Detection and Quantitation of Phencyclidine in Blood by Use of $[^2\text{H}_5]$Phencyclidine and Select Ion Monitoring Applied to Non-Fatal Cases of Phencyclidine Intoxication

Dan S. Pearce

I describe a method for the qualitative and quantitative determination of phencyclidine in blood. Pentadeuterophencyclidine is used as an internal standard. The analyses are conducted with a gas chromatograph–mass spectrometer by use of select-ion monitoring. Results of 26 case samples, including a brief resumé of representative examples, are discussed.

Additional Keyphrases: gas chromatography–mass spectrometry • drug abuse • "street" drugs • drug assay • multiple ion detection • mass fragmentography • toxicology

Phencyclidine [PCP, Sernyl®, 1-(1-phencyclohexyl) piperidine] is a potent analgesic originally developed as an intravenous anesthetic. Deemed unsuitable for human use, it is currently marketed legitimately as a primate tranquilizer. The illegitimate use of phencyclidine has increased drastically throughout the United States since its “discovery” on the West Coast by the so-called drug culture in the late 1960’s (1). The relative ease with which phencyclidine may be synthesized (2) has certainly added in some measure to the drug’s widespread use.

Illicit phencyclidine preparations are sold under various street names, the most common being “THC,” “cannabiol,” and “crystal joints” (marijuana or parsley cigarettes sprinkled with the drug). Phencyclidine is generally present as the free base, the hydrochloride salt, or the hydrobromide salt. The purity of these preparations varies from essentially 100% crystalline base to less than 5%. The latter is often in combination with “TCP,” 1-[1-(2-thienyl) cyclohexyl] piperidine. Phencyclidine preparations are self-administered by smoking, insufflation, oral ingestion, and injection.

The effects of phencyclidine have been found to be highly dose dependent. Three stages of intoxication have been described. Even in low doses (5–10 mg), the drug has considerable effect on behavior and motor functions (1). The pronounced effects of phencyclidine present definite medicolegal problems, of greatest import for individuals who are involved in violent crimes, operating motor vehicles, or presenting to emergency medical personnel.

Data on concentrations of phencyclidine in the blood have been obtained primarily from fatal cases. In cases where the primary cause of death has been attributed to an overdose of the drug, the reported postmortem concentrations range from 0.3 to 7.0 mg/liter. In instances where the cause of death is attributed to other factors (trauma, drowning, etc), the postmortem concentrations found range from 0.3 to 0.5 mg/liter (3, 4). Surprisingly, one blood concentration of 3.7 mg/liter was reported in a non-fatal overdose (1). Somewhat lower concentrations have recently been reported for non-fatal overdoses, ranging from 49 μg/liter (blood) to 171 μg/liter (serum), by a method similar to that reported here (5).

Urinary phencyclidine concentrations cover a much broader range. In fatal cases, the concentrations range from 0.6 to 330 mg/liter. Samples from living individuals range from 0.03 to 10.5 mg/liter for chronic users, and from 0.4 to 92 mg/liter in non-fatal overdoses (3).

The low phencyclidine concentrations in blood associated with intoxication as well as the limited quantity of blood available from living individuals necessitated the development of a specific, sensitive assay for phencyclidine. One recently developed technique suited to this problem is performed with a gas chromatograph interfaced to a mass spectrometer. This technique—variously called mass fragmentography, multiple-ion detection, and select ion monitoring—consists of using the mass spectrometer as a detector for one or more ions, as opposed to scanning a given m/e range (δ). The quantitative accuracy of this method, as well as the sensitivity, may be increased through the use of an isotopically labeled counterpart of the compound of interest. Isotopically labeled material added to the original sample as the internal standard acts as a carrier for the unlabeled compound during the purification process and analysis. Furthermore, the partition coefficients between various solvents will be essentially

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identical for the two compounds, thus providing a compensating internal standard. This technique has been successfully applied in a variety of analyses including morphine and codeine (7), amphetamine (8), and tetrahydrocannabinol (9) in blood. Recently, a method similar to that described here was developed for phencyclidine (5).

We have developed such a procedure for identifying and measuring phencyclidine in blood. The procedure is based on the use of phencyclidine, carrying a pentadeuterated phenyl moiety, as the internal standard. A known quantity of this internal standard is added to a measured amount of blood, and to aqueous phencyclidine solutions of appropriate concentrations. Each sample is then carried through a three-step separation. The resulting extract is taken to dryness, reconstituted, and two aliquots subjected to analysis, one for quantitation, the other for positive identification.

