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Ultrasensitive Determination of Phencyclidine in Body Fluids by Surface Ionization Organic Mass Spectrometry

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Gas chromatography (GC)/surface ionization organic mass spectrometry (SIOMS) has been found to give much higher sensitivity for measurements of phencyclidine (PCP) than the conventional GC/electron impact (EI)-mass spectrometry (MS). Thus, we have established a detailed procedure for measurements of PCP in body fluids by both mass chromatography and selected-ion monitoring (SIM) of SIOMS using pethidine as an internal standard (IS). Good linearity was found in the range of 0.25−10 ng/mL of whole blood or urine, when measured by mass chromatography, and in the range of 0.025−1.0 ng/mL of whole blood by SIM. The recoveries of PCP and IS spiked to whole blood were 106 ± 17% at 1 ng/mL and 113 ± 11% at 5 ng/mL; that of IS was 97.8 ± 10.4% at 5 ng/mL. The detection limits (signal-to-noise ratio = 3) were estimated to be 0.05 ng/mL of whole blood or urine by mass chromatography and 0.01 ng/mL of whole blood by SIM. The coefficients of intraday and interday variations were not greater than 10.3%. We could detect PCP from rat whole blood 2 h after subcutaneous injection of PCP (1 mg/kg) by mass chromatography. The mean PCP concentration in rat blood was 47.7 ± 6.2 ng/mL (mean ± SD, *n* = 4).

Phencyclidine (PCP), a hallucinogen developed in the 1950s, was first used as an anaesthetic for animals and then for humans for a short period. The hallucinogenic property of PCP gained popularity as an abused drug in the early 1970s. PCP is known by street names of "angel dust" and "crystal".[1−4] Since PCP is sometimes misrepresented as LSD, mescaline, or Δ^9-THC, its true exposure may be underestimated.[1,3] Although illicit PCP use declined from 1979 to 1993,[4] it still remains as an important drug of abuse in many metropolitan areas and among certain socio-demographic groups.[3,4]

Surface ionization (SI), a phenomenon in which neutral atoms or molecules are converted to ions on an incandescent metal surface, was used for a detector of gas chromatography (GC);[5,6] we succeeded in detecting PCP in body fluids by this GC-surface ionization detection (SID) with high sensitivity.[7,8] Recently, Fujii has devised a combination of SI and a quadrupole mass spectrometer and named this system a surface ionization organic mass spectrometer (SIOMS),[9,10] which is highly selective and sensitive for compounds containing tertiary amino groups. In this paper, we describe how PCP in human body fluids can be determined by SIOMS with extremely high sensitivity and specificity. This is the first report of detection of an abused drug by SIOMS.

EXPERIMENTAL SECTION

Materials. 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. Bond Elut Glass columns were obtained from Varian (Harbor City, CA) and an Rtx-5MS Guard fused silica capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm) from Restek (Bellefonte, PA). Human blood and urine samples were obtained from healthy volunteers.

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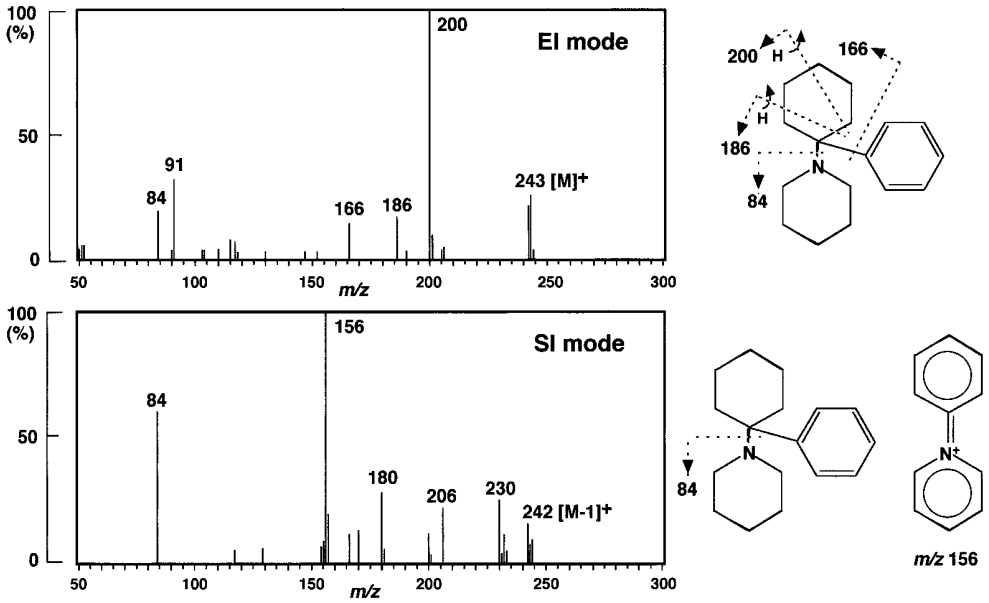

