the detection of PCP, ketamine, metabolites, and selected emerging analogs in blood, offering a practical tool for forensic toxicology laboratories. This method demonstrated acceptable quantitative performance using a simple liquid-liquid extraction and can support the identification of emerging NPS substances related to PCP and ketamine in forensic toxicology casework.

ACKNOWLEDGEMENTS

Thank you to Sam Houston State University, including the Department of Forensic Science, for funding and support.

Scan here for electronic poster

Department of Forensic Science
College of Criminal Justice
forensics.shsu.edu