Materials and Methods

Preparation of 1-(1-Phenyl-[2H5]-cyclohexyl) Piperidine (Pentadeuterophencyclidine) (2)

Magnesium (0.9 g, 34 mmol) was suspended with vigorous stirring in 25 ml of anhydrous diethyl ether under a nitrogen atmosphere. To this was added a solution of 5.0 g (31 mmol) of [2H5]bromobenzene (Aldrich Chemical Co., San Leandro, Calif. 94577) in about 25 ml of anhydrous diethyl ether, during 30–40 min, with intermittent heating. The reaction was begun by adding a few milligrams of iodine. After the addition was complete, the mixture was stirred vigorously with occasional heating for about 30 min. After this time, no further reaction was noted (some unreacted magnesium remained). 1-Piperidinocyclohexane carbonitrile (3 g, 16 mmol) (Aldrich Chemical Co.) in 25 ml of benzene was added during 30 min, with gentle heating. The reaction mixture was allowed to stand overnight, with occasional stirring. Fifteen milliliters of HBr (2 mol/liter) was then added to the reaction mixture; the effluent gas from the system passed through a strong aqueous NaOH scrubbing solution. (The presence of HCN was quite evident by its odor.) The following day, the mixture was neutralized with solid Na2CO3 and the crude product purified first by pH then HCl ion pair extraction. Recrystallization of the hydrochloride (CHCl3/toluene) gave 1.7 g (39%), mp 218–220 °C (lit. (5): 219–20 °C). A second crop of 0.35 g was obtained from the mother liquors (total yield: 46%). The pentadeuterophencyclidine thus obtained showed a slight amount of impurity (<1%) by gas chromatography. The infrared and ultraviolet spectra were consistent with the assigned structure. The electron impact mass spectrum showed no undeuterated phencyclidine to be present.

Extraction Procedure

SpectrAR® spectral grade chloroform (Mallinckrodt Chemical Co., Los Angeles, Calif. 90058) and AR grade diethyl ether (Mallinckrodt) were used in the extraction; no appreciable interference was noted in extracted blanks. Aqueous solutions of phencyclidine and [2H5]phencyclidine were prepared in de-ionized water; the pH was adjusted to slightly acid with dilute HCl or H2SO4.

I added 0.50 ml of an aqueous solution of [2H5] phencyclidine (50 µg/liter) to 0.25 ml of whole blood in
a 12-ml conical centrifuge tube. Ether (5 ml) and 2 drops of NaOH (5 mol/liter) were added, the sample was vortex-mixed for 15–20 s, then centrifuged. The organic layer was aspirated and passed through a disposable pipette containing a plug of glass wool and 50–100 mg Na₂SO₄, into a second centrifuge tube. The dried ethereal extract was then extracted with 1 ml of H₂SO₄ (0.1 mol/liter). After vortex-mixing for 15–20 s, the organic phase was again aspirated and discarded. Chloroform (5 ml) and one drop of saturated NaOH were then added. The sample was vortex-mixed for 15–20 s and the organic layer was passed through a disposable pipette containing a plug of glass wool, into a third centrifuge tube (the pipette, glass wool, and tube were previously rinsed with CHCl₃). A few drops of HCl in ethanol were added, and the chloroform taken to dryness under an air stream in a hot water bath at about 50 °C. Suitable aqueous phencyclidine standards and a blank were carried through the extraction.

Analysis

The analyses were performed with a Model 5982A mass spectrometer, under the control of a Model 5933A data system (Hewlett-Packard Co., Palo Alto, Calif. 94304). The data were acquired and manipulated by use of standard software routines. The ion source temperature was maintained at 175–180 °C and operated at 70 eV. The gas chromatograph used was a Model 5710 (Hewlett-Packard Co.) equipped with a 120-cm glass column containing 3% OV-17 on 100–120 Gas Chrom Q. The helium flow rate was 40 ml/min. The injection port and column temperatures were 200 and 190 °C, respectively. The gas chromatograph was interfaced to the mass spectrometer with a jet separator, maintained at 200 °C.

Immediately before the analysis, the residue (preceeding section) was reconstituted in 10–20 µl of absolute ethanol. One to five microliters of this solution was then injected into the gas chromatograph. The phencyclidine was quantitated by monitoring the ions at m/e 205 ([²H₄]phencyclidine) and 200 (phencyclidine). The concentration of phencyclidine was calculated by normalizing the area of the response at m/e 205 to 100%.

Applying the factor thus obtained to the area of the response at m/e 200 produced directly by the concentration of phencyclidine in µg/liter. A second injection of a similar volume was used to confirm the presence of phencyclidine. The ions at m/e 243 (M+), 242, 200, and 186 were monitored and the calculated ratios of the responses compared to that of a standard sample (Figure 1).

Results and Discussion

Blood phencyclidine concentrations are calculated by assuming equivalent responses for the labeled and unlabeled compounds, an assumption verified by analysis of whole-blood samples prepared by adding phencyclidine to phencyclidine-free blood. The average value obtained for 25 µg/liter samples was 25.4 µg/liter, with a standard deviation of 1.0 µg/liter (n = 9); the average value obtained for 100 µg/liter samples was 102 µg/liter, with a standard deviation of 2.7 µg/liter (n = 10); the average value obtained for 500 µg/liter samples was 498 µg/liter, with a standard deviation of 17 µg/liter (n = 10).

The lower limit for the reliable determination of phencyclidine is about 10 µg/liter. For smaller concentrations one or more milliliters of blood may be successfully used for the analysis.

Twenty-six actual case samples were analyzed by the above-described procedure. Values obtained ranged from 6 to 240 µg/liter, as phencyclidine hydrochloride. The standard deviation between duplicate analyses was 3.0 µg/liter.

