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Note

Comparison of methods of analysis for phencyclidine

ROBERT D. BUDD
Los Angeles County Medical Examiner-Coroner, Los Angeles, CA 90033 (U.S.A.)
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Phencyclidine (PCP, Angel Dust) is frequently abused[1,2] and its users frequently find themselves in trouble with the police, injured in emergency rooms, or occassionally even dead. Forensic laboratories, hospital emergency room laboratories and toxicology laboratories are frequently requested to analyze biological specimens for its presence. Procedures utilizing gas chromatography (GC)[3–6], enzyme multiplied immunoassay technique (EMIT)[7], radioimmunoassay (RIA)[8–10], thin-layer chromatography (TLC)[10–12], and gas chromatography–mass spectrometry (GC–MS)[13,14] have been developed for the analysis of phencyclidine. This study will attempt to show systematically the advantages and disadvantages of each of these five classes of methods so that laboratories can more easily choose the best method for their needs.

MATERIALS AND METHODS

The GC analyses were performed using equipment from Hewlett-Packard (Fullerton, CA, U.S.A.). Phencyclidine can be analyzed from any biological specimen[6] as follows.

A volume of 5 ml of biological fluid or 10 ml of tissue homogenate (tissue–H_2O, 1:1), 50 μl of internal standard solution (chlorpheniramine, 0.1 μg/ml), 1 ml of 10 *M* sodium hydroxide, and 15 ml of extraction solvent (*n*-butyl chloride–hexane–isoamyl alcohol, 400:50:2.5) are added to a 50-ml centrifuge tube. The mixture is shaken, then centrifuged. The solvent layer is transferred to another tube containing 3 ml of 0.5 *M* hydrochloric acid. This mixture is shaken and centrifuged. The solvent (upper) layer is aspirated and discarded. The acid layer is transferred to a 12-ml centrifuge tube containing 0.3 ml of 10 *M* sodium hydroxide and 0.1 ml of chloroform. The mixture is vortexed and centrifuged. An aliquot of the chloroform layer is then injected into a gas chromatograph equipped with a 6 in. × 2 mm (I.D.) glass column packed with 3% OV-17 operated at 210°C and equipped with a nitrogen sensitive detector. The retention times for phencyclidine and chlorpheniramine are 2.4 and 4.0 min, respectively. Phencyclidine concentrations as low as 0.002 μg/ml can be detected.

The EMIT phencyclidine analyses[7] were performed using equipment and reagents from Syva (Palo Alto, CA, U.S.A.). The analysis is performed as follows.

To a 2.0-ml disposable beaker are added 50 μl of urine specimen or standard

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solution, 250 μl of a 0.055 *M* Tris–HCl buffer (pH 8.0), and 50 μl of an antibody–substrate mixture (phencyclidine and substrate: glucose-6-phosphate and nicotinamide adenine dinucleotide). To this mixture 50 μl of enzyme-labeled phencyclidine solution is added and the mixture is immediately aspirated into the photocell (30°C) of a spectrophotometer set at 340 nm. The optical density of the solution is measured at 15 sec and at 45 sec after the reagents are added to the photocell. The difference between the two readings is then recorded. Sample results are compared to standard results. Samples with differences (Δ values) greater than the cut-off standard are considered positive; others, negative.

The RIA analyses[8] were performed using a gamma scintillation counter from Tracor Analytic (Elk Grove Village, IL, U.S.A.) and reagents from Roche Diagnostics (Nutley, NJ, U.S.A.). The RIA analysis was done as follows.

To a tube are added 0.5 ml of biological fluid or tissue homogenate and 0.25 ml of physiological saline. The mixture is vortexed, then centrifuged. After centrifugation 0.125 ml of the supernatant fluid is transferred to a second tube containing 0.200 μl of ^{125}I-labelled phencyclidine antigen and 0.200 μl of phencyclidine antibody. The tube is vortexed and then allowed to incubate at room temperature for 1 h. After incubation, 0.5 ml of saturated ammonium sulfate solution is added and the tube is vortexed and allowed to incubate at room temperature for 15 min. The tube is then centrifuged and the supernatant fluid is transferred to a third tube which is counted in a gamma scintillation counter for 1 min. The sample results are compared with those of the standards.