Figure 1. Mass spectra of PCP in the EI (upper panel) and SI (lower panel) modes and its probable fragmentation pathways. Five nanograms of PCP was injected in the EI mode and 500 pg injected in the SI mode.

Extraction with Bond Elut Glass Columns. We followed the previous methods in purifying PCP and pethidine (internal standard, IS) in body fluids by solid-phase extraction using Bond Elut Glass columns with slight modifications.[7,11] In brief, 8 mL of distilled water and 1 mL of 1 M $NaHCO_3$ solution were added to 1 mL of body fluids with or without PCP and pethidine (IS). The mixture was loaded onto a pretreated column. After washing the column with 20 mL of distilled water, PCP and IS were eluted with 3 mL of chloroform−methanol (9:1, v/v). The organic layer was evaporated to dryness under a stream of nitrogen, and the residue was dissolved in 50 μL of methanol. A 1-μL aliquot was subjected to GC/MS analysis.

GC Conditions. GC analyses were performed on a Shimadzu GC-17A instrument with a split-splitless injector (Kyoto, Japan) coupled with a mass spectrometer. The chromatograph was fitted with an Rtx-5MS Guard fused silica capillary column. The column temperature was maintained at 100 °C for 1 min and then programmed at 20 °C/min to 300 °C; the injection temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.8 mL/min. The samples were injected at the splitless mode, and the splitter was opened 1 min after the completion of the injection.

MS Conditions. The instrument used was a Shimadzu QP-5050A quadrupole mass spectrometer (Kyoto, Japan) which was modified to be usable in both electron impact (EI) and SI modes. For an SI ion source, the direct inlet (DI) probe was remodeled to a detachable SI assembly; a rhenium filament was placed on the tip of the DI probe and was inserted to the center of an EI ion source chamber. The SI assembly consisted of a ceramic insulator, two tantalum poles, and a rhenium ribbon (0.8 × 12 mm, thickness 0.025 mm); two tantalum poles were connected, through the ceramic insulator, to the rhenium ribbon by spot welding. The rhenium filament was heated by current of about 1.6 A. Oxygen gas was supplied to the filament to keep the rhenium surface stable. The partial pressure of oxygen was kept to (2−3) × 10^{-3} Pa in the chamber.

MS conditions were as follows: interface temperature, 260 °C; ionization current, 60 μA; electron energy, 70 eV; and detector voltage, 1.5 kV. Scan speed was 1000 amu/s.

Animal Experiments. Male Wistar rats, weighing about 250 g, were used. PCP-HCl dissolved in saline (1 mg/kg) was injected into 4 rats, and saline only into 2 rats subcutaneously. Two hours after the injection, the animals were anesthetized using ethyl ether; they were rapidly subjected to laparotomy and about 5 mL of blood was drawn from the abdominal aorta. The animals were thus led to death by exsanguination. The blood samples were heparinized and stored at 4 °C; PCP and IS were extracted from the samples on the same day.

