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

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Abstract

PCP was developed in the 1950s as an anesthetic; however, due to its side effects, its use in the medical field was discontinued. Although it was discontinued for human medical use, PCP was still being used by veterinarians. Some of the side effects experienced with PCP were confusion and delirium, paranoia, image distortion, illusions and hallucinations. The pure form of PCP is a white crystalline powder that readily dissolves in water or alcohol. It has a distinctive bitter taste. Common street names include: Angel Dust, Hog, Lovely, Embalming Fluid, Wack, and Rocket Fuel.

A new analytical method was validated for the determination of PCP in human blood using liquid chromatography-tandem mass spectrometry. The gold standard method of detection for PCP in blood is gas chromatography-mass spectrometry, which has a lower limit of quantification of 20 ng/mL at Aegis. The purpose of transitioning the GC-MS method to an LC-MS/MS method was to decrease the lower limit of detection and quantification of PCP and to decrease analysis time. A lower limit of detection is needed because PCP-laced cigarettes are becoming prevalent. When these cigarettes are smoked, not all of the PCP is inhaled or ingested leading to lower concentrations of PCP in the blood. The new method involved the use of solid-phase extraction for sample preparation, followed by liquid chromatography-tandem mass spectrometry for separation and analysis. The instrumentation used was a Pinnacle DB C_{18} column, with an AB Sciex Triple Quad™ 4500

single channel tandem mass spectrometer. PCP was quantified using multiple-reaction-monitoring with deuterium labeled PCP (PCP-d_5) as an internal standard. The LC-MS/MS method was validated for linearity, precision, accuracy, interferences and parallel studies. The lower limit of quantitation (LLOQ) was determined to be 1 ng/mL and the upper limit of quantitation (ULOQ) was determined to be 125 ng/mL based on the linearity studies that were done. The low control (1 ng/mL), calibrator (5 ng/mL), and high control (125 ng/mL) were precise within 10% of each replicate and accurate within 20% of the target concentration. No interferences were seen with commonly abused drugs, based on the study or within the matrix itself.

PCP can be now be quantified in postmortem human blood at lower concentrations (LOQ= 1 ng/mL) than gas chromatography-mass spectrometry. Due to the revival of PCP on the drug- market, this analytical method provides an improved process to extract and analyze blood samples that can aid in determining cause and manner of death in medical examiner investigations.

A. Introduction

Phencyclidine (PCP) is a hallucinogen that is classified as a dissociative anesthetic. A dissociative anesthetic "produces analgesia and amnesia without respiratory depression, resulting in a state in which the patient appears dissociated from his environment but not necessarily asleep" (Levine). PCP was synthesized in the 1950s primarily as an anesthetic; however, due to the side effects produced, it was discontinued for human medical use. The side effects that were being seen were delirium, paranoia, hallucinations, and euphoria (Levine). PCP is currently a Scheduled II drug in the United States.

In the pure form, PCP is a white crystalline powder that is easily dissolved in water or alcohol. PCP has a distinctive bitter taste. PCP is structurally similar to ketamine. The structure of PCP can be found in Figure 1. It is an antagonist of the N-methyl-D-aspartate (NMDA) ionotropic receptor in the central nervous system. This causes an inhibition of de-polarization of neurons and subsequent interferences with cognitive and other functions of the brain (Jickells).

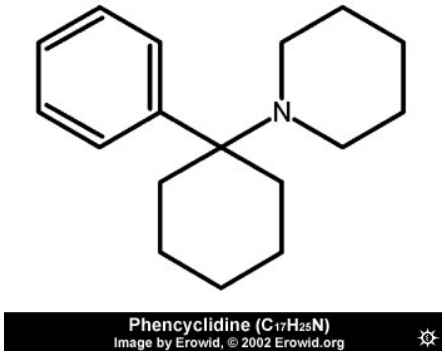


Figure 1. Structure of PCP

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 dosing levels range from 10–200 ng/mL. A single dose of PCP is approximately 5 mg, with a range of 1-10 mg (Levine). A study was performed where human volunteers were given PCP via oral administration. The study found that the maximum serum PCP concentrations ranged between 2.7 and 2.9 ng/mL after 1 mg of PCP was administered orally (Levine). Moderate use effects of PCP include: detached feelings such as hallucinations and image distortion, loss of coordination, profuse sweating and slurred speech. Reported lethal levels of PCP in postmortem blood range from 300–2,500 ng/mL. At these high doses, 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 and this is 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 cutoff limit 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.

