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SIMULTANEOUS DETERMINATION OF PHENCYCLIDINE AND MONOHYDROXYLATED METABOLITES IN URINE OF MAN BY GAS CHROMATOGRAPHY—MASS FRAGMENTOGRAPHY WITH METHANE CHEMICAL IONIZATION

EDWARD J. CONE*, WILLIAM BUCHWALD and DAVID YOUSEFNEJAD

National Institute on Drug Abuse, Division of Research, Addiction Research Center, Lexington, KY 40583 (U.S.A.)

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SUMMARY

Phencyclidine and monohydroxy metabolites were measured in human urine using gas chromatography—mass fragmentography with methane chemical ionization. Samples were extracted either untreated or following acid hydrolysis, derivatized with heptafluorobutyric anhydride, separated on a 3% SE-30 column and analyzed by mass fragmentography. The assay was sensitive to ca. 0.01 μg/ml for phencyclidine and ca. 0.05 μg/ml for the metabolites. Urine samples from five human subjects enrolled in a methadone maintenance program who had ingested phencyclidine were analyzed. The phencyclidine concentration ranged from 0.3 to 23.7 μg/ml. The concentrations of metabolites ranged from 0 to 1.8 μg/ml. A new monohydroxy metabolite was detected in the samples, but its structure was not fully elucidated. The specificity of the assay was examined.

INTRODUCTION

Phencyclidine (PCP) was developed in the 1950s for use as an anesthetic agent in man. Its therapeutic usefulness was curtailed, however, when extremely disturbing side-effects were noted in postsurgical patients. These effects included drunkenness, blurring of vision, delusions and a general impairment of mental function [1].

PCP appeared later in the drug subculture of the late sixties as the "Peace Pill". The ready availability of starting materials and the ease of synthesis of PCP and derivatives, together with the lure of substantial financial gains, prompted its widespread illicit synthesis and distribution. As a consequence, PCP has become a major drug of abuse in the United States. Recent reports indicate a rising number of overdose cases [2—5] and PCP-related deaths [6—9].

Detection of drug usage is essential in the treatment of overdosage since PCP can produce a psychosis resembling that of schizophrenia [1,10,11]. Of interest also is the role of pharmacologically active metabolites in the overall spectrum of effects of PCP. The monohydroxylated metabolites [4-phenyl-4-piperidinocyclohexanol (PPC) and 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (PCHP), see Fig. 1] have generally been assumed to be inactive [6,9, 12,13], based on the work by McCarthy and Potter [14] who reported the absence of PCP-like activity for PPC and PCHP in the monkey. Recently, however, studies in this laboratory [15,16] and elsewhere [17] have indicated that both PPC and PCHP exhibit significant biological activity.

The assays that have been developed for measurement of PCP include use of gas chromatography (GC) with flame-ionization detection [7,8], GC with a nitrogen-sensitive detector [5,6], gas chromatography—mass spectrometry (GC—MS) [18], mass fragmentography (MF) [4,8,19—21] and radioimmunoassay [22]. At present, however, there is no assay which also measures the hydroxy metabolites. Consequently, we sought to develop an assay for PCP, PPC and PCHP which would be applicable for the simultaneous measurement of these compounds in human urine. This report describes an MF assay specific for PCP, PPC and PCHP and its application to the analysis of human urine from subjects who had smoked or ingested an unknown quantity of illicit PCP.

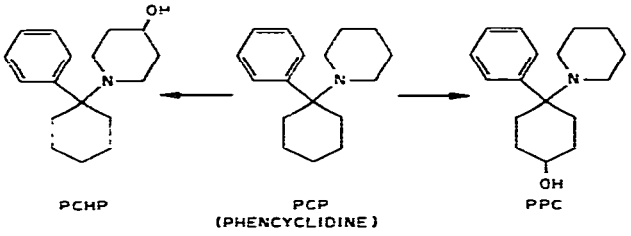

Fig. 1. Metabolism of phencyclidine (PCP) to 4-phenyl-4-piperidinocyclohexanol (PPC) and 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (PCHP).

