Dissociation by Design: LC-MS/MS Detection and Validation of PCP, Ketamine, Metabolites, and Novel Structural Analogs in Blood and Urine

Andrea Bernal, BS; Britni Skillman, PhD, F-ABFT

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

Sam Houston State University

INTRODUCTION

In recent years, novel psychoactive substances (NPS) have continued to be produced and sold throughout the world. With evolving drug trends, detection and identification of NPS in forensic casework can be difficult. From 2024-2025, the Center for Forensic Science Research and Education (CFSRE) reported that NPS stimulants and hallucinogens accounted for an average of 8-17% of NPS drug identifications in the United States[1,2]. Several analogs of phencyclidine (PCP) and ketamine have been increasing in prevalence to include analytes such as 3-methyl PCP, 3-methoxy PCP, and 2-fluoro-2-oxo PCE. As a result, new toxicological methodologies need to be developed and validated for the identification of these substances in casework.

MATERIALS & METHODS

Calibrators and controls were prepared by fortifying blood or urine 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 for both matrices.

LLE

1. Fortify 0.5 mL blood or urine:
   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)
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

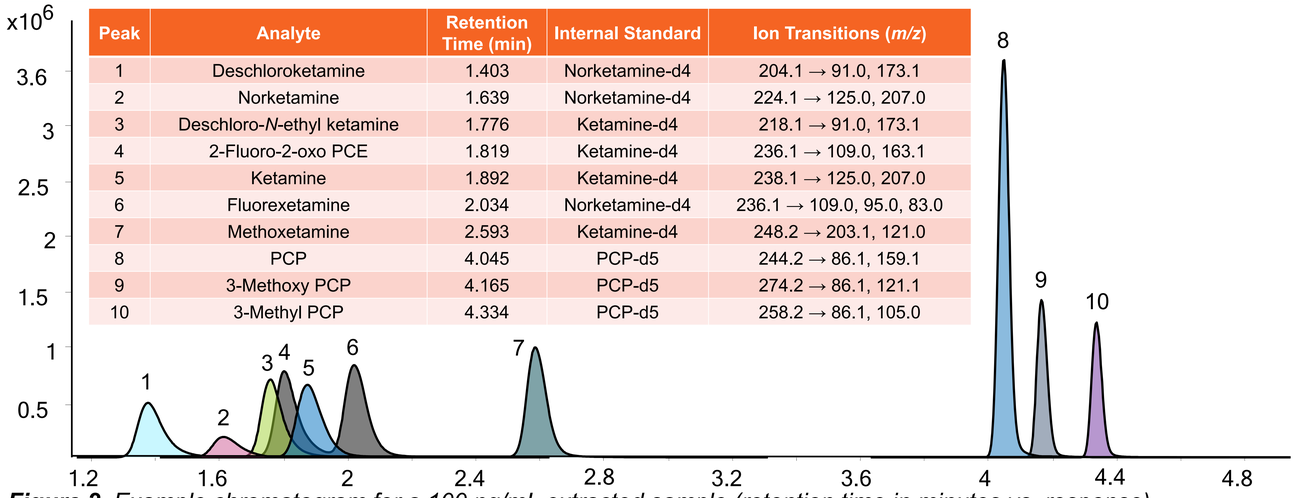

Figure 2. *Example chromatogram for a 100 ng/mL extracted sample (retention time in minutes vs. response)*

Table 2. *Validation Summary Results*

Parameter	Results
Matrix Effects (%)	Acceptable for all analytes within ± 13.3% (BL) or ± 11.0% (UR)
Limit of Detection	0.1 ng/mL (BL), 0.5 ng/mL (UR)
Carryover (CO)	Acceptable for BL & UR; Average %CO of LOQ < 3.5% (BL)
Interferences	No interferences from the matrix, internal standard, commonly encountered drugs of abuse or prescription drugs were detected
Calibration Model	Quadratic 1/x weighted model for all analytes, 0.5 – 500 ng/mL (BL); $R^2 > 0.997$
Grand Bias (%)	All values at three blood concentrations (1.5, 125, and 400 ng/mL) within ± 7.1%
Precision (%CV)	Between-run %CV ≤12.1% all analytes (BL) Within-run %CV ≤17.1% all analytes (BL)
Limit of Quantitation	0.5 ng/mL (BL)
Dilution Integrity	Values at 5X, 10X, and 25X dilution were all within ± 11.1% of expected blood concentration
Processed Sample Stability (72 hours)	All analytes stable; within ± 7.7% of t_0 concentration in blood

REFERENCES

[1]Krotulski AJ, Walton SE, DeBord J, et al (2024) NPS Stimulants and Hallucinogens in the United States: Trend Report – Q1 2024. Center for Forensic Science Research and Education.
[2]Krotulski AJ, Walton SE, DeBord J, et al (2025) NPS Stimulants and Hallucinogens in the United States: Trend Report – Q2 2025. Center for Forensic Science Research and Education.

MATERIALS & METHODS

For chromatographic separation and detection, an LC-MS/MS method was optimized on an Agilent 1290 Infinity II LC coupled to 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 and urine, offering a practical tool for forensic toxicology laboratories. The 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.

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