B. Experimental

1-[1-(Phenyl)cyclohexyl] piperidine hydrochloride (PCP-HCl) was obtained from Grace (Mt. Pleasant, TN). A 1 mg/mL solution of phencyclidine was obtained from Cerilliant (Round Rock, TX). The deuterated internal standard, PCP-d_5, was purchased from Cerilliant (Round Rock, TX).Drug free porcine blood was purchased from Biochemed (Winchester, VA). HPLC grade methanol was used to make all the spiking solutions. All other chemicals were of analytical grade.

LC-MS/MS

An AB Sciex Triple Quad™ 4500 Single channel mass spectrometer (Framingham, MA)used for all analyses. The LC system was a Shimadzu LC Controller equipped with a CTC PAL Autosampler. The mass spectrometer was operated in the positive ionization mode.

Liquid chromatographic separation was achieved on a Pinnacle DB C_{18} column. Mobile phase A consisted of 10 mM ammonium acetate with 0.1% formic acid. Mobile phase B was comprised of 0.1% formic acid in acetonitrile. Table 1 shows the settings for the LC and Table 2 shows the concentration gradient used for the mobile phases. Figure 2 is a graph showing the concentration gradient for the duration of the analysis. Multiple reaction monitoring in positive mode was used for the mass spectrometer. Table 3 shows the MS/MS parameters that were used. Table 4 shows the mass fragments that were monitored during analysis.

Table 1. LC Parameters

Parameter	Setting
Run Time	4.206 min
Binary Flow	0.5 mL/min Start with 15% Pump B
Injection Volume (µL)	12.00
Injection Speed (µL)	5.00
Loop Volume 1 (µL)	100
Loop Volume 2 (µL)	100

Table 2. Concentration Gradient

Time	Event	Parameter
0.20	Pump B Conc	15
0.50	Pump B Conc	15
3.00	Pump B Conc	70
3.01	Pump B Conc	95
3.50	Pump B Conc	95
3.51	Pump B Conc	15
4.20	Stop	

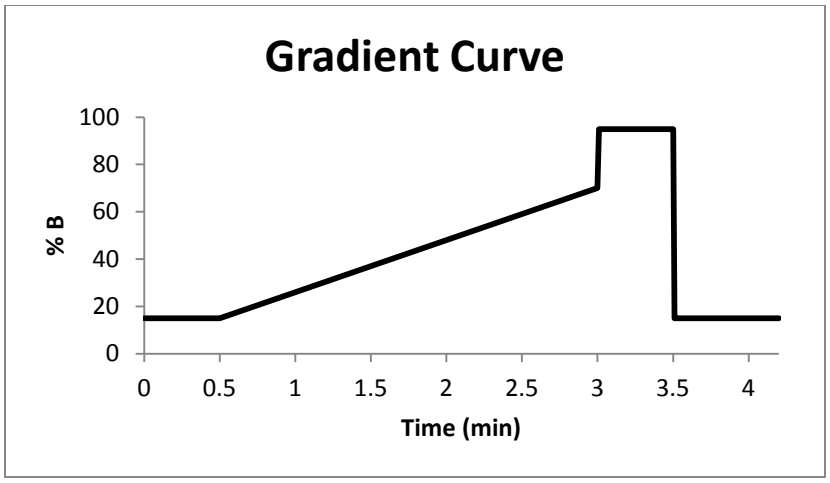


Figure 2. Graph of the concentration gradient used.