EXPERIMENTAL

Standards and reagents

PCP, PCC and 1-(1-phenylcyclohexyl)morpholine (PCM, internal standard) were obtained from the Research Technology Branch, Division of Research, National Institute on Drug Abuse, Rockville, MD, U.S.A. Their structural identity and purity were confirmed by thin-layer chromatography, GC and GC—MS.

Heptafluorobutyric anhydride (HFBA) was purchased from Pierce, Rockford, IL, U.S.A. All other chemicals were of reagent grade quality.

Instrumentation

A Finnigan Model 4021 automated gas chromatograph—mass spectrometer—data system operating in the chemical ionization (CI) mode was used. Methane was used as reagent and carrier gas at a flow-rate of 16 ml/min. The gas chromatograph consisted of a glass column (1.83 m × 2 mm I.D.) packed with 3%

SE-30 on Gas-Chrom Q (100—120 mesh) and was coupled to the mass spectrometer by a glass-lined stainless-steel tube and a venting valve. After sample injection, the venting valve was opened for 90 sec, allowing solvent and volatile substances to escape without entering the ion source. The temperatures of the injector, column, interface oven, and ion source were maintained at 165, 190, 250 and 250°C, respectively. The electron energy was set at 70 eV and the multiplier voltage at 1.35 kV. The ions selected for monitoring were *m/e* 242.1, 243.1, 245.1 and 455.1. Under these conditions selective responses for the compounds of interest were obtained at the following relative retention times: PCP, 0.79; PCM, 1.0; PPC, 1.56; PCHP, 1.79. Fig. 2A shows a typical mass fragmentogram of PCP, PCM, PPC and PCHP.