The TLC analyses were performed as follows: 15 ml of urine specimen, 2 ml of 2 *M* Tris buffer solution, and 20 ml of 5% isopropanol–chloroform are added to a 50-ml centrifuge tube. The mixture is shaken at low speed for 15 min, and the aqueous layer is aspirated and discarded. The remaining organic layer is evaporated to dryness under nitrogen in a 60°C waterbath after 3 drops of 1% hydrochloric acid–methanol are added. After concentration the sample residues are reconstituted with 100 μl of methanol, then spotted on a 20 × 20 cm Merck silica gel G plate which is developed in a solvent system of ethyl acetate–methanol–diethylamine (90:10:1.6) or methylene chloride–*n*-butanol–concentrated ammonia (85:15:0.5). After development, the plate is air dried then sprayed with acidified iodoplatinate to visualize the purple-gray spots for phencyclidine[10–12].

The gas chromatography–mass spectrometry analyses were performed using an instrument from Finnigan (Costa Mesa, CA, U.S.A.). The analyses were performed as follows.

An aliquot of the chloroform layer of an extraction residue (such as one from the described GC procedure) is injected into a GC–MS system equipped with a 6 in. × 2 mm (I.D.) glass column packed with 3% OV-1 on Gas Chrom Q, 100–120 mesh, operated at 225°C. The major ion for phencyclidine is at *m/z* 159.

METHOD COMPARISON

Each of the general classes of methods for the analysis of PCP presented has advantages and disadvantages in regards to specificity, sensitivity, analysis time, cost, accuracy, etc. (Tables I and II). In selecting a method to employ, each laboratory will have to make their decision based on their particular needs and budget. It is

TABLE I

COMPARISON OF METHODS

	TLC	*GC*	*RIA*	*EMIT*	*GC-MS*
PCP detectable	Yes	Yes	Yes	Yes	Yes
Metabolites detectable	Yes	Yes	Poorly	Poorly	Yes
Differentiable from PCP	Yes	Yes	No	No	Yes
Related drugs detectable	Yes	Yes	Yes	Yes	Yes
Differentiable from PCP	Yes	Yes	No	No	Yes
Other drugs detectable	Yes	Yes	No	No	Yes
Differentiable from PCP	Yes	Yes	Yes	Yes	Yes
Interference from other drugs	Occ.	Occ	No	No	No
False positives	Few	Rare	Very rare	Rare	Very rare
False negatives	Rare	No	No	No	Rare
Minimum concentration		0.001 (N/P)			
Detectable (μg/ml)	0.1	0.1 (FID)	0.0005	0.15	0.005
Equipment cost	Low	Mod.	High	High	Very high
Reagent cost	Low	Low	High	High	Low
Objectivity in result interpretation	No	Yes	Yes	Yes	Yes
Adaptability for mass screening	Yes	Limited	Yes	Yes	No
Analysis time/1000 samples	Mod.	Slow	Mod. fast	Mod.	Very slow
Analysis time/1 sample	Mod. slow	Mod. fast	Slow	Fast	Mod.
Specimen required	All (Urine best)	All	Biological fluids	Urine	All

generally advisable that two different methods be employed to minimize the possibility of a false positive result.

As a laboratory faces the decision of choosing the best method for PCP analysis, several factors must be considered:

(1) the concentration minimum to be detected (ranging from overdoses to trace amounts),

(2) the type of samples to be analyzed (urine, blood, serum, and/or other tissues),

(3) the volume of samples to be analyzed,

(4) the time requirements for analysis,

(5) expense limitations,

(6) whether the analysis is to be qualitative or quantitative,

(7) whether other basic drugs are to be simultaneously detected.

In practice, one must choose between EMIT, RIA, TLC, GC, and GC–MS, as UV is too insensitive and non-specific to be of much value and color tests are of limited value even for pure powdered specimens and are totally worthless for sample extracts.

In an emergency room situation, a rapid method is required, as well as one that can be at least roughly quantitative and able to detect other related drugs (in case PCP itself is not the culprit). EMIT (when the machine is kept on and calibrated) can give a semiquantitative PCP result for a urine specimen in just a matter of minutes. TLC can give a semiquantitative result for PCP and many other basic drugs in about an hour and a half. GC is probably the method of choice in this situation. It can give a quantitative result for PCP and many other basic drugs in about 15 minutes for a urine specimen and in about half an hour for a blood specimen.