Table 3. MS/MS Parameters

Source Parameter	Valve
Curtain Gas	30
Collision Gas	6
Nebulizer Current	4500
Temperature	650
Ion Source Gas 1	75
Ion Source Gas 2	60

Table 4.MS/MS Parameters – MRM Table

	Q1 Mass (Da)	Q3 Mass (Da)	Time (msec)
PCP-1	244.2	86.2	150
PCP-2	244.2	159.2	150
PCP-3	244.2	91.2	150
PCP-D5	249.2	86.2	150

Sample Preparation

All controls, calibrators, and samples were prepared in 500 µL of negative porcine blood. All were extracted using solid phase extraction with a double elution. The columns were conditioned using methanol, water, and buffer. Each reagent was allowed to flow through completely before adding the next. Once the column was conditioned, the samples were added to the column and allowed to flow through completely via gravity. The columns were rinsed to

make sure no contaminants were left behind. Samples were eluted. The columns were rinsed. Samples were eluted for a second time with an elution buffer. Samples were dried at 37°C under N_2 at ≤ 2 psi. Once dry, the samples were reconstituted in 200 µL of mobile phase A. Samples were then transferred to the LC-MS/MS and analyzed. For all batches a retention time standard (RTS), low control, calibrator, high control, and negative were run for analysis.

Linearity

Linearity studies were performed using different concentrations ranging from 1 ng/mL to 500 ng/mL. Spiking solutions were made in order to obtain these concentrations. Table 5 shows the concentrations that were made from each spiking solution. The solutions were spiked into 500 µL of negative porcine blood and extracted as stated above in the Sample Preparation. A minimum of three sets were extracted and ran.

Table 5. Linearity Concentrations

Target Concentration (ng/mL)	Volume (µL)	Spiking Solution Volume Taken From (ng/mL)	Acceptable Range (within 20%)	
1	10	50	0.8	1.2
2	20	50	1.6	2.4
5	10	250	4	6
10	20	250	8	12
30	60	250	24	36
50	100	250	40	60
125	250	250	100	150
250	10	12500	200	300
500	20	12500	400	600

Accuracy and Precision

Accuracy and precision were analyzed by running two batches containing multiple samples of the low control, calibrator, and high control. A total of 10 repeats for each control and calibrator were used for data analysis. The method is deemed accurate if the mean value is ±

20% of the target concentration. For the method to be precise, the mean must be ± 20% of the target concentration and the CV should be ± 10%.

Drug Interference

The interference of PCP with similar or associated drugs was analyzed. This was accomplished by spiking a 10 ng/mL calibrator. Table 6 shows the drugs that were analyzed for interferences.

Table 6. Drugs and their Concentrations Tested for Interferences

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

Matrix Interference

The matrix itself was analyzed for interferences. Eight random postmortem blood samples were obtained and put into three aliquots. The samples were analyzed for each different matrix and the results were compared to a batch calibrator response: (1) analytes and IS fortified at calibrator level, (2) IS only fortified, and (3) neither analyte nor IS fortified (matrix blank).

Parallel Study

Parallel analyses were completed to assess the new procedure's performance against that of a validated method. Known PCP positive samples were obtained. The samples were extracted and analyzed using LC-MS/MS. The results obtained were compared to the original GC-MS results.

C. Results and Discussion

Linearity

The linear range was determined to be 1 ng/mL to 125 ng/mL. Therefore, the lower limit of quantitation is 1 ng/mL and the upper limit of quantitation is 125 ng/mL. Originally, 500 ng/mL was investigated as the upper limit; however, consistent results were not obtained at this high of a concentration. During the accuracy and precision study, 250 ng/mL proved to not be accurate leading the upper limit being decreased to 125 ng/mL Table 7 shows the combined results for the linearity study. Figure 3 shows the calibration curve for the linear range. Figure 4 shows the chromatogram for 1 ng/mL.