RESULTS AND DISCUSSION

Mass Spectra and Mass Chromatograms in the SI and EI modes. Figures 1 and 2 show mass spectra of PCP and pethidine (IS), respectively, in the EI (upper panels) and SI (lower panels) modes, together with probable fragmentation pathways. The base peaks appeared at m/z 200 in the EI mode and at m/z 156 in the SI mode for PCP. In the SI mode, $[M - 1]^+$ ion, which is characteristic for surface ionization,[10,12] appeared at m/z 242 for PCP. We propose an aromatized structure for explaining the peak at m/z 156 (Figure 1, lower panel); similar phenomena were observed by Fujii and other researchers.[13,14] Some higher mass-range peaks at m/z 180, 206, and 230 could not be explained; this may be due to some complicated thermal dissociation process with skeletal rearrangements.[15] In the case of pethidine (IS),

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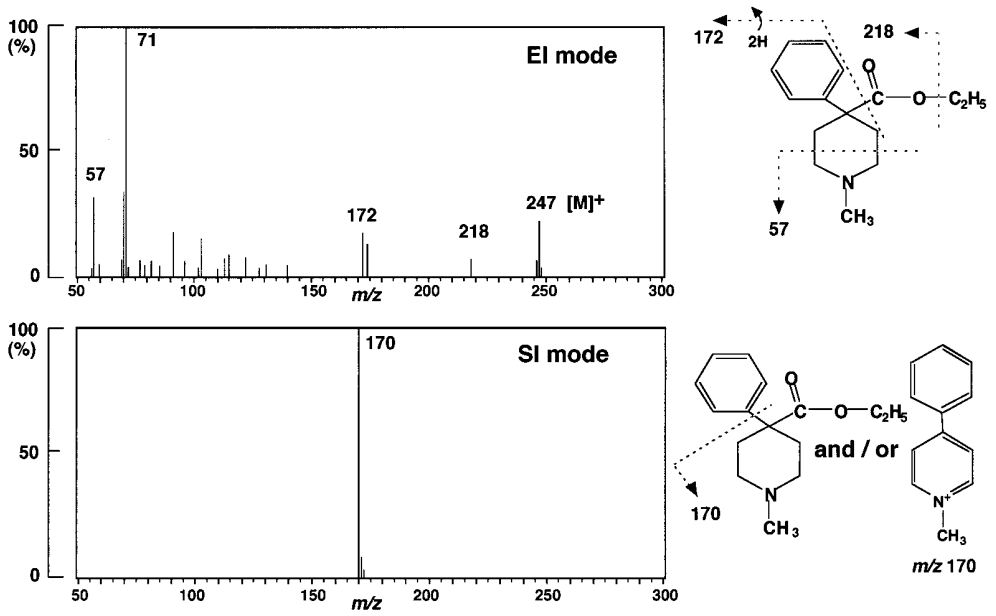

Figure 2. Mass spectra of pethidine (IS) in the EI (upper panel) and SI (lower panel) modes and its probable fragmentation pathways. The amounts of pethidine injected were the same as those of PCP described in Figure 1.

