Journal of Chromatography, 276 (1983) 433—437
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1746

Note

Measurement of phencyclidine and two hydroxylated metabolites by selected ion monitoring

B.R. KUHNERT*, B.S. BAGBY and N.L. GOLDEN

Department of Obstetrics and Gynecology, Pediatrics, and the Perinatal Clinical Research Center of Cleveland Metropolitan General Hospital/Case Western Reserve University, 3395 Scranton Road, Cleveland, OH 44109 (U.S.A.)*

(First received January 24th, 1983; revised manuscript received March 29th, 1983)

Phencyclidine (PCP) is a commonly abused street drug, especially among adolescents — often the same adolescents with unplanned pregnancies. However, the extent of PCP use during pregnancy is unstudied and thus its pharmacology and effect on the mother, fetus, and neonate are largely unknown. Nevertheless, PCP has well defined effects in children [1] and adults [2] and a recent case report suggested that it may be teratogenic [3].

In order to undertake a study of the use of PCP during pregnancy and its pharmacology during the peripartum period, a sensitive method for the analysis of PCP and its hydroxylated metabolites in samples from asymptomatic mothers and neonates was needed. Previous assays for PCP have been developed based on thin-layer chromatography [4], gas—liquid chromatography (GLC) with flame ionization [5] or nitrogen—phosphorus detectors [2, 6, 7], GLC with capillary columns [8], gas chromatography—mass spectrometry (GC—MS) [9, 10], and radioimmunoassay [11, 12]. Most of these assays have been either nonspecific, insensitive, require too large a sample size, are not applicable to the metabolites, or have extraction procedures that are too time consuming.

We would like to report an easy, rapid and sensitive technique using selected ion monitoring GC—MS for the analysis of PCP in plasma and PCP and its hydroxylated metabolites in urine.

MATERIALS AND METHODS

Reference compounds

Reference crystals of phencyclidine hydrochloride (PCP), 4-phenyl-4-

0378-4347/83/$03.00

piperidinocyclohexanol (PPC), 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (PCHP), and the deuterated internal standards, [phenyl-$^2H_5$]-1-(1-phenylcyclohexyl)piperidine and [phenyl-$^2H_5$]-1-(1-phenylcyclohexyl)-4-hydroxypiperidine were all provided by Research Triangle Institute (Research Triangle Park, NC, U.S.A.), through the Research Technology Branch of the National Institutes of Drug Abuse. Stock solutions of all compounds were prepared with methanol to yield concentrations equivalent to 1 μg/ml free base.

Apparatus

A Hewlett-Packard 5995A quadrupole table-top mass spectrometer equipped with a direct probe inlet was used to obtain 70-eV electron impact mass spectra of the reference compounds. For selected ion monitoring the gas chromatograph was interfaced to the mass spectrometer with a glass jet separator. The chromatograph was fitted with a 1.3 m × 2 mm I.D. AW DMCS treated glass coil packed with 2% OV-17 coated on 80—100 mesh Supelcoport (Applied Sciences Labs., Bellefonte, PA, U.S.A.). The instrument conditions for PCP analyses were: carrier gas flow-rate 20 ml/min; injection port temperature 200°C; and oven temperature 200°C, the analyzer and ion source temperatures were set at 180°C and 150°C, respectively, to avoid thermal degradation of PCP occurring with temperatures above 200°C [9]. Ion intensities at *m/z* 200 and 205 were monitored for PCP and its deuterated internal standard, respectively, with a window width of 0.10 a.m.u. Total run time was 2.1 min. Ions 91 and 96 in addition to 200 and 205 could also be included for additional confirmation of PCP.

Changes in the conditions for analysis of PPC and PCHP were: injection port temperature 250°C; oven temperature 224°C; analyzer and ion source temperature both 276°C. Ions selected for monitoring were *m/z* 96, *m/z* 200 and *m/z* 288 for the trimethylsilyl derivatives of the internal standard, PPC and PCHP, respectively, with a run time of 2.2 min. For both procedures the optics of the mass spectrometer were optimized by autotuning at *m/z* 502 and the GC column was treated daily with Silyl-8 [13] (Pierce, Rockford, IL, U.S.A.).

Procedure

Urine samples were obtained from asymptomatic pregnant patients attending the obstetrical clinics of Cleveland Metropolitan General Hospital. Those with positive drug histories for PCP use were followed throughout pregnancy by urine analysis at each clinic visit. At delivery both maternal and cord blood samples were drawn. In addition, six 6-h urine collections were attempted and maternal and neonatal blood samples were drawn at approximately 24, 48 and 72 h post partum. Blood samples were separated by centrifugation and the plasma and urine were stored frozen until analyzed by GC—MS.