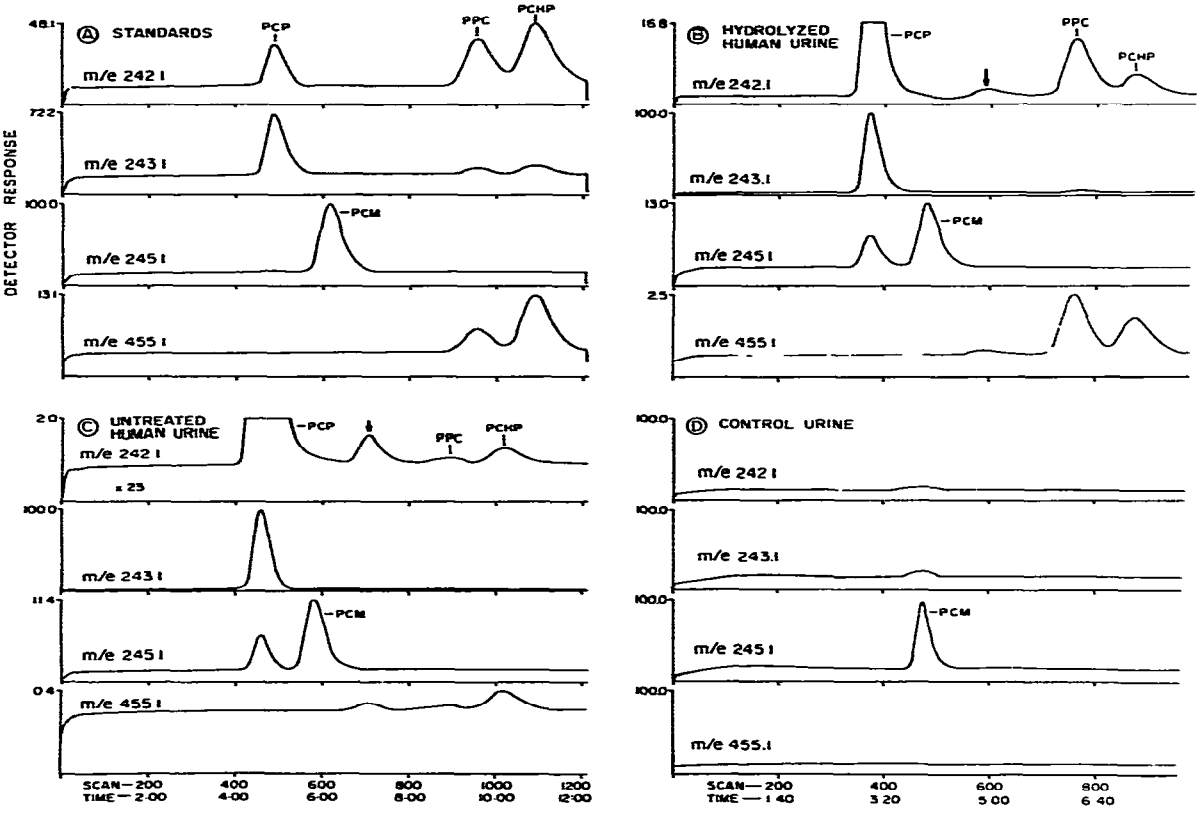

Fig. 2. Mass fragmentograms of phencyclidine (PCP) urine extracts to which 10 μg of 1-(1-phenylcyclohexyl)morpholine (PCM) were added. (A) Normal urine with PCP, 4-phenyl-4-piperidinocyclohexanol (PPC) and 1-(1-phenylcyclohexyl-4-hydroxypiperidine (PCHP) added. (B) Acid-hydrolyzed human urine from subject who ingested an unknown amount of PCP. (C) Same sample as (B) without acid hydrolysis. (D) Control urine. Arrow in (B) and (C) indicates presence of new hydroxy metabolite of PCP.

Human urine samples

Urine specimens from subjects who had taken PCP were obtained from the Veterans Administration Regional Drug Screening Laboratory, Veterans Ad-

ministration Medical Center, Sepulveda, CA, U.S.A. The subjects were enrolled in a methadone maintenance program at the time of sample collection.

Sample preparation and extraction

An aliquot (5—10 ml) of each urine specimen was mixed with internal standard (10 μg of PCM) and extracted either untreated or following acid hydrolysis. These extracts contained the free drug and metabolite fraction or total drug which included both free and conjugated drug species liberated by acid hydrolysis. The samples to be hydrolyzed were treated with a 10% volume of conc. hydrochloric acid and autoclaved at 115°C at 15 p.s.i. for 30 min.

Prior to extraction the pH of all samples was adjusted to ca. pH 10, and 3 ml of 3 *N* potassium carbonate were added to give a final pH of 10.5. Sodium chloride (0.5 g) and hexane (12 ml) were added and the contents were shaken and centrifuged. An aliquot (10 ml) of the organic phase was transferred to a clean tube containing 5 ml of 2 *N* hydrochloric acid. The tubes were shaken, centrifuged and the organic phase was discarded. The pH was adjusted as before to a final value of 10.5. Sodium chloride (0.5 g) and hexane (12 ml) were added and the contents were shaken and centrifuged. An aliquot (10 ml) of the organic phase was transferred to a clean, conical tube and evaporated to dryness under nitrogen at 40°C. Benzene (0.1 ml) and HFBA (20 μl) were added and the tube was sealed and heated at 85—90°C for 60 min. Ammonium hydroxide solution (1 ml of 5% v/v) was added and the tube was shaken and centrifuged. Benzene (50 μl) was added and the aqueous layer was discarded. An aliquot (1—3 μl) of the organic layer was removed for analysis.

Calibration curves and quantitative analysis

Standard curves were constructed from the analysis of standard solutions. The solutions were prepared by adding known amounts of drug and metabolites (0—8 μg) to control urine containing PCM (10 μg) added as internal standard. The samples were hydrolyzed, extracted, derivatized and analyzed by MF in the same manner as that described for the drug urines. Linear relationships for peak intensity ratios of drug or metabolite to internal standard versus concentration were observed throughout the concentration range. Correlation coefficients (r) were typically $\geqslant 0.98$. The lower limits of the assay were ca. 0.01 μg/ml for PCP and 0.05 μg/ml for PPC and PCHP. There was sufficient volume for duplicate analyses of some of the drug urines and the mean ± standard error of the analyses is reported.