TABLE II

COMPARISON OF METHODS

Method	*Advantages*	*Disadvantages*
TLC	Detects and differentiates PCP, PCP metabolites, PCP-related drugs, and other basic drugs Good for large sample volumes No false negatives All biological samples can be tested Only standard laboratory equipment needed Low reagent cost	Occasional problems with tissue homogenates and badly decomposed blood samples; best for urine Limited sensitivity Fairly slow for a single sample Possible interferences from other substances
GC	Detects and differentiates most basic drugs (PCP, PCP metabolites, PCP-related drugs, and other basic drugs) Best for quantitation Excellent sensitivity No false negative Rare false positives Lowest reagent cost Quite fast for a single sample Good for all biological samples	Slow for many samples Occasional interferences from other substances (particularly decomposed products) Moderate equipment cost
RIA	Detects PCP and closely structurally related compounds Best for large sample volumes All biological fluids can be tested Best sensitivity No false positives No interference from unrelated drugs or substances No false negatives in blood or urine	Very slow for single samples High equipment cost High reagent cost Occasional problems with bile and stomach contents samples No differentiation between PCP, metabolites, and related drugs Limited quantitative range
EMIT	Detects PCP and closely structurally related compounds No false negatives Fastest for a single sample Good for large sample volumes No interference from other unrelated drugs	Only urine can be analyzed High equipment cost High reagent cost Poorest sensitivity Occasional false positives No differentiation between PCP, metabolites and related drugs Not strictly quantitative
GC–MS	Detects and differentiates PCP, PCP metabolites, PCP-related drugs, and other basic drugs Good for quantitation Good sensitivity No false positives Low reagent cost All biological samples can be tested Can be very specific for PCP	Slow for even one sample; extremely slow for several samples; impossible for large sample volumes Highest equipment cost Possible false negatives at high concentrations — overloading of mass spectrometer system Greatest potential for equipment problems

In large scale analysis situations, a mass screening method is generally needed: EMIT, RIA, and TLC can all be readily adapted. EMIT is reasonably rapid and quite specific for PCP, but is limited to urine, not particularly sensitive, and is rather expensive. RIA is extremely sensitive, can analyze any fluid, and is quite specific for PCP, but it is the most expensive of the three methods. TLC is reasonably rapid, can analyze any fluid (though best for urine), can detect other basic drugs and PCP-related drugs (differentiating many of them, depending on the solvent system), and is the most inexpensive method, but is only slightly more sensitive than EMIT. All of these methods are semiquantitative at best, and are best coupled with a GC or GC–MS method to confirm positives, eliminate false positives, and provide a quantitative result.

In a general (smaller-scale) laboratory situation, a pair or (even better) a trio of different methods should be employed. For a quantitative result one of the mass screening methods can be combined with GC or GC–MS or, if the workload is small enough, GC and GC–MS themselves can be paired. For a qualitative result EMIT or RIA can be paired with TLC. The following illustrations should provide greater clarity of the situation and workable solutions.

In a 600 urine sample per day laboratory situation where it was necessary to detect other drugs (all at concentrations of 0.1 μg/ml), specimens were screened by TLC. PCP positives were confirmed by RIA and TLC with a completely different solvent system (Budd and Leung[10]). If other drugs had not been important, screening could have been accomplished by RIA followed by confirmation with both TLC solvent systems.

In a 70 sample per fortnight forensic laboratory situation where PCP alone was to be detected at 0.001 μg/ml concentrations, excellent results were found by screening the biological fluids and tissue homogenates by RIA followed by confirmation with GC with nitrogen–phosphorus detection (N/P GC). Alternatively these samples could be analyzed by N/P GC and confirmed by GC–MS or N/P GC using a completely different column.

In a 70 sample per week forensic laboratory situation where PCP and other basic drugs were to be detected in blood specimens at 0.01 μg/ml concentrations, the blood samples were screened by N/P GC and confirmed by GC–MS.

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