Prior to extraction, urine samples (0.2—2 ml) were mixed with 1 ml 0.1 *M* sodium acetate buffer (pH 5) and 0.1 ml β-glucuronidase preparation (Sigma, St. Louis, MO, U.S.A.) per 1 ml urine and incubated for 19 h at 37°C in order to deconjugate the hydroxylated metabolites. Following the addition of 25 ng of both deuterated internal standards, plasma and urine samples and spiked blank urine or plasma standards were made basic with 0.5 ml of 2 *M* sodium carbonate solution saturated with sodium chloride. After extraction with 5 ml

diethyl ether, the samples were centrifuged and following flash freezing with methanol and carbon dioxide, the organic layer was transferred to a 5-ml reactivial (Pierce). The ether was carefully evaporated under nitrogen at room temperature and the extract reconstituted with 30 μl of benzene. A 2-μl aliquot of the final solution was injected into the GC—MS system for PCP analysis. Standard curves were prepared and the samples quantitated using the Hewlett-Packard software for automatic quantitation of selected ion monitoring data by area normalization on the *m/z* 205 peak for deuterated PCP. Standard curves ranged from 1.56 to 200 ng/ml.

In order to measure PPC and PCHP, the urine samples from the final step (above) were evaporated to dryness. They could be allowed to sit overnight at this point. Trimethylsilyl derivatives were formed by adding 10 μl pyridine and 100 μl bis(trimethylsilyl)trifluoroacetamide (Pierce) under dry nitrogen, and without heat to the reactivials containing the sample residue. These were sealed immediately. If desired, plasma samples could also be evaporated and silylated using only 5 μl pyridine and 50 μl BSTFA. Samples were then heated at 80°C in a reactivial heating block (Pierce) for 1 h and allowed to return to room temperature. Aliquots (2 μl) were then injected into the GC—MS system with conditions as described above for the metabolites. Samples were quantitated by normalization on the *m/z* 96 internal standard peak. Standard curves ranged from 1.56—200 ng/ml.

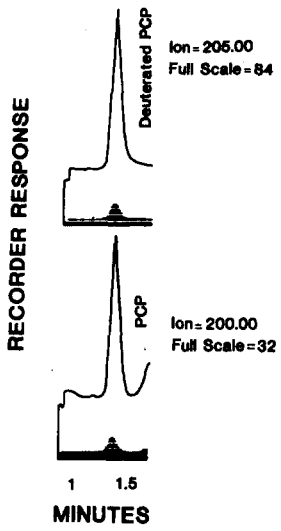
RESULTS

The mass spectra of PCP, the trimethylsilyl derivatives of PPC and PCHP were identical to those published previously using electron impact ionization [9]. The spectra for the two silylated deuterated internal standards, PCP and PCHP, were comparable with the base peaks being *m/z* 205 and 96, respectively.

The selected ion chromatogram of a urine extract containing 12.5 ng/ml PCP and 25 ng/ml internal standard is shown in Fig. 1. The retention times for PCP and deuterated PCP are approximately 1.44 and 1.42 min, respectively. Full scale refers to the amplification required to plot all chromatograms the same size (the higher the number the less required). Fig. 2 is the same urine extract following silylation and reinjection at the higher temperatures. The sample contained 12.5 ng/ml of both PPC and PCHP and 25 ng of the deuterated internal standard. Retention times were approximately 1.40, 1.33 and 1.41 min for the internal standard, PPC and PCHP, respectively. Problematic interference from other metabolites was not noted.

Calibration curves were linear to 500 ng/ml for both PCP and the two metabolites. The least-squares linear regression line which describes a typical PCP curve is $y = 3.251x - 0.674$. Typical curves for PPC and PCHP, respectively, are $y = 8.756x - 3.37$ and $y = 1.821x - 1.542$. Using these curves, samples containing less than 200 pg/ml of PCP, less than 2 ng/ml PPC and less than 1 ng/ml PCHP in urine could be quantitated. In plasma, this method is sensitive to 6.25 ng/ml; sensitivity can be increased with an acid back-extraction step.

The precision of the method was determined by repeat analysis of spiked urine samples containing low (6.25 ng/ml) and high (50 ng/ml) concentrations



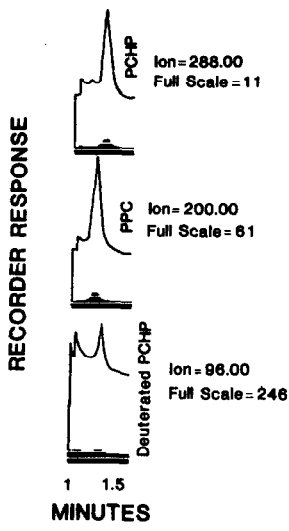


Fig. 1. Selected ion chromatogram of a urine extract.

Fig. 2. Selected ion chromatogram of a urine extract following silylation.

