Journal of Chromatography, 137 (1977) 135–143
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 9914

SEPARATION AND DETECTION OF PHENCYCLIDINE IN URINE BY GAS CHROMATOGRAPHY

PETER M. FROEHLICH* and GAIL ROSS

Department of Chemistry, Trace Analysis Research Centre, Dalhousie University, Halifax, Nova Scotia B3H 4J3 (Canada)

(Received December 28th, 1976)

SUMMARY

A rapid, sensitive and selective analytical procedure for phencyclidine and one of its metabolites in urine has been developed. Three techniques have been studied for extraction of the drug from the biological matrix: (*a*) reversed-phase XAD resin, (*b*) charcoal absorption, and (*c*) solvent extraction using chloroform. Temperature-programmed gas chromatography was used to quantitate the illicit drug. Solvent extraction appears to offer the most efficient separation of the drug and its metabolite, as the recovery was 94% and the technique required only 7–8 min per sample. Reversed-phase column extraction is also quite useful; although more time-consuming for an individual sample, it would be useful for screening purposes.

INTRODUCTION

Phencyclidine, 1-(1-phenylcyclohexyl)piperidine (PCP) was developed as an anaesthetic in the 1950s and marketed as Sernyl in 1958. Although the drug appeared to be useful in certain types of surgery[1–8] and as a possible remedy for some psychological disorders[9], its use was limited by observations that some patients exhibited severe degrees of manic behaviour[1], hallucinations and delirium[2] after its administration. It was noted that the psychotic reaction was most frequently encountered in young and middle-aged males, the most violent forms following 20-mg doses, and that the excitation was not noted in elderly patients[2]. As a result of these adverse reactions to PCP, the drug was not approved for general usage, and is now used only for animals.

In recent years, PCP has become a serious drug of abuse, often being sold under such names as LBJ, Angel Dust and the Peace Pill[10]. Several instances of the use of PCP have led to overdose intoxication and death, and PCP is frequently sold in combination with (or in place of) other illicit drugs such as mescaline, LSD, THC and amphetamines[11–13]. The common use of PCP may be related to the relative ease

* To whom correspondence should be addressed. Present address: Department of Chemistry, North Texas State University, Denton, Texas 76203, U.S.A.

of preparation[14] of the drug; to exemplify this point, the Royal Canadian Mounted Police seized more than 100 kg of the drug and 900 kg of precursors from five underground laboratories in Canada during the first six months of 1976 (see ref. 15).

The serious consequences of ingestion of PCP would suggest that an analytical procedure for determining the drug in body fluids would be of great value for emergency-room personnel; since the victim may be unaware that the abused drug is PCP, such an analytical procedure would be even more useful. To date, several procedures (all based on gas chromatography) have been reported for determining the drug in dosage forms. Lin *et al.*[16] have developed an analytical procedure for determining the drug in urine or blood by gas chromatography–chemical ionization mass spectrometry and a recent report indicates that the drug may be quantified by gas chromatography after extraction from urine[17].

We report here the development of a rapid analytical procedure for determining PCP and one of its metabolites in urine. Urine was chosen because the levels therein are notably higher than those in blood or serum. The fact that a metabolite, 1-(1-phenylcyclohexyl)piperidin-4-ol (PCP·OH) is readily observable should provide useful confirmation of the presence of PCP. An additional advantage of the detection of the metabolite is the possibility of determining the presence of the drug in those cases where much of it has already been metabolized.

EXPERIMENTAL

Chemicals

Phencyclidine (Lot CI-395, Parke Davis & Co., Brockville, Canada), ketamine hydrochloride (Lot C430738, Parke Davis & Co.) 1-(1-phenylcyclohexyl)piperidin-4-ol (Lot Q, Parke Davis & Co., Detroit, Mich., U.S.A.), caffeine and 1-phenylcyclohexene (Aldrich, Milwaukee, Wisc., U.S.A.) were used as received.