Table 7. Linearity Range for Calibration Curve

Target Concentration (ng/mL)	Acceptable Range (within 20%)		Calculated Concentration (ng/mL)
1	0.8	1.2	1.07
2	1.6	2.4	2.16
5	4	6	5.60
10	8	12	9.98
30	24	36	29.33
50	40	60	53.46
125	100	150	120.19

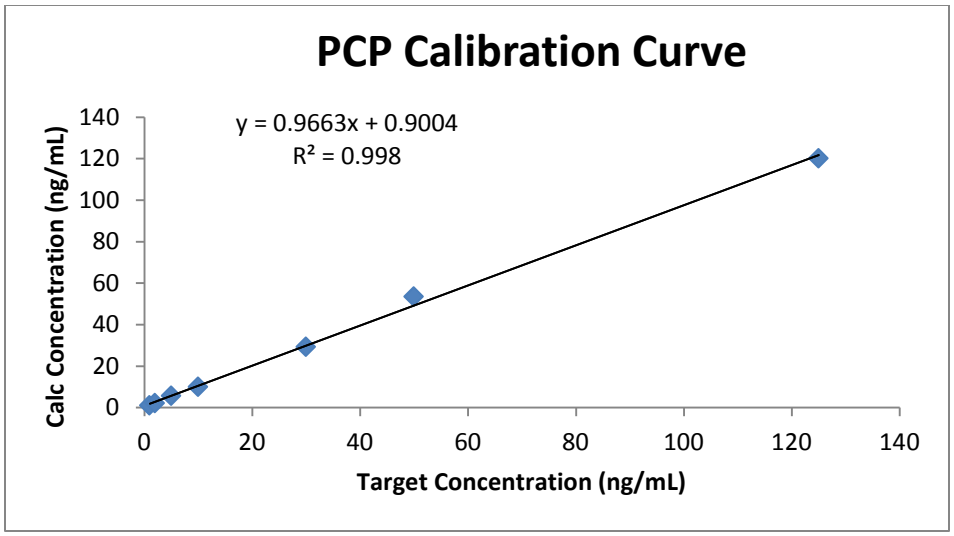


Figure 3. PCP calibration curve

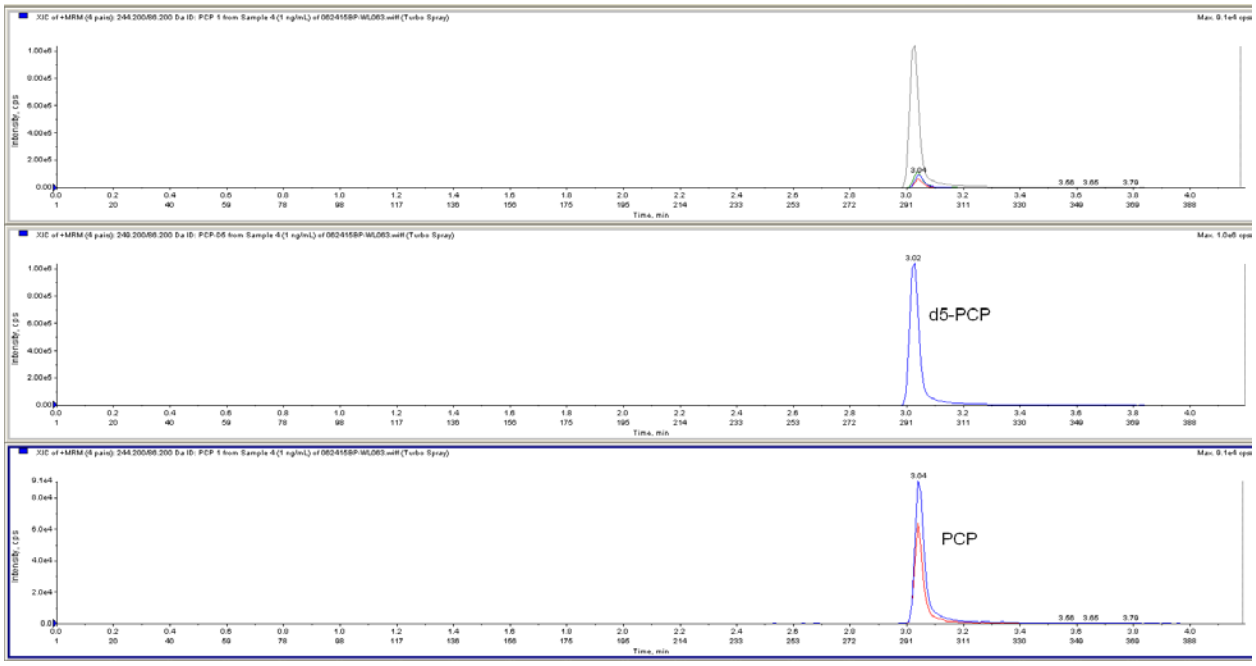


Figure 4.Liquid chromatogram of the LOQ quality control. Peak Identification: PCP (Rt = 3.04), d5- PCP (Rt= 3.02)

Accuracy and Precision

The low control, calibrator (5 ng/mL), and high control were analyzed for accuracy and precision. In order for the method to be accurate, the percent difference must be ± 20% of the target concentration. The method is precise if the percent difference is ± 20% of the target

concentration and the CV is ±10%. The low control percent difference was 2.67% with a CV of 9.70%. The calibrator percent difference was 3.74% with a CV of 9.56%. The high control percent difference was 5.07% with a CV of 7.34%. Based on this data, all of the percent differences fall