Recovery studies

The addition of PCP, PPC and PCHP to urine samples at a concentration of 10 μg/ml resulted in overall recoveries ± S.E. of 65.8 ± 4.5%, 70.2 ± 3.7% and 73.4 ± 2.6%, respectively, for the analysis of free drug and 64.4 ± 1.7%, 69.4 ± 2.3% and 69.8 ± 2.9%, respectively, for the acid-hydrolyzed samples.

Assay specificity

In an attempt to identify substances that might interfere in the assay for PCP and metabolites, a number of compounds commonly encountered in toxicological screens were processed under the same conditions as those de-

TABLE I

RELATIVE GC RETENTION DATA ON 3% SE-30 (190°C) OF COMPOUNDS COMMONLY ENCOUNTERED IN TOXICOLOGICAL SCREENS

Retention times are reported relative to the internal standard, PCM. Relative retention times were reproducible under different operating conditions, i.e. changes of venting time, column temperature. Values in parentheses represent uncorrected retention times in minutes.

Compound	Relative retention time*
Methadone	2.0 (6.4)
Methadone metabolites	1.0 (3.2), 1.3 (4.2)
Morphine	—
Codeine	—
Propoxyphene	0.8 (2.6), 0.9 (2.8), 2.3 (7.4)
Cocaine	2.3 (7.3)
Methaqualone	1.9 (6.0)
Amphetamine	0.1 (0.3)
Diazepam	1.9 (6.1)
Chlordiazepoxide	1.3 (4.1)
Glutethimide	0.7 (2.3)
Phenobarbital	—
Pentobarbital	—
Amobarbital	—
Secobarbital	—
Chlorpromazine	—
Ethchlorvynol	—
Acetylsalicylic acid	—
Doxepin	—
Dextromethorphan	1.8 (5.9)
Meprobamate	—
Ketamine	0.7 (2.2)
1-[1-(2-Thienyl)cyclohexyl]piperidine (TCP)	0.2 (0.6), 0.8 (2.6)
N-Ethyl-1-phenylcyclohexylamine (PCE)	0.2 (0.7)
1-(1-Phenylcyclohexyl)pyrrolidine (PCPY)	0.6 (2.0)
PCP	0.8 (2.7)
PPC	1.5 (4.8)
PCHP	1.7 (5.3)
PCM (internal standard)	1.0 (3.2)

*— indicates that the compound did not elute from the column under these conditions.

scribed for PCP. Mass spectral data were obtained for those substances which eluted from the gas chromatograph with similar retention times to the compounds of interest (see Table I). Limited mass scans were analyzed for possible interferences.

Identification of phencyclidine and metabolites

Total ion current chromatograms were obtained routinely for urine extracts from each subject. PCP and the hydroxy metabolites, PPC and PCHP, were identified by comparison of their spectra (Fig. 3) with those of authentic standards (Table II). A new hydroxy metabolite was detected in extracts from a number of subjects (Fig. 2, relative retention time = 1.2); however, there was not enough sample to obtain total ion scans with sufficient intensity for structural assignment.