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**Table 1. Summary of Phencyclidine Intoxication Cases**

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>µg/liter</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>F</td>
<td>8.7</td>
<td>Rape victim, no symptoms reported.</td>
</tr>
<tr>
<td>29</td>
<td>M</td>
<td>7</td>
<td>Hit and run driver, blood alcohol (1.0 g/liter).</td>
</tr>
<tr>
<td>*</td>
<td>M</td>
<td>23</td>
<td>Arrested for narcotics use.</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>26</td>
<td>Hit and run driver.</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>27</td>
<td>Driver, cause of traffic accident. Blood alcohol (0.10 g/liter).</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>43</td>
<td>Arrested for narcotics use.</td>
</tr>
<tr>
<td>*</td>
<td>M</td>
<td>46</td>
<td>Drunk for narcotics use.</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>49</td>
<td>Arrested for driving under the influence of drugs.</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>57</td>
<td>Arrested for driving under the influence of drugs.</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>59</td>
<td>Driver, cause of traffic accident.</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>63</td>
<td>Arrested for driving under the influence of drugs.</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>67</td>
<td>Subject unconscious in vehicle.</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>76</td>
<td>Arrested for driving under the influence of drugs.</td>
</tr>
<tr>
<td>26</td>
<td>M</td>
<td>77</td>
<td>Arrested for driving under the influence of drugs. Secobarbital and amobarbital* 1.5 to 2 mg/liter; methaqualone 1 to 1.5 mg/liter.</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>80</td>
<td>Driver, cause of traffic accident.</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>82</td>
<td>Hit and run driver.</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>110</td>
<td>Arrested for narcotics use. No morphine found.</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>115</td>
<td>Arrested for driving under the influence of drugs.</td>
</tr>
<tr>
<td>*</td>
<td>M</td>
<td>115</td>
<td>Arrested for narcotics use.</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>125</td>
<td>Arrested for drunk driving.</td>
</tr>
</tbody>
</table>

* not available

* blood concentration
The following compilation of six representative examples includes brief summaries of the individuals' behavior as described in the associated police report. Table 1 gives pertinent data for the remaining 20 cases. Unless otherwise indicated, the samples were screened and found negative for barbiturates, benzodiazepines, carbamates, methaqualone, ethchlorvynol, and ethanol (10).

1. A 21-year-old man was arrested for driving under the combined influence of alcohol and drugs. He was extremely nervous, agitated, and talkative to the point of incoherence. When asked to recite the alphabet, he did so correctly, then recited unrelated letters, then again rapidly recited the alphabet. His eyes were glasy, the pupils "dilated" and "pulsating," and his eyes showed severe lateral nystagmus. His eyes did not properly converge when tested. His blood alcohol concentration was 100 mg/liter; phencyclidine 34 µg/liter; no amphetamine or methamphetamine was found.

2. An 18-year-old man was stopped for speeding and erratic driving. He was unsteady on his feet, exhibited a "fixed, glassy stare," and contracted pupils. A field sobriety test was administered. He had "great difficulty" in performing the heel-to-toe walk, almost lost his balance, and was not able to maintain his balance on one foot for more than 4 s. He could not recite the alphabet. Phencyclidine was found in his possession. His blood phencyclidine concentration was 50 µg/liter.

3. A 19-year-old man was observed swerving from lane to lane and scraping the curbing while operating an automobile. The subject staggered considerably; his speech was slurred, and his eyes had a "thick stare." The subject almost fell when asked to perform a balance test. He could not balance on one foot, and staggered considerably with wide steps during the heel-to-toe walk. Marijuana was in his possession.

His blood phencyclidine concentration was 44 µg/liter.

4. An adult male pedestrian, 20 years of age, was observed staggering along a roadway. The subject responded to questions by attempting to whistle. His mouth appeared dry; thick saliva was on his lips. His eyes were bloodshot and watery; he had difficulty standing and swayed considerably.

His blood phencyclidine concentration was 150 µg/liter.

5. A male pedestrian, 17 years of age, was observed staggering, walking into cement walls, and falling into bushes. The subject's speech was slurred and incoherent; his eyes were reddened and watery, and his mouth appeared dry. He was not able to understand questions and any responses were unintelligible.

His blood phencyclidine concentration was 180 µg/liter.

6. A 19-year-old man was observed kneeling, beating his head on the ground, and screaming. The subject was unresponsive, was not able to walk, and was not cognizant of what was occurring. The subject's pupils were unresponsive to light and contracted; mucous was apparent about his nose and mouth. The subject had phencyclidine in his possession. The blood phencyclidine concentration found was 240 µg/liter.

The most commonly encountered symptoms in these cases include a "glassy" expression, ataxia, and, at higher drug concentrations, incoherence and disorientation. An excited state was noted in one case. Unfortunately, because of the nature of the samples involved, the possible contribution of other, undetected drugs to the behavior cannot be dismissed. However, the symptoms are consistent with those generally attributed to phencyclidine intoxication (I, 9).

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References


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