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ORIGINAL ARTICLE

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Simple and sensitive detection of phencyclidine in body fluids by gas chromatography with surface ionization detection

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Abstract Phencyclidine (PCP) can be detected in body fluids with very high sensitivity by gas chromatography (GC) with surface ionization detection (SID) using pethidine as internal standard. PCP was extracted with Sep-Pak C_{18} cartridges from whole blood and urine samples, which gave clean extracts. The calibration curve for spiked whole blood was linear in the range 1.25–20 ng/ml. The detection limit of PCP was approximately 15 pg on-column (0.75 ng/ml sample), which was much lower than by GC-nitrogen phosphorus detection. The recovery of PCP and pethidine from spiked whole blood or urine samples was above 85%. This method seems very useful for the determination of PCP in forensic and clinical toxicology.

Key words Phencyclidine · Pethidine · Gas chromatography · Surface ionization detection · Sep-Pak C_{18} cartridges

Introduction

Phencyclidine (PCP), a hallucinogenic drug developed in the 1950s, was first used as an anaesthetic for animals and for a short time as an anaesthetic in humans. It gained popularity as an abused drug in the early 1970s and is known by street names such as "angel dust" and "crystal". Since PCP is sometimes misrepresented as LSD, mescaline or Δ^9-THC, the true exposure may be underestimated [1, 2].

In 1985 Fujii and Arimoto developed the method of surface ionization detection (SID), a new detection technique specific for tertiary amines to be coupled with gas chromatography (GC) [3, 4]. In this report we show that PCP in biological samples can be determined with very high sensitivity by GC-SID. A rapid solid-phase extraction procedure before GC-SID is also given.

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Materials and methods

Materials

Phencyclidine (PCP)-HCl was a generous gift from Dr. T. Nishikawa (National Institute of Mental Health, Tokyo, Japan). Pethidine-HCl (internal standard, IS) was purchased from Tanabe Seiyaku, Osaka, Japan. Other chemicals used were of analytical grade. Chemical structures of PCP and pethidine are given in Fig. 1. Sep-Pak C_{18} cartridges were obtained from Waters Associates (Milford, Mass.) and a DB-1 fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm) from J & W Scientific (Folsom, Calif.).

Extraction with Sep-Pak C_{18} cartridges

Pretreatment of Sep-Pak C_{18} cartridges was carried out by washing with 10 ml of chloroform, 10 ml of methanol and 10 ml of distilled water. This procedure was repeated a minimum of 3 times. Following the pretreatment, 1 ml of whole blood or urine, with or without PCP and pethidine (IS), was mixed with 8 ml of distilled water and 1 ml of 1 M $NaHCO_3$, and loaded onto a Sep-Pak C_{18} cartridge. After washing the cartridge with 20 ml of distilled water,

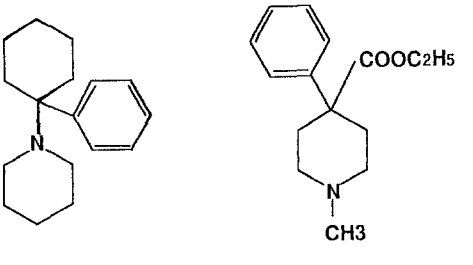


Fig. 1 Chemical structure of phencyclidine and pethidine (internal standard)

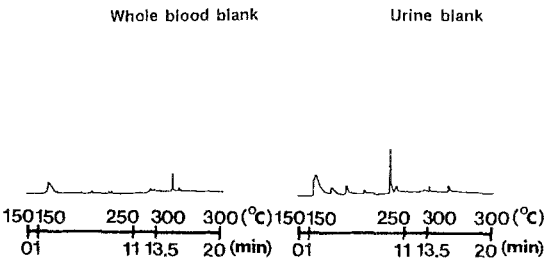
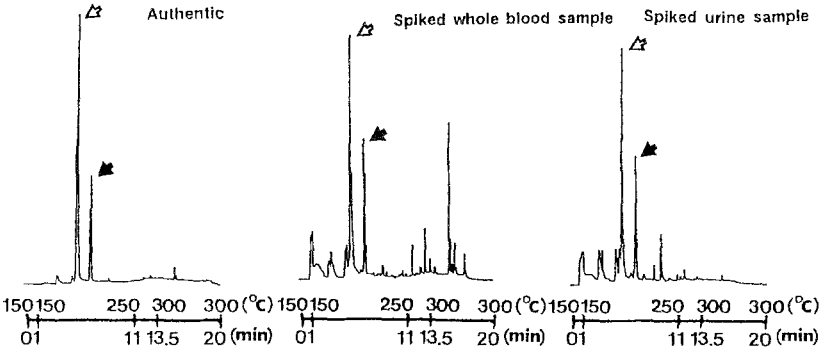
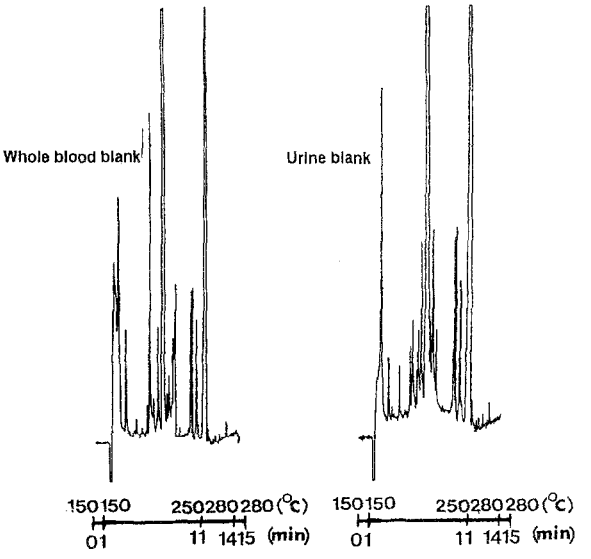


