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Note

Capillary gas chromatography with a nitrogen detector for measurement of phencyclidine, ketamine and other arylcycloalkylamines in the picogram range

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Bailey *et al.*[1], Alatorre and Simpson[2] and Yago *et al.*[3] have developed similar gas chromatography (GC)–nitrogen detector methods for the determination of phencyclidine (PCP); detector response is linear from 1 to 1000 ng/ml. These procedures are based on general methods of extraction of basic drugs from plasma and differ only in the technical details of extraction and the internal standard used (meperidine, mepivacaine and the morpholine derivative of PCP, respectively). In clinical-laboratory studies on PCP users, we found many subjects with less than 1 ng/ml in blood, several of whom had very high (200–500 ng/ml) levels of PCP in urine collected simultaneously; others had low levels in urine, but admitted recent and/or remote use of PCP. We found that we could regularly detect, and quantitatively measure with the computer data system, 0.5 to 1.0 ng/ml of PCP added to blank human plasma; we could regularly detect, and often quantitate, 0.1 to 0.5 ng/ml.

ANALYTICAL METHODS

Reagents

PCP and its analogues were purchased from Applied Science Labs. (State College, Pa., U.S.A.). All other chemicals were of reagent grade and were obtained from Sigma (St. Louis, Mo., U.S.A.).

Gas chromatography

A Varian (Palo Alto, Calif., U.S.A.) automated gas chromatograph (Model 3711) equipped with a chromatography data system, with a capillary column kit, with Varian thermionic specific (nitrogen- or phosphorus-selective) detectors and with a Varian strip-chart recorder was used for sample analysis.

A glass column (2 m × 2 mm I.D.) packed with 3% of OV-17 on 80–100 mesh Chromosorb W HP (Varian) was used for detection and measurement of PCP and other arylcycloalkylamines at sample concentrations of 0.1 to 1000 ng/ml. Chromatographic conditions were: column initially at 220° (held for 8 min and programmed to increase 50°/min to 270° (held for 1 min for column cleanup); injector 210°; detector 300°; carrier gas, nitrogen (30 ml/min); air, 220 ml/min; hydrogen, 2.2 ml/min;

detector bias voltage "4"; detector bead voltage "5.5"; attenuation $8 \cdot 10^{-12}$ A/mV; 3 μl of sample injected. Under these conditions, the retention time was 2.6 min for PCP, 3.6 min for PCP–morpholine, 2.17 min for TCP–pyrrolidine, 3.44 min for TCP–morpholine, and 3.05 min for ketamine.

A 25-m capillary glass column packed with OV-101 (Chrompack, Middelburg, The Netherlands) was used for detection and measurement of PCP and other aryl-cycloalkylamines at sample concentrations of 5 to 1000 pg/ml. Glass capillary GC conditions were: column initially at 150° (held for 5 min) and programmed at 10°/min to 200° (held for 1 min); injector 210°; detector 310°; carrier gas, nitrogen (2 ml/min); detector make-up, 28 ml/min; air, 170 ml/min; hydrogen, 4.5 ml/min; detector bias voltage "4"; detector bead voltage "6.5"; attenuation $8 \cdot 10^{-12}$ A/mV; splitless-injection technique of 8 μl with 20 sec split delay, and split ratio 10^3:1. The retention time was 9.2 min for PCP and 9.8 min for PCP–morpholine.

Sample preparation

To 2 or 3 ml of sample (plasma, serum, urine, saliva, tissue extract or aqueous standard solution) in a 5-ml conical glass centrifuge tube with a ground-glass stopper were added 1 ml of 167 m*M* NaCl, 1 ml of internal standard (morpholine analogue of PCP or others), 400 μl of 1.5 *M* Tris–HCl (pH 9) and 100 μl of *n*-heptane–isoamyl alcohol (98.5%, v/v). With the ground-glass stopper in place, the sample was then vigorously mixed for 1 min (Vortex-action mixer) and then centrifuged at 2000 *g* for 6 min. The lower aqueous phase was removed by careful aspiration, and the 100 to 150 μl remaining were centrifuged again for 6 min at 2000 *g*. Then 100 μl of the organic phase were recovered from the conical tip of the centrifuge tube with a micropipette and transferred to a stoppered glass tube (50 × 4 mm) for storage and subsequent sampling.

RESULTS

Packed-column GC

Recovery of PCP and/or PCP–morpholine by extraction from aqueous standard solutions and biological samples was 95–99%, and the percentage recovery was identical for PCP and the PCP–morpholine internal standard from the same sample. Calibration graphs were linear for PCP and PCP–morpholine from 0.5 to 1000 ng/ml for extractions from aqueous standard solutions and from normal biological samples with added PCP and PCP–morpholine.

The reproducibility of the assay was tested by making 10 injections of 3 μl of *n*-heptane–isoamyl alcohol extract of the same plasma sample. The mean for samples containing 10 ng/ml was: 10.00 ng/ml $\pm$ 0.27 (S.D.). The reproducibility of the extraction method was tested by making extractions of 10 separate 2-ml aliquots of the same normal plasma with added PCP and PCP–morpholine. The mean values for 1, 10, and 100 ng/ml, respectively, were: 1.00 ng/ml $\pm$ 0.08, 10.04 ng/ml $\pm$ 0.65, and 100.05 ng/ml $\pm$ 2.5.

Glass capillary GC

Recovery of PCP and its analogues was quantitative by this technique, and the calibration graph was linear from 5 to 1000 pg/ml (original sample concentration). The reproducibility of the assay was excellent. Ten consecutive aliquots of 8 μl of extract from plasma containing 100 pg/ml were injected and gave mean values of

100.13 pg/ml ± 5.9. The reproducibility of extraction was also excellent; 10 consecutive extractions of 10 aliquots (2 ml) of plasma containing 100 pg/ml gave 101.58 pg ± 2.0.

DISCUSSION

These capillary GC methods for measurement of PCP, ketamine and other arylcycloalkylamines in the picogram range are highly sensitive, specific, reliable, and, unlike many other methods for measuring trace quantities of substances, technically relatively simple to perform. Capillary GC requires technical experience, but is easily learned by anyone accustomed to GC work.

These methods are important because their development is essential to efforts to comprehend fully the pharmacokinetics of PCP and its analogues. Some of these compounds are used legitimately as anesthetic agents, and many more are used illicitly as the most common drugs of abuse in what has been repeatedly and accurately termed an increasing epidemic[4]. Methods based on UV absorption and high-performance liquid chromatography[5], GC with flame ionization detection[6,7] and radioimmunoassay[8] have lowest detection limits of 100–200 ng/ml. These compounds are so lipophilic, however, that blood levels fall very rapidly to 10 ng/ml or less while the experimental animal or human patient is still grossly intoxicated. In the rat, at 1, 2 and 12 h after i.p. injection of PCP (50 mg/kg), all absorbed PCP is in the lipid tissues, with accumulation in brain lipids[9]; no long-term studies of the probable very slow clearance of these compounds from brain and other organs with considerable lipid content has so far been published. Domino[10] could find only 9.5 mg (1.9%) of a 500-mg load of PCP in the urine of a comatose patient treated vigorously with forced diuresis and acidification for 65 h to the point of recovery of consciousness; presumably, the unexcreted 95–98% remained in the brain and body lipids at the end of that phase of treatment, to be excreted very slowly over the succeeding weeks and months. These re-excretion pharmacokinetics, and probable re-excretion pharmacological effects, require study, and this will necessitate the more sensitive analytical methods described in this report.

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