All solvents used were Fisher certified-reagent grade (Fisher Scientific, Pittsburgh, Pa., U.S.A.). Norit A (Fisher) was used as a decolorizing charcoal. Amberlite resins were obtained from Mallinkrodt [St. Louis, Mo., U.S.A. (XAD-2)] and BDH [Toronto, Canada (XAD-4 and XAD-7)]. Pre-packaged XAD-2 columns were obtained from a Drug-Skreen Kit (Brinkman Instruments, Rexdale, Canada).

Urine was pooled from healthy individuals and stored at 4° for periods up to 7 days.

Ketamine hydrochloride (KET·HCl) was used as internal standard.

Drug extraction from urine

Using a macro-reticular resin. Resins were cleaned by stirring four times with four bed-volumes of acetone, three times with three bed-volumes of methanol and three times with three bed-volumes of distilled water as previously recommended[18,19]. The resins were stored for at least a week at 4° under distilled water to ensure optimum hydration and then packed into glass chromatography columns (25 × 0.7 cm I.D.) to a height of 10 cm (approximately 1.3 g of dry resin).

A 100-μl sample of PCP and internal standard was mixed with 10 ml of urine, the pH was adjusted to 9 with 5 *N* sodium hydroxide, and the sample was mixed and centrifuged for 2 min. The supernatant was then placed on the XAD column and the flow was controlled; when the flow ceased, suction was applied to the tip to remove

residual urine. The column was then eluted with 20 ml of methanol–chloroform (1:9), the eluate was mixed with 2 ml of 0.5 *N* sodium hydroxide (back-extraction), and the separated organic layer was made acidic with 1 drop of concentrated hydrochloric acid–methanol (1:1) and evaporated to dryness under nitrogen in a water bath at 55°. The residue from the methanol–chloroform extraction was reconstituted in 100 μl of acetone, and 1 μl was injected into the chromatograph.

Using a universal absorbent (charcoal). Approximately 100 mg of charcoal were mixed with 1 ml of buffer solution (0.4 *M* Na_2CO_3 and 0.1 *M* $NaHCO_3$, pH 11) to ensure that it was completely wetted; 10 ml of distilled water were added to the residue. The charcoal–water mixture was shaken vigorously and centrifuged, and the aqueous layer was discarded. Then 2.5 ml of methanol–chloroform (1:9) were added, and the sample was placed on a vortex-type mixer for approximately 30 sec. The organic layer was removed, and filtered through glass wool to remove traces of charcoal (the glass wool was washed with an additional 0.5 ml of elution solvent, which was added to the filtrate). The solvent system was removed in the same way as described above.

Using micro-phase solvent extraction. Solid potassium chloride (5%, w/v) was added to 5 ml of urine containing PCP and internal standard, and the pH was adjusted to 9 with 0.5 *N* sodium hydroxide, then 100 μl of chloroform were added to the tube, which was shaken and centrifuged. A 1-μl portion of the organic phase was withdrawn through the aqueous layer and injected into the gas chromatograph.

Gas chromatography

A Tracor model 550 gas chromatograph (Tracor, Austin, Texas, U.S.A.) equipped with a flame ionization detector was used; it was fitted with a column (1.7 m × 2.0 mm I.D.) containing 5% of SE-30 plus 1% of Carbowax 20M on Chromosorb W HP (80–100 mesh). The column temperature was programmed from 150 to 230° at 7.5°/min, the injection-port temperature was held at 210° and the detector was kept at 270°.

RESULTS AND DISCUSSION

The development of an analytical scheme for PCP and its metabolites in body fluids involves separation of the compounds of interest from the matrix before quantitation. We have considered three common procedures for the separation of the drug and its metabolites from urine: (a) reversed-phase extraction using a macro-particulate resin[18,19], (b) charcoal absorption[20] and (c) solvent extraction[21]. Several criteria were considered in the choice of suitable pre-concentration procedures, including the fraction recovered, the presence of compounds that would interfere with the detection step, the simplicity of the procedure, and the amount of time required.