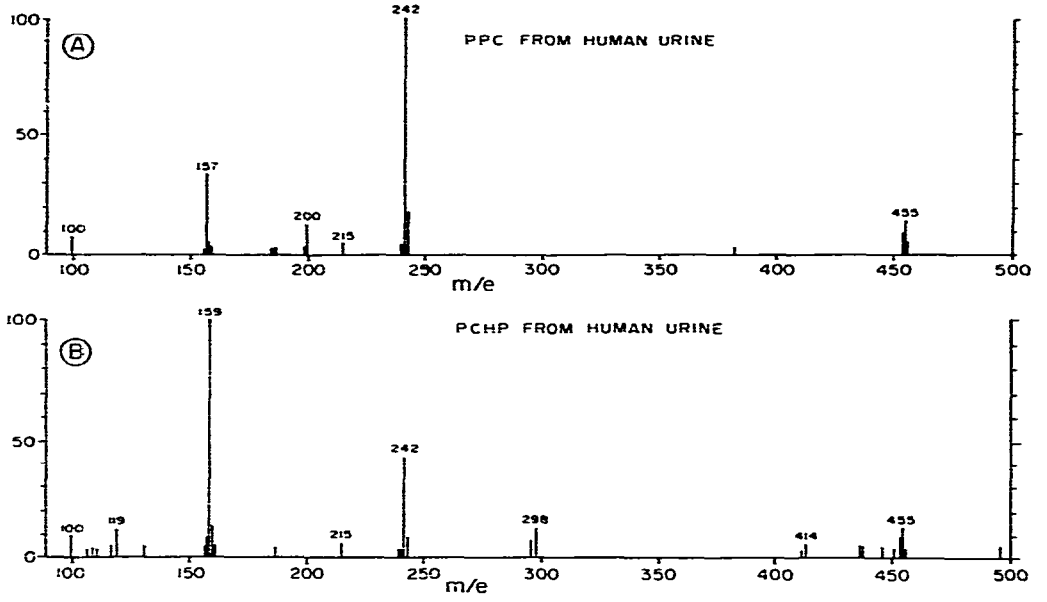

Fig. 3. Methane chemical ionization spectra of metabolites of phencyclidine from human urine identified as: (A) 4-phenyl-4-piperidinocyclohexanol (PPC); (B) 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (PCHP).

TABLE II

METHANE CI SPECTRA OF PHENCYCLIDINE, METABOLITES (HEPTAFLUOROBUTYRYL DERIVATIVES) AND THE INTERNAL STANDARD, PCM [1-(1-PHENYLCYCLOHEXYL)MORPHOLINE]

Compound	Molecular weight	Methane CI spectra (*m/e*)*			
		$[M+H]^+$	M^+	$[M-H]^+$	Prominent ions**
PCP	243	244 (28)	243 (100)	242 (48)	166 (8), 159 (68), 119 (6)
PPC	455	456 (4)	455 (19)	454 (30)	243 (30), 242 (100), 200 (5), 157 (42)
PCHP	455	456 (7)	455 (22)	454 (9)	298 (19), 296 (10), 243 (16), 242 (86), 215 (7), 187 (11), 160 (13), 159 (100), 158 (5), 157 (9), 119 (9)
PCM	245	246 (6)	245 (31)	244 (13)	160 (11), 159 (100), 116 (11)

*Percentage abundance in parentheses.
**Only ions ≥ 5% abundance are reported.

RESULTS AND DISCUSSION

A mass fragmentographic (MF) assay was developed for the determination of PCP and the hydroxy metabolites, PPC and PCHP, in human urine. The GC—MS system was operated in the CI mode using methane as the reagent gas. Urine samples were extracted with hexane, derivatized with HFBA, and analyzed by MF. Quantitation was performed by the internal standard method

using PCM, an analog of PCP, for standardization. Standard curves were generated on a daily basis and were linear throughout the ranges tested.

Human urine samples were analyzed either untreated or following acid hydrolysis. The acid-hydrolysis procedure was used to cleave conjugated metabolites and to allow the estimation of their amount in urine. The possibility of loss of PCP or metabolite from this treatment was considered; however, studies showed that there were no differences in recoveries by either method.

The extraction procedure involved extraction with hexane, back-extraction into acid solution followed by re-extraction at high pH with hexane. Recoveries by this method were in the range of 64—73%. Although it was found that recoveries could be improved by extraction with chloroform, the use of hexane provided cleaner extracts with reduced background.

