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Journal of Chromatography B, 000 (2001) 000–000

JOURNAL OF CHROMATOGRAPHY B

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Sensitive determination of pethidine in body fluids by surface ionization organic mass spectrometry

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Abstract

We have presented a simple and sensitive method for determining pethidine, a narcotic analgesic drug in body fluids by gas chromatography (GC)/surface ionization organic mass spectrometry (SIOMS). Good linearity was obtained in the range of 0.625–25 ng/ml of whole blood and urine by mass chromatography, and in the range of 0.05–2 ng/ml of whole blood by selected ion monitoring (SIM). Pethidine and diphenylpyraline (internal standard) were extracted from body fluids with Bond Elut Certify cartridges; their recoveries were above 95%. The detection limits (signal-to-noise ratio=3) were estimated to be 0.2 ng/ml of whole blood or urine by mass chromatography, 0.02 ng/ml of whole blood by SIM.

Keywords: Pethidine

1. Introduction

Pethidine (meperidine) is a narcotic analgesic drug, which is widely prescribed, in therapeutic practice. Pethidine is readily absorbed after oral administration, and rapidly metabolized to norpethidine or pethidinic acid [1–3]. Pethidine was widely and most frequently abused by persons working in anaesthesia units [4].

Surface ionization (SI), a phenomenon in which neutral atoms or molecules are ionized on an incandescent metal surface, was applied as a detector for gas chromatography (GC) [5,6]. Our group succeeded in detecting pethidine in body fluids with high sensitivity by this GC–surface ionization detector (GC–SID) [7]. Recently, Fujii has devised a combination of SI and a quadrupole mass spectrometer; he named this mass spectrometric system a surface ionization organic mass spectrometer (SIOMS) [8,9]. SIOMS is highly sensitive and selective for compounds containing tertiary amino groups. We have developed new and sensitive methods for determining PCP [10], pentazocine [11] in human body fluids by SIOMS. In this paper, we have

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0378-4347/01/$ – see front matter
PII: S0378-4347(01)00049-4

presented that pethidine in body fluids can be determined with very high sensitivity.

2. Experimental

2.1. Materials

Pethidine hydrochloride and diphenylpyraline hydrochloride (internal standard, I.S.) were purchased from Tanabe Seiyaku Co. Ltd. (Osaka, Japan) and Sigma Chemical Co. (St. Louis, MO, USA), respectively. Other chemicals used were of analytical grade. Bond Elut Certify columns were obtained from Varian (Harbor City, CA, USA), 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, USA). Human blood and urine samples were obtained from healthy volunteers.

2.2. Extraction of compounds with Bond Elut Certify columns

We extracted pethidine and I.S. with Bond Elut Certify columns. The procedure was as follows. Four millilitres of distilled water and 1 ml of 1 *M* sodium phosphate buffer (pH 5.5) were added to 1 ml of whole blood or urine sample with or without pethidine and I.S. In the case of whole blood, the mixture was spun down at 3500 rpm for 15 min, and the supernatant was loaded onto a pretreated column. In case of urine, the mixture was applied to a column without centrifugation. After washing the column with 1 ml of 1 *M* acetic acid and 6 ml of methanol, the compounds were eluted with ethyl acetate containing 2% ammonium hydroxide. The eluents were evaporated to dryness in vacuo. The residue was dissolved in 50 μl of methanol, and 1-μl aliquot was injected to the GC port.

2.3. 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 column. The column temperature was maintained at 100°C for 1 min and then programmed at 20°C/min to 280°C; the injection temperature was 260°C. Helium (99.999%) was used as a carrier gas at a flow-rate of 1.5 ml/min. The samples were injected at the splitless mode, and the splitter was opened 1 min after the completion of the injection.

2.4. MS conditions

The instrument used was a Shimadzu QP-5050A quadrupole mass spectrometer (Kyoto, Japan) which was modified to use in both electron impact (EI) and SI modes. For an SI ion source, the direct inlet (DI) probe was remodelled; 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 was described in our [10] and Fujii's [8] previous papers. The rhenium filament was heated by current of about 1.65 A. The partial pressure of oxygen in the chamber was kept to $(2–3)\times10^{-3}$ Pa.

MS conditions were as follows: interface temperature, 270°C; ionization current, 60 μA; electron energy, 70 eV; and detector voltage, 1.3 kV. The scan speed was 1000 amu/s.

2.5. Drug administration

Two healthy male volunteers (52 and 38 years old) administered 10 mg of pethidine orally. Blood and urine samples were obtained 2 and 6 h after the oral administration. The samples were frozen at −80°C until analysis.

3. Results and discussion

3.1. Mass spectra and mass chromatograms in the SI mode

SI mass spectra and probable fragment ions for pethidine and diphenylpyraline are shown in Fig. 1. For pethidine, the intense base peak appeared at m/z 170. We propose an aromatized structure for explaining the peak at m/z 170; we and other groups observed similar phenomena [12–14]. For diphenylpyraline, the base peak was m/z 94, which

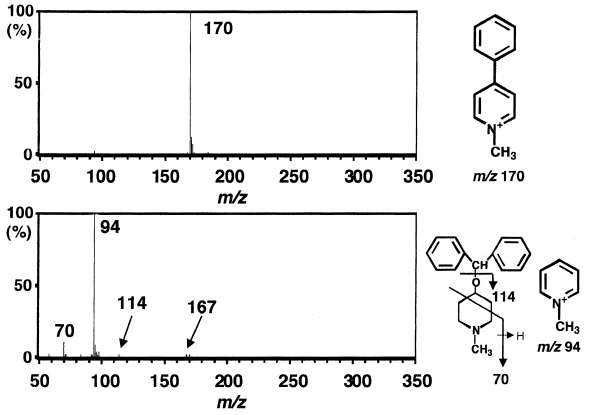

Fig. 1. SI mass spectra and their probable fragmentation pathways for pethidine and diphenylpyraline (IS). Five hundred picograms of pethidine and I.S. were injected.