within the ± 20% of the target concentration and the CV percents are below 10%, meaning that the method is accurate and precise. Table 8 shows the combined results for the low control, calibrator, and high control.

Table 8. Accuracy and Precision Results

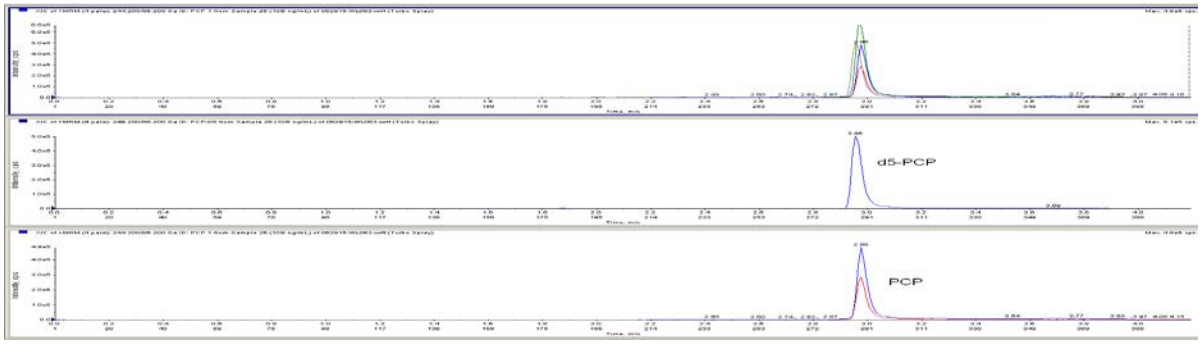
	Combined		
	PCP QC-LC	**PCP CAL**	**PCP QC-HC**
Target	1.00	5.00	125.00
AVE	0.9733	5.1872	118.6618
SD	0.0944	0.4958	8.7078
%CV	9.70	9.56	7.34
%Diff	2.67	3.744	5.0706
Min	0.839	4.50	110.46
Max	1.131	6.253	138.01

Drug Interference

Table 6 shows the drugs that were tested for interferences. Based on the analysis, no interferences were seen with any of the drugs. Figure 5 shows the liquid chromatograms of the samples that were spiked with the drugs used for the interference study. The retention time for PCP was not affected by any drugs that were spiked into the samples and no other peaks were identified where PCP elutes. The concentration of PCP that was targeted in the samples was 10 ng/mL. All sample concentrations fell in the acceptable range.

A.

B.



C.