Reversed-phase solvent extraction with macro-reticular resin

The use of micro-reticular resins such as XAD has become popular for the separation of weakly acidic, weakly basic and neutral drugs in urine[18,19,22–24]. Various factors were studied in order to maximize the efficiency of this method, including flow-rate, the use of back-extraction procedures, and the method of solvent removal

TABLE I

EFFECT OF FLOW-RATE AND BACK-EXTRACTION ON RECOVERY OF PCP·HCl AND KET·HCl FROM XAD-2 RESIN

Type of extraction	*Recovery* (%)*		*Flow-rate (ml/min)*
	PCP·HCl	*Ket·HCl*	
Simple	60 ± 10	93 ± 4	5.6–8.5
Simple	68 ± 5	106 ± 5	4.0–6.6
Simple	58**	89	2
Back-extraction	58 ± 2	92 ± 3	5.6–8.5
Back-extraction	84 ± 7	101 ± 3	2

* Based on peak-height comparisons with a drug standard.

** Average of three determinations (all other values are the average ± S.D. of results for six samples).

before injection into the gas chromatograph. The importance of control of the flow-rate can be seen from Table I and Fig. 1. Use of gravitational flow (4–8 ml/min) leads to poor recovery of PCP and a high background. Incorporation of back-extraction into the procedure reduced the urine background considerably, but did not increase the rate of recovery. Control of the urine flow (2 ml/min) through the resin resulted in chromatograms having a high background, which again resulted in poor quanti-

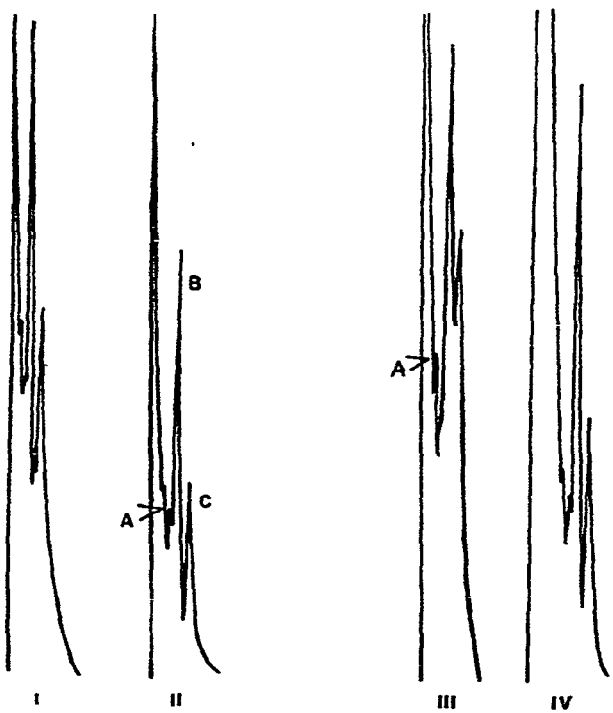

Fig. 1. Effect of flow-rate and extraction on drug recovery: I, gravitational flow, no back-extraction; II, gravitational flow, back-extraction; III, flow controlled (2 ml/min), no back-extraction; IV, flow controlled (2 ml/min), back-extraction. A = Caffeine; B = ketamine; C = PCP.

tation. The recovery could be significantly improved by including the back-extraction. This is not to suggest that the extraction actually increased the recovery; what happens is that the background in the chromatogram (see Fig. 1) is reduced, so that the quantitation is improved upon back-extraction.

The effect of the choice of solvent may be seen from Table II. In this respect, it might be pointed out that, although we used 20 ml of the methanol–chloroform (1:9), about 80% of the PCP and more than 98% of the ketamine used as standard were eluted within the first 10 ml.

TABLE II

EFFECT OF VARIOUS ELUTION SOLVENTS ON RECOVERY OF PCP·HCl AND KET·HCl FROM XAD-2 RESIN

Elution solvent	*Recovery (%)*		*Comments*
	PCP·HCl	*Ket·HCl*	
Chloroform	63*	100	Low background; good drug-peak shape and separation
Methanol–chloroform (1:9)	94	104	As above; somewhat higher background
Methanol–chloroform (1:3)	26	36	Poor drug separation from high background; unsuitable for use
Hexane	—	—	No apparent recovery
Isopropanol–chloroform (1:9)	54	96	Background similar to use of CH_3OH–$CHCl_3$ (1:9); good peak profiles
Isopropanol–chloroform (1:3)	24	32	Relatively low background, but small broad drug peaks; unsuitable for use
Isopropanol–ethyl acetate–dichloroethane (5:9:6)	12	30	Highly coloured residues; little drug recovery; unsuitable for use

* Values are the average for duplicate samples.