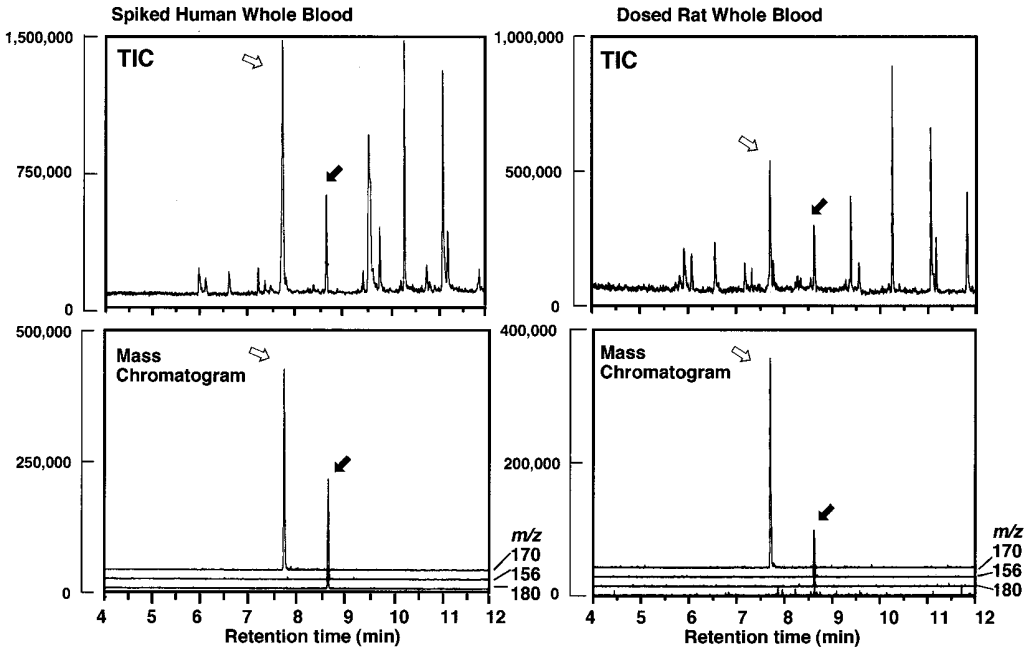

Figure 3. Total-ion chromatogram (TIC) (left upper panel) and mass chromatograms (left lower panel) of PCP and IS extracted from spiked human whole blood (12.5 ng/mL each), and the TIC (right upper panel) and mass chromatograms (right lower panel) for PCP and IS extracted from 0.1 mL of rat whole blood obtained 2 h after subcutaneous administration of PCP (1 mg/kg) in the SI mode. In each panel, filled and open arrows show the peaks of PCP and IS, respectively.

almost only a single peak appeared at m/z 170 in the SI mode (Figure 2).

Figure 3 shows the total ion chromatogram (TIC) and mass chromatograms of PCP and IS extracted from spiked human whole blood (12.5 ng/mL each) in the SI mode (left panels). PCP and IS gave intense peaks in both TIC and mass chromatograms at 7.7 and 8.6 min, respectively. Background noises were very small in the mass chromatograms. The TIC and mass chromatograms for the same sample were obtained in the EI mode. In the TIC, PCP and pethidine peaks were overlapped by large impurity peaks and, thus, not discernible; in EI mass chromatograms, the peaks of PCP and IS could be observed, but suffered from many noises (data not shown).

These data show that SIOMS is much superior in sensitivity to the conventional EI-MS for detection of PCP in body fluids.

Selected-Ion Monitoring (SIM). On the basis of the above data showing that SIOMS for PCP is much more sensitive than EI-MS, we have tried to quantitate PCP in whole blood with even higher sensitivity by selected-ion monitoring (SIM) of SIOMS. Figure 4 shows SIM chromatograms for extracts of whole blood

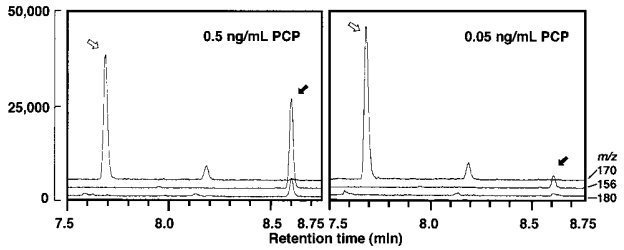

Figure 4. Selected-ion monitoring (SIM) for two different concentrations (left panel, 0.5 ng/mL and right panel, 0.05 ng/mL) of PCP in whole blood measured in the SI mode. Filled and open arrows show the peaks of PCP and IS, respectively. The vertical scale of the right panel is the same as that of the left panel.

spiked with two different concentrations of PCP; it shows that as little as 0.05 ng/mL of PCP could be easily determined.