TABLE I

DRUG LEVELS IN SPOT URINE COLLECTIONS FROM ASYMPTOMATIC PREGNANT PATIENTS

Patient	Concentration ng/ml (ng/mg creatinine)				Metabolite/total product ratio
	PCP	PPC	PCHP	Total products	
(1) RS*	20.0 (81.0)	9.4 (37.6)	6.6 (26.7)	36.0	0.44
(2) PB	57.4 (67.2)	35.6 (41.6)	10.9 (12.7)	103.9	0.45
(3) MM	512.0 (300.7)	56.0 (32.9)	15.0 (8.8)	583.0	0.14
(4) NB	138.6 (76.6)	28.6 (15.8)	10.2 (5.6)	177.4	0.28
(5) JB*	99.1 (311.7)	14.7 (46.3)	9.7 (30.4)	123.5	0.20
(6) LC	25.4 (11.1)	9.1 (4.0)	—**	34.6	0.26

*Admitted ingestion 24 h earlier.
**Below detection limits.

of PCP, PPC and PCHP in urine. For PCP the relative standard deviation (coefficient of variation) was 4.01 and 3.81 for ten high and low samples, respectively. Repeatability of high and low concentrations for PPC resulted in a 7.19% and 9.46% relative standard deviation and with 7.59% and 9.11% values for PCHP. The precision for PCP in plasma was 6.60% for the 50-ng sample. Day-to-day repeatability was determined using 50 ng/ml frozen urine samples. The values were 2.94% for PCP, 7.69% for PPC and 7.66% for PCHP.

Using the method described, PCP, PPC and PCHP were quantitated in urine from asymptomatic pregnant patients during routine prenatal screening. The values for six of these patients are shown in Table I. Furthermore, PCP and both metabolites have been quantitated in maternal and neonatal urine post partum.

DISCUSSION

This method is very sensitive, quantitative, easy to perform, and it utilizes relatively inexpensive MS equipment increasingly common in hospital chemistry and other laboratories. In addition, the automatic quantitation feature of the HP 5995A system greatly decreases analysis time; one technician can extract, analyze and quantitate 30 samples and standards in one day. Finally, the ability to easily quantitate PPC and PCHP in urine is of more than academic interest because recent studies have suggested that these compounds are pharmacologically active [14].

This method can be used in conjunction with a routine screening procedure such as the Emit® Phencyclidine screening test (Syva, Palo Alto, CA, U.S.A.). However, the reported sensitivity of this method is only 75 ng/ml. We found that urine values higher than 10—15 units above the "blank" often warranted further analysis by GC—MS.

As reported previously [15], we also noted that diphenhydramine can interfere with the analysis of PCP. It has significant ion intensities at *m/z* 200 and a similar retention time. However, the presence of PCP and not diphenhydramine can be verified by also monitoring the ratio of *m/z* 91/200 and quantitating the PCP metabolites.

ACKNOWLEDGEMENTS

This work was supported in part by NIH USPHS Grant No. 5M01-RR-00210 and NIDA Grant No. 1 R01 DA02903.

REFERENCES

1 M.J. Welch and G.A. Correa, Clin. Pediatr., 19 (1980) 510.
2 D.N. Bailey, R.F. Shaw and J.J. Guba, J. Anal. Toxicol., 2 (1978) 233.
3 N.L. Golden, R.J. Sokol and I.L. Rubin, Pediatr., 65 (1980) 18.
4 R.A. van Welsum, J. Chromatogr., 78 (1973) 237.
5 R.C. Kammerer, E.D. Stefano and D. Schmitz, J. Anal. Toxicol., 4 (1980) 293.
6 D.N. Bailey and J.J. Guba, Clin. Chem., 26 (1980) 437.
7 J.N. Miceli, D.B. Bowman and M.K. Aravind, J. Anal. Toxicol., 5 (1981) 29.
8 F.N. Pitts, Jr., L.S. Yago, O. Aniline and A.F. Pitts, J. Chromatogr., 193 (1980) 157.
9 D.C.K. Lin, A.F. Fentiman, Jr., R.L. Foltz, R.D. Forney, Jr. and I. Sunshine, Biomed. Mass Spectrom., 2 (1975) 206.
10 E.J. Cone, W. Buchwald and D. Yousefnejad, J. Chromatogr., 223 (1981) 331.
11 B. Kaul and B. Davidow, Clin. Toxicol., 16 (1980) 7.
12 R.D. Budd and W.J. Leung, Clin. Toxicol., 18 (1981) 85.
13 D. Legult, J. Chromatogr. Sci., 20 (1982) 228.
14 E.F. Domino, in R.C. Petersen and R.C. Stillman (Editors), Phencyclidine (PCP) Abuse: An Appraisal, NIDA Research Monograph No. 21, Rockville, MD, 1978, p. 18.
15 F.A. Ragan, Jr., M.S. Samuels, S.A. Hite and R. Ory, Clin. Chem., 26 (1980) 785.