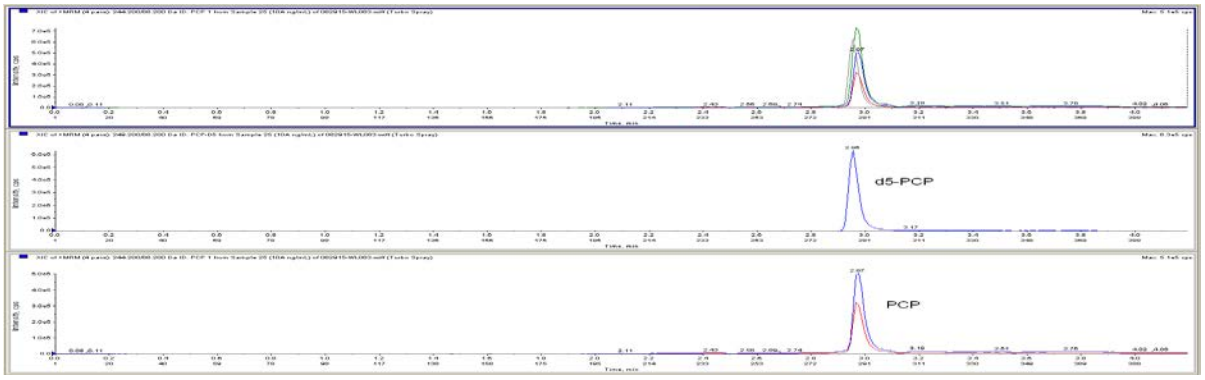


Figure 5.Liquid chromatograms of the calibrators that were spiked with possible interference drugs.A. 10 ng/mL sample was spiked with ketamine, diphenhydramine, and dextromethorphan. B. 10 ng/mL sample spiked with benzodiazepines. C. 10 ng/mL sample spiked with opiates and other remaining drugs. No peaks other than PCP were detected at the retention time of PCP and the retention time was not affected.

Matrix Interference

For the assessment of matrix interference, 8 random blood samples were obtained and split into three aliquots: (1) at the calibrator concentration plus internal standard, (2) internal standard only, and (3) no fortification. In drug fortified samples, all qualitative acceptance criteria must be met. In internal standard only fortified samples, no alternate peak should be detected that would be reported as a presumptive positive. In the blank, or non-fortified samples, the peak area of any peak at the internal standards retention time must be <5% of the peak areas for the calibrator. Table 9 shows the results for the matrix interference study. The samples with both calibrator and internal standard were spiked at the calibrator concentration of 5 ng/mL. Therefore, the acceptable range of concentrations is 4 ng/mL to 6 ng/mL. Sample 1 is slightly lower than 4 ng/mL, while sample 4 is higher than 6 ng/mL. All other samples met the acceptable criteria. For the entire internal standard only fortified samples, no peaks were seen that would be reported as a presumptive positive. The concentration for all samples was below the lower limit of quantitation of 1 ng/mL. The non-fortified samples all had percent calibration peak areas of less than 5 percent.

Table 9. Blood Matrix Interference Data

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

Parallel Study

Five samples that were previously analyzed for PCP were obtained. The samples were extracted and analyzed using the new method and compared to the original results that were obtained with an already validated method. Surprisingly, the results obtained by LC-MS/MS were not what were expected. The results showed a positive bias, which led to a methanol: acetonitrile crash being added before the solid-phase extraction. Work is continuing to assess linearity, precision and accuracy, drug interference, and matrix interference.

D. Conclusions

The new method proposed in this paper offers a lower limit of quantitation of 1 ng/mL and an upper limit of quantitation of 125 ng/mL for PCP in postmortem blood. The method is deemed accurate and precise based on the repeat measurements of the low control, calibrator, and high control. No interferences were seen with structurally similar drugs, associated drugs, or other drugs. The matrix itself did not show any interference with the quantitation of PCP.

PCP can be now be quantified in postmortem human blood at lower concentrations (LOD= 1 ng/mL) than gas chromatography-mass spectrometry. Due to the revival of PCP on the drug-market, this analytical method provides an improved process to extract and analyze blood samples that can aid in determining cause and manner of death in medical examiner investigations.

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