Fig. 2 GC-SID for the extracts of human whole blood and urine samples spiked and not spiked with 10 ng of PCP (*filled arrow*) and 10 ng of pethidine (IS, *open arrow*)





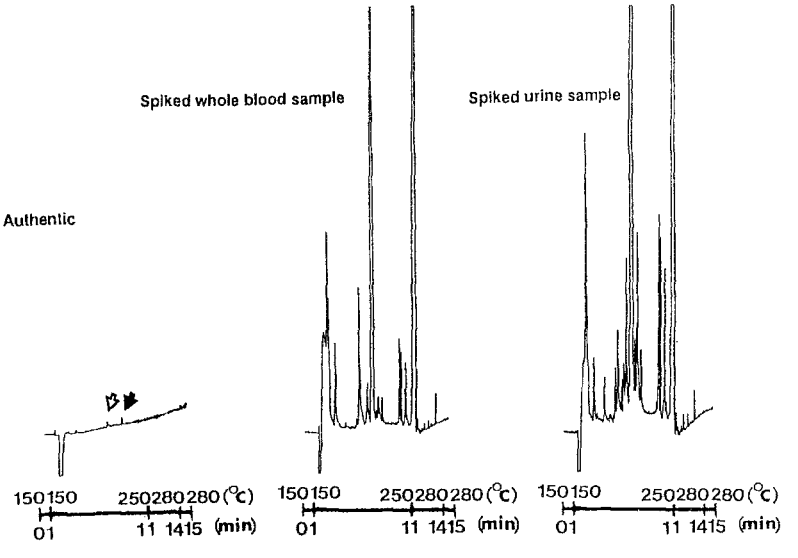


Fig. 3 GC-NPD for the extracts of human whole blood and urine spiked and not spiked with 25 ng of PCP (*filled arrow*) and 25 ng of pethidine (IS, *open arrow*)

the drugs were eluted with 3 ml of chloroform/methanol (9:1). The organic layer was evaporated to dryness under a stream of nitrogen and the residue was dissolved in 100 µl of methanol. Aliquots of 2 µl were subjected to GC analysis.

GC conditions

GC analyses were carried out on a Shimadzu GC-15B instrument equipped with an SID system and on a Hewlett Packard 5860 Series II instrument equipped with a nitrogen-phosphorus detection (NPD) system. A DB-1 fused silica capillary column and a split-splitless injector were used for both instruments. The GC conditions were: column temperature, 150–300°C (1 min hold at 150°C, 10°C/min from 150–250°C and 20°C/min from 250–300°C) for GC-SID or 150–280°C (1 min hold at 150°C, 10°C/min from 150–280°C) for GC-NPD; injection and detection temperatures, 230 and 280°C, respectively; helium flow rate, 3 ml/min. The SID conditions were: heating current through the platinum emitter 2.2 A; emitter temperature about 600°C; and ring electrode bias voltage +200 V with respect to the collector electrode.

Results

Figure 2 shows GC-SID profiles for 10 ng of PCP-HCl and pethidine-HCl, which were added to 1 ml of whole blood or urine samples and extracted with Sep-Pak C_{18} cartridges. The peaks of pethidine and PCP were found at 7.0 and 8.3 min, respectively. There were no impurity peaks which interfered with the peaks of PCP and pethidine.

Figure 3 shows GC-NPD profiles for 25 ng of PCP-HCl and pethidine-HCl in 1 ml of whole blood or urine, which were extracted by the same method, for comparison with those by GC-SID (Fig. 2). We stopped heating the column at 280°C because NPD became unstable above 280°C. We could detect small PCP and pethidine peaks only in the authentic sample, and many impurity peaks interfered with those of PCP and pethidine for the spiked whole blood and urine samples.