A water bath was used to remove solvent from the eluted sample. We found that the recovery of both PCP and standard was maximized by the addition of one drop of concentrated hydrochloric acid–methanol (1:1) before evaporation of the methanol–chloroform solvent system. The drug recovery fell if the tube containing the residue was not removed shortly after the solvent was completely evaporated; for example, recovery of the drug was 43% if the sample was removed from the heat 5 min after dryness was achieved; this value fell to 21% if the interval was 30 min. In the presence of the hydrochloric acid, quantitative recoveries of both PCP and standard were obtained, even with a 30-min period of heating following total evaporation of solvent.

The resin technique was extended by including the drug metabolite PCP·OH to the urine; the recovery of metabolite was maximized by increasing the pH of the urine from 6 to 9 before application to the resin bed[23].

The flow-rate of the methanol–chloroform eluent was fairly slow; it could be improved by adding 1 ml of acetone to the column before the eluent or by the use of pressure by placing the palm of the hand over the top of the column reservoir. The use of XAD-7 resin led to a flow-rate that had to be controlled at 2 ml/min.

To determine the useful range of the method, 4 μg of PCP, 5 μg of PCP·OH and 1.6 μg of ketamine were extracted from 10, 20, 30 and 40 ml of urine; recovery of both drugs was greater than 90% at all four concentrations. A sample chromatogram is shown in Fig. 2; the PCP·OH is resolved only poorly, and a total loss of peak separation for PCP·OH was noted at 0.17 ppm due to the presence of unknown impurities. For PCP, the technique is suitable to the 0.1-ppm level, or lower, and appears to be limited only by the inability to obtain a suitably clean extract*.

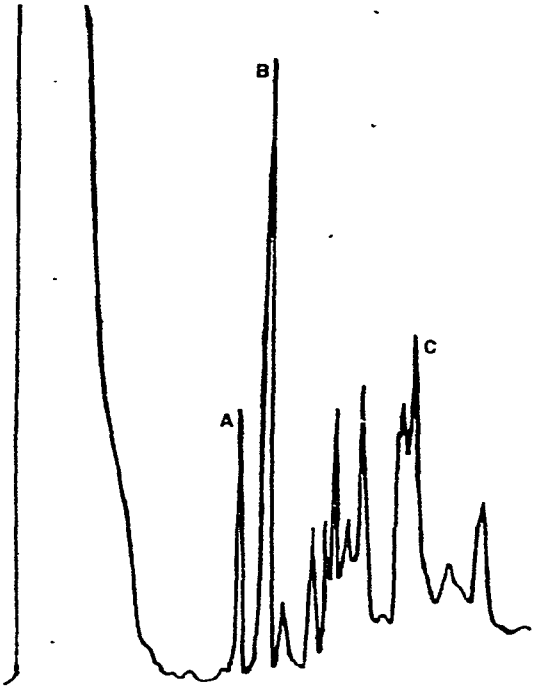

Fig. 2. Chromatogram of residue from resin extraction: A = 0.4 ppm of PCP; B = 1.6 ppm of ketamine; C = 0.5 ppm of PCP·OH.

The use of pre-packaged absorbent cartridges containing XAD-2 resin was considered. Flow-rates approaching that of XAD-7 could be achieved if the cotton plugs used for stabilization and support of the column were replaced by glass-wool. Although urine flow-rates were not controlled, the extraction efficiency for PCP was 100% and the corresponding efficiency for ketamine was 96%; recovery of PCP·OH was 89%. We observed a generally higher background at the temperature required for elution of PCP·OH on the chromatogram; this we attribute to extraction of plasticizers from the column (no additional peaks were observed). It is possible that some other solvent system would overcome the problem.