Reliability of the SIOMS Method. We tried to quantitate PCP spiked to human whole blood by mass chromatography of SIOMS; the peak area ratio of m/z 156 to 170 was used for quantitation. Calibration curves for PCP in whole blood or urine were drawn by plotting six different concentrations using 5 ng/mL of IS. It gave good linearity in the PCP range of 0.25−10 ng/mL of whole blood or urine. The equations and r values for the curve were $y = 0.0519x - 0.000879$ and $r = 0.997$ for whole blood and $y = 0.0651x - 0.000340$ and $r = 0.998$ for urine. Its detection limit giving a signal-to-noise ratio of 3 was estimated to be 0.05 ng/mL (1 pg on-column) for both whole blood and urine.

Similar experiments for a calibration curve were made using SIM for PCP in whole blood with 6 plots in the range of 0.025−1.0 ng/mL; the equation and r value were $y = 1.64x - 0.134$ and $r = 0.960$. The detection limit was estimated to be 0.01 ng/mL.

In the previous reports,[16−21] quantitation methods for PCP by mass chromatography and by SIM of the conventional EI-MS were reported. The detection limits were 25 pg on-column,[21] 0.25 ng/mL (urine),[16] 0.47 ng/mL (urine),[19] 0.58 ng/mL (urine),[18] and 10 ng/mL (urine).[17] Therefore, the sensitivity (detection limit = 0.05 ng/mL) of the present method by mass chromatography is 5−200 times higher than the previous methods by SIM and that by the present SIM (detection limit = 0.01 ng/mL) is 25−1000 times higher.

As stated in the Introduction part, SIOMS is selective for compounds containing tertiary amino groups; it is therefore applicable only for limited numbers of compounds. However, SIOMS is very advantageous for sensitive determination of these compounds. Especially, tertiary amino compounds with ring structures such as PCP seem to give intense base peaks by SIOMS, which are suitable for sensitive quantitation; this was also true for MPTP.[22]

According to Javitt and Zukin,[23] PCP-induced psychosis could occur at a serum PCP concentration of less than 0.02 μM (5 ng/mL). In more than 10% of patients with a clinical diagnosis of "pure" PCP intoxication, their serum PCP levels were below 5 ng/mL.[24] Our ultrasensitive method (both by mass chromatography and SIM) for PCP determination meets the demand to measure such very low PCP concentrations in body fluids.

The recoveries of PCP and IS were determined by mass chromatography of SIOMS; peak areas of whole blood spiked with known amounts (1 or 5 ng/mL for PCP and 5 ng/mL for IS) of the compounds were compared with peak areas of the authentic compounds observed by direct injection. The recoveries of PCP from whole blood were 106 ± 17% (mean ± SD, $n = 5$) at 1 ng/mL and 113 ± 11% ($n = 6$) at 5 ng/mL; that for IS was 97.8 ± 10.4% ($n = 11$) at 5 ng/mL. The recoveries exceeding 100% can be accounted for by the possibility that certain substances in whole blood may prevent PCP from adsorbing to the GC column and/or from degrading during exposure to heat as in the case of bromisovalum[25] or phenothiazines.[26]

To check reproducibility of the present method, we quantitated 1 and 5 ng/mL of PCP spiked to whole blood using each calibration curve by mass chromatography. The coefficients of intraday variations were 9.1% at 1 ng/mL and 4.8% at 5 ng/mL; those of interday variations were 10.3 and 10.2%, respectively ($n = 6$ for each experiment).

Quantitation of PCP in Rat Blood. To validate our method, we quantitated PCP in rat blood 2 h after subcutaneous administration of PCP (1 mg/kg) by mass chromatography. Because the concentrations of the rat blood were too high, we used only 0.1 mL of the blood. The TIC and mass chromatograms of PCP and IS in the rat blood were shown in Figure 3 (right panels). The concentration was 47.7 ± 6.2 ng/mL ($n = 4$, mean ± SD). In the saline-injected animals, any peak of PCP was not found (data not shown).

CONCLUSION

To our knowledge, this is the first report for measuring an abused drug in body fluids by SIOMS. Our method is recommendable for use in forensic and clinical toxicology because of its high sensitivity and specificity.

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