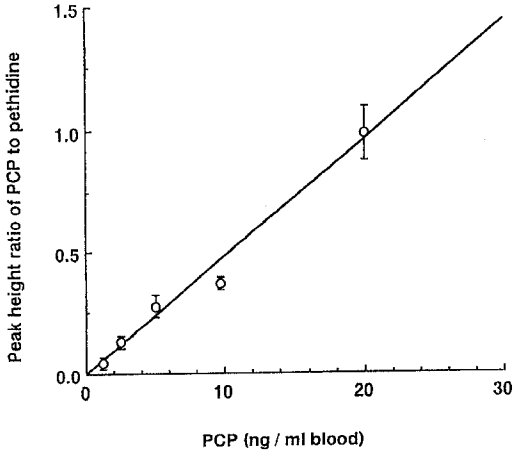


Fig. 4 Calibration curve for PCP-spiked human whole blood where 1.25–25 ng of PCP and 10 ng of pethidine were added to 1 ml of whole blood and extracted with Sep-Pak C_{18} cartridges. A 2-µl aliquot was subjected to GC-SID (25–400 pg on column if recovery is 100%). Each point represents the mean and SEM of triplicate experiments

For urine, the recoveries of PCP measured by GC-SID were 92.5 ± 22.5% (mean ± SEM, $n = 4$) at 2 ng/ml, and 105 ± 13.8% ($n = 4$) at 10 ng/ml; the recovery of pethidine was 110 ± 18% ($n = 7$) at 10 ng/ml urine. For blood, the recoveries of PCP were 137 ± 25% ($n = 3$) at 2 ng/ml and 88.2 ± 11.3 ($n = 4$) at 10 ng/ml, and that of pethidine 94.3 ± 8.9% ($n = 8$) at 10 ng/ml. The within-run and day-to-day coefficients of variation in quantitating 10 ng PCP/ml blood were 14.7 ($n = 6$) and 10.0% (4 days), respectively.

Figure 4 shows a calibration curve for PCP-spiked whole blood samples using pethidine (10 ng/ml) as IS with good linearity in the range 1.25–20 ng/ml (25–400 pg on-column if recovery is 100%). The equation and the regression coefficient were: $y = 0.0482x - 0.00240$ and $r = 0.995$, respectively. The detection limit (signal-to-noise ratio = 3) was 0.75 ng/ml (15 pg on-column).

Discussion

To our knowledge, this is the first report showing that PCP can be determined by GC-SID with very high sensitivity. As PCP is one of the most important drugs of abuse, there are many reports for its detection by GC-flame ionization detection (FID) [5, 6], GC-NPD [7–12], GC-mass spectrometry (MS) [13–19], high-performance liquid radiochromatography [20], radioimmunoassay [21] and enzyme multiplied immunoassay techniques [12, 22]. The detection limits of PCP in these reports were 20–50 ng/ml in GC-FID [5, 6], 0.5–10 ng/ml in GC-NPD [8–11], 0.25–15 ng/ml in GC-MS [14, 15, 17, 19], and 0.5 ng/ml in radioimmunoassay [21]. The present result showed comparable sensitivity to those of GC-MS [18, 19]. From the data shown in Figs. 2 and 3 it is clear that GC-SID is far superior to GC-NPD and on average the sensitivity of GC-SID is 10–100 times higher than that of GC-NPD [23].

Solid-phase extraction by Sep-Pak C_{18} was very useful for purifying PCP and pethidine from biological samples as the recoveries were above 85%. However, the recoveries of PCP at 10 ng/ml urine and at 2 ng/ml blood, and that of pethidine at 10 ng/ml urine, exceeded 100%. Because of the lack of impurity peaks overlapping those of PCP or pethidine, this phenomenon can be accounted for by the possibility that certain factors in biological fluids may prevent absorbance onto the column and/or from degrading during exposure to heat as in the case of bromosovalum [24] or phenothiazines [25].

After a single oral dose of 5 mg of PCP, the serum concentration of the unchanged drug was 0.01–0.1 µM (2.5–25 ng/ml), which could cause psychotic symptoms [26]. About 73% of the dose is excreted into urine, about 16% is unchanged, and 30% is present as conjugated and/or hydroxylated metabolites [27]. ElSohly et al. [16] reported that 5-[*N*-(1′-phenylcyclohexyl)-amino]pentatonic acid, an amino acid metabolite of PCP was found in urine of PCP users, but the amount of unchanged PCP in urine of 31 abusers ranged from 7.9–75.2% (26–75.2 ng/ml) [16]. As our method can detect such low concentra-

tions of unchanged PCP it could be very useful in forensic and clinical toxicology.

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