Prior to sample analysis the extracts were derivatized with HFBA in benzene at elevated temperatures. This procedure produced mono-acyl derivatives of the hydroxy metabolites, PPC and PCHP. Although PPC, PCHP and PCP can be separated by GC on SE-30 without derivatization [23], an improvement in peak shape and resolution of the metabolites was achieved by derivatization. The derivatives also exhibited good stability. Derivatized extracts that were 1—5 days old and stored at 0—5°C showed minimal decreases in intensity of response when reanalyzed. PCP and PCM were unaffected by the derivatization process.

For assaying by MF, the ion selected for monitoring for PCP, PPC and PCHP was *m/e* 242.1. This represents the $[M - H]^+$ ion for PCP and the $[M - OOCCF_2CF_3]^+$ ions for PPC and PCHP. The molecular ion (M^+, *m/e* 245.1) was monitored for the internal standard. The ions at *m/e* 243.1 and *m/e* 455.1 (M^+ ions for PPC and PCHP) were monitored for confirmation (see Fig. 2). Standard methane CI spectra are presented in Table II for PCM, PCP and the HFBA derivatives of PPC and PCHP.

Using the procedures described, the concentrations of PCP and hydroxy metabolites were measured in urine from five human subjects who had self-administered PCP (Table III). The amount of PCP was found to be highly variable from subject to subject, ranging from 0.3 to 23.6 μg/ml. Only traces of PPC and PCHP were detected in untreated urine, whereas considerably more was usually present following acid hydrolysis. Apparently PPC and PCHP are excreted for the most part in conjugated form. The amount of PPC ranged from 0.03 to 1.79 μg/ml with a mean of 0.9 μg/ml for the acid-hydrolyzed samples. The amount of PCHP following acid hydrolysis was generally less than that of PPC and ranged from 0 to 0.48 μg/ml with a mean of 0.2 μg/ml. Total ion chromatograms were used for final verification of structure of the metabolites.

A new metabolite of PCP was detected in each of the urines of the five subjects. This metabolite had a relative retention time of 1.2 (see Fig. 3B and C) and an apparent molecular weight equal to that of PPC or PCHP. The metabolite formed a mono-acyl derivative which suggests that the metabolite is a new hydroxylated metabolite of PCP. The molecular weight assignment was based on the presence of the ion at *m/e* 455.1. Unfortunately, there were insufficient amounts of the new metabolite for further spectral work and the assignment of position of hydroxylation awaits further study.

TABLE III

CONCENTRATIONS OF PCP AND MONOHYDROXYLATED METABOLITES IN URINE OF HUMAN SUBJECTS

Values are expressed in μg/ml.

Subject No.	PCP		PPC		PCHP	
	UT*	AH**	UT	AH	UT	AH
8	6.30±0.05	5.94±0.74	Trace	1.79±0.34	0.02±0.0	0.48±0.06
19	0.33	0.37±0.03	0	0.03	0	0.07±0.01
29	4.10	4.13	0	0.10	0	0
39	—	1.70	—	0.81	—	0.10
46	23.65	24.68	0	1.53	0.05	0.28
Mean ± S.E.	8.60±5.2	7.4±4.4	0	0.9±0.4	0.02±0.01	0.2±0.1

*UT = untreated samples.
**AH = acid-hydrolyzed samples.

It is likely that the urine samples contained other drugs in addition to PCP since they were obtained from subjects who were enrolled in a methadone maintenance program at the time of sample collection. Both methadone and metabolites were identified in the total ion chromatogram of several of the urine extracts; however, the specificity of the MF assay was sufficient to measure PCP, PPC and PCHP in the presence of these substances without interferences. In addition, a number of other drugs which are commonly encountered in toxicological screens were added to the assay to determine if they presented interferences (see Table I). Although there was an occasional overlap of retention time, none of these drugs interfered in the MF assay. The procedure is sufficiently sensitive and specific for the determination of PCP and hydroxy metabolites in human urine and can provide the basis for further investigation of the pharmacological roles of PCP metabolites.

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