* PCP·OH would be found as the glucuronide in the urine of individuals who have ingested PCP. Lin *et al.*[16] have detected the complexed metabolite in urine and hydrolyze it to PCP·OH with a β-glucuronidase preparation at pH 5. Following hydrolysis, the pH would be adjusted to 9, and PCP·OH would be extracted into the organic phase.

Extraction with a universal absorbent

We observed that the use of wetted charcoal led to extremely clean residues if the solvent system suggested by Cordova and Banford[20] [chloroform–diethyl ether–isopropanol (50:54:10)] was used; unfortunately, recovery of the compounds of interest was low (PCP 22 ± 5%, PCP·OH 35 ± 8% and ketamine 47 ± 7%). When the elution solvent that was successful for the resin-extraction procedure was used with charcoal, recoveries were increased (PCP 72 ± 11%, PCP·OH 75 ± 7% and ketamine 30 ± 3%), but the background increased to a point at which the procedure was useless for quantitation.

Microphase solvent extraction

The recovery of PCP, PCP·OH and the standard by solvent-extraction procedures depends on the relative solubility of the compounds in the two solvents. At the natural pH of urine (approximately 6), chloroform recovered about 74% of the added PCP, whereas methanol–chloroform (1:9) extracted about 85%. If chloroform was used to extract the compounds of interest, it was found that an increase in the pH of the urine to 9 enhanced recovery of PCP to 94%, with quantitative recovery of PCP·OH and the standard. Fig. 3 shows a chromatogram derived from the extraction of urine containing PCP, its metabolite and the standard. The lower limit of sensitivity for PCP is about 0.3 ppm. Addition of the salt to the urine was found to eliminate formation of emulsions, which led to large deviations in drug recovery.

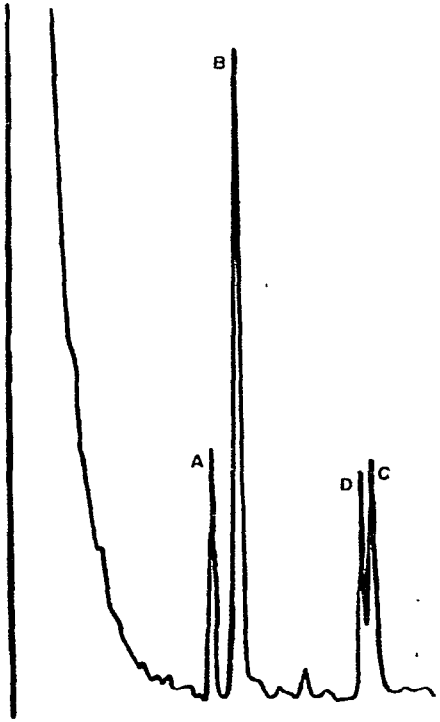

Fig. 3. Chromatogram of chloroform extract: peaks A, B and C as in Fig. 2; D = unknown.

Gas chromatography

It was determined that isothermal operation would permit detection of PCP at 155° with ease; the metabolite was not observable under such circumstances, probably because of the higher polarity of the hydroxy-piperidine ring.

CONCLUSIONS

We have developed a fairly rapid and sensitive procedure for determining PCP and one of its metabolites in urine. Although several assays for the drug in dosage forms have been reported[25–30], only a few useful procedures have been reported for the drug in biological samples. Gupta *et al.*[17] have developed a procedure involving chloroform extraction, but their assay takes longer than 30 min and does not include the metabolite.

A gas chromatography–chemical ionization mass spectroscopy technique that is more sensitive than our procedure has been reported[16]; however, we believe that the use of this procedure is limited by the availability of the instrument and the internal standard (pentadeuterated PCP).

We believe that the analyst would be best served by either the XAD resin extraction procedure or the chloroform extraction procedure. Although the reversed-phase resin procedure is lengthier and slightly less sensitive for the metabolite, it permits a large number of samples to be processed at the same time, which would be useful in a screening programme. For individual samples, the chloroform extraction procedure is recommended as being the fastest and providing a very clean background.

