Detection and Quantification of Phencyclidine (PCP) in Postmortem Blood for the Application of Death Investigation

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ABSTRACT

Phencyclidine (PCP) was developed as an anesthetic but was discontinued due to its side effects. PCP is a drug of abuse and is often combined with other agents. There is an increased incidence of individuals dipping cigarettes into PCP *(dippers). This has resulted in a need for increased sensitivity for PCP detection. The purpose of this study was to develop an LC-MS/MS method with a lower limit of detection for determining and quantifying PCP in postmortem blood, while decreasing extraction and analysis time. The experimental linear range for PCP detection in our studies was 1 ng/mL to 125 ng/mL. In summary, we have established an LC-MS/MS method that is more sensitive and rapid than standard methods for PCP detection.

INTRODUCTION

Phencyclidine (PCP) was developed in the 1950s as an anesthetic, however, due to the hallucinogenic effects observed it was discontinued for human medical use. In the pure form, PCP is a white crystalline powder that rapidly dissolves in water or alcohol and has a distinctive bitter taste.

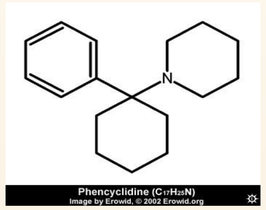

Figure 1. Structure of Phencyclidine (PCP)

On the market, PCP is available in tablets, capsules, colored powders, and liquid form. PCP is usually ingested orally, snorted, smoked, or injected. Common street names include: Angel Dust, Lovely, Wack, Embalming Fluid, and Rocket Fuel.

In humans, therapeutic levels range from 10–200 ng/mL. Moderate use effects of PCP include: detached feelings such as hallucinations and image distortion, loss of coordination, profuse sweating and slurred speech. Reported lethal concentrations of PCP in postmortem blood range from 300–2,500 ng/mL. At these high concentrations, patients can exhibit physiological effects of vomiting, a significant drop in blood pressure, seizures, coma and eventually death.

Most PCP-related deaths occur due to injury or suicide during PCP intoxication. Although PCP use started to decline after the 1980s, it is still prevalent in the United States. PCP–laced cigarettes are becoming widespread, leading to an increase in violent activity and death cases. When these cigarettes are smoked, not all of the PCP is ingested, leading to lower concentrations within the blood. This, along with the half-life being 7-46 hours, makes detection and quantification of low concentrations difficult. Detection by GC-MS has a lower limit of detection of 20 ng/mL at Aegis. Therefore, postmortem cases with lower concentrations cannot be reported. The transition to LC-MS/MS analysis allows for a decrease in acquisition time and greater sensitivity at the lower limit of detection and quantification.

METHODS

Samples

Linearity, accuracy and precision, and interference studies were all performed with porcine blood. Eight random postmortem blood samples were obtained for matrix interference. Postmortem blood samples previously analyzed and confirmed for PCP were selected and analyzed as parallel studies.

Extraction Method

- Internal standards added
- Extraction approach
  - Solid-phase extraction with double elution
- Solvent evaporated to dryness
- Reconstitution: 200 µL mobile phase A
- Single point calibrator, -/+ controls with each batch

LCMSMS Parameters

- Shimadzu LC Controller
- Pinnacle DB C18 Column
- CTC PAL Autosampler
- AB Sciex Triple Quad™ 4500 Mass Spectrometer
- Mobile Phase A: 10 mM ammonium acetate, 0.1% formic acid
- Mobile Phase B: 0.1% formic acid in acetonitrile

RESULTS & DISCUSSION

Table 1. Linear Range of Postmortem Blood Samples

Target Concentration (ng/mL)	Calculated Concentration (ng/mL)
1	1.07
2	2.15
5	5.59
10	9.97
30	29.32
50	53.46
125	120.18

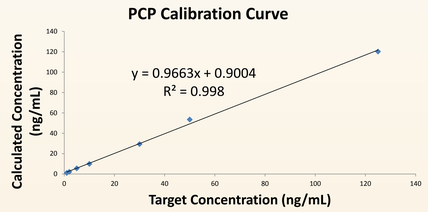

Figure 2. PCP Linear Range

Figure 3. LCMSMS chromatogram of LOQ quality control. Peak Identification: PCP (Rt = 3.04) , d5- PCP (Rt= 3.02)

Table 2. Accuracy and Precision of Controls and Calibrator

Low Control (1 ng/mL)

Sample ID	PCP (ng/mL)
QC-LC 1	0.89
QC-LC 2	0.84
QC-LC 3	0.91
QC-LC 4	1.05
QC-LC 5	1.00
QC-LC 6	0.93
QC-LC 7	1.13
QC-LC 8	1.03
QC-LC 9	0.90
QC-LC 10	1.06
Target	1.00
AVE	0.97
SD	0.09
% CV	9.70
% Target	2.67

Calibrator (5 ng/mL)

Sample ID	PCP (ng/mL)
Cal 1	5.00
Cal 2	4.50
Cal 3	4.53
Cal 4	6.25
Cal 5	5.45
Cal 6	5.08
Cal 7	5.18
Cal 8	5.38
Cal 9	5.32
Cal 10	5.18
Target	5.00
AVE	5.19
SD	0.50
% CV	9.56
% Target	3.74

High Control (125 ng/mL)

Sample ID	PCP (ng/mL)
QC-HC 1	119.02
QC-HC 2	112.65
QC-HC 3	110.46
QC-HC 4	119.41
QC-HC 5	129.90
QC-HC 6	114.41
QC-HC 7	115.12
QC-HC 8	113.85
QC-HC 9	138.01
QC-HC 10	113.78
Target	125.00
AVE	118.66
SD	8.71
% CV	7.34
% Target	5.07

Table 3. Blood Matrix Interference with PCP

Sample	Calibrator (5 ng/mL) and IS	IS only	Blank		
	PCP (ng/mL)	PCP (ng/mL)	IS Peak Area	IS Peak Area for Cal	% Cal Peak Area
1	3.757	0.045	541.652	1066310.380	0.05
2	5.315	0.037	832.594	591783.796	0.14
3	4.822	0.027	414.217	502054.188	0.08
4	6.645	0.022	343.397	403538.402	0.08
5	5.798	0.024	693.427	220768.074	0.31
6	4.285	0.063	296.026	625714.397	0.04
7	4.404	0.02	N/A	463680.022	N/A
8	4.875	0.021	245.325	725090.474	0.03

Table 4. Potential Interfering Drugs with PCP

Analytes Tested at 12.5 ng/mL	
Narcotic Analgesics	Fentanyl , Norfentanyl
Analytes Tested at 100 ng/mL	
Benzodiazepines	Alprazolam, Clobazam, Clonazepam, Chlordiazepoxide, Demoxepam, Desalkylflurazepam, 7-aminonitrazepam, 7-aminoflunitrazepam, 7-aminoclonzaepam, N-desmethylclobazam, N-Desmethyldiazepam, Diazepam, Estazolam, Flunitrazepam, Flurazepam , Hydroxyehtylflurazepam, a-Hydroxyalprazolam, a-Hydroxytriazolam, a-Hydroxymidazolam, Lorazepam, Midazolam, Nitrazepam , Oxazepam, Prazepam , Temazepam, Triazolam
Analytes Tested at 1000 ng/mL	
Opiates	Morphine, Oxymorphone, Hydromorphone , Dihydrocodeine, Norcodeine , Codeine , Noroxycodone, Oxycodone , Norhydrocodone , Hydrocodone
Muscle Relaxants	Meprobamate, Carisoprodol
Analytes Tested at 5000 ng/mL	
Narcotic Analgesics	Methadone, EDDP

CONCLUSIONS

- LLOQ of 1 ng/mL
- ULOQ of 125 ng/mL
- Controls and calibrator accurate within 20% of target concentration
- Controls and calibrator precise within 10%
- No interferences due to the matrix itself
- No interferences with other drugs, based on study
- **FUTURE AIM:** Finish parallel studies for validation
- **FUTURE AIM:** Detection of metabolites of PCP (4-Phenyl-4-piperidinylcyclohexanol and 1-(1-phenylcyclohexyl)-4-hydroxypiperidine)
- **FURTURE AIM:** Investigate metabolites as possible interfering substances

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