ACKNOWLEDGEMENT

We thank the National Research Council of Canada for support of this work. Special thanks for assistance are due to Dr. W. A. Aue and Dr. S. Kapila of Dalhousie University.

REFERENCES

1 F. E. Greifenstein, M. De Vault, J. Yoshitake and J. B. Gajewski, *Anaesth. Analgesia*, 37 (1958) 283.
2 M. Johnstone, V. Evans and S. Baigel, *Brit. J. Anaesth.*, 31 (1959) 433.
3 A. J. Catenacci, D. P. Grove, W. A. Weiss, S. M. Fisher, A. M. Sismondo and J. H. Moyer, *Antibiotic Med. Clin. Ther.*, 6 (1959) 145.
4 M. E. Riddoch, *Anaesthesia*, 17 (1962) 419.
5 B. J. Muir, V. Evans and J. J. Mulcahy, *Brit. J. Anaesth.*, 33 (1961) 51.
6 J. G. Camilleri, *Anaesthesia*, 17 (1962) 422.
7 R. Y. Gool and H. L. Clarke, *Anaesthesia*, 19 (1964) 265.
8 J. M. Riffin, *J. Med. Soc. New Jersey*, 57 (1960) 15.
9 T. Bodi, I. Share, H. Levy and J. H. Moyer, *Antibiotic Med. Clin. Ther.*, 6 (1959) 79.
10 F. H. Meyers, A. J. Rose and D. E. Smith, *J. Psychedelic Drugs*, 1 (1967) 139.
11 J. M. Rainey, Jr. and M. K. Crowder, *J. Amer. Med. Ass.*, 230 (1974) 824.
12 J. A. Marshman and R. J. Gibbins, *Ont. Med. Rev.*, 37 (1970) 429.
13 R. C. Baselt, L. J. Casarett and N. E. Winn, *Drug Forum*, 1 (1965) 263.
14 V. H. Maddox, E. F. Godefroi and R. F. Parcell, *J. Med. Chem.*, 8 (1965) 230.
15 Anon., *Halifax (N.S.) Mail Star*, May 26, 1976.

16 D. C. K. Lin, A. F. Fentiman, Jr., R. L. Foltz, R. D. Fortney, Jr. and I. Sunshine, *Biomed. Mass Spectrom.*, 2 (1975) 206.
17 R. C. Gupta, I. Lu, G.-L. Oei and G. M. Lundberg, *Clin. Toxicol.*, 8 (1975) 611.
18 S. J. Mulé, M. L. Bastos, D. Jukofsky and E. Saffer, *J. Chromatogr.*, 63 (1971) 289.
19 M. L. Bastos, D. Jukofsky, E. Saffer, M. Chedekel and S. J. Mulé, *J. Chromatogr.*, 71 (1972) 549.
20 J. F. Cordova and T. A. Banford, *J. Forensic Sci.*, 20 (1975) 58.
21 J. Ramsey and D. B. Campbell, *J. Chromatogr.*, 63 (1971) 303.
22 W. L. Miller, M. P. Kullberg, M. G. Banning, L. D. Brown and B. P. Doctor, *Biochem. Med.*, 7 (1973) 145.
23 M. P. Kullberg, W. L. Miller, F. J. McGowan and B. P. Doctor, *Biochem. Med.*, 7 (1973) 323.
24 M. P. Kullberg and C. W. Gorodetzky, *Clin. Chem.*, 20 (1974) 177.
25 J. E. Lindgren, C. G. Hammer, R. Hessling and B. Holmstedt, *Amer. J. Pharm.*, 141 (1969) 86.
26 N. C. Law, V. Aandahl, H. M. Fales and G. W. A. Milne, *Clin. Chim. Acta*, 32 (1971) 221.
27 J. K. Brown, L. Shapazian and G. D. Griffin, *J. Chromatogr.*, 64 (1972) 129.
28 A. Sperling, *J. Chromatogr. Sci.*, 10 (1973) 268.
29 R. A. van Welsum, *J. Chromatogr.*, 78 (1973) 237.
30 C. Shaler, J. M. Parker and A. Sage, *Clin. Toxicol.*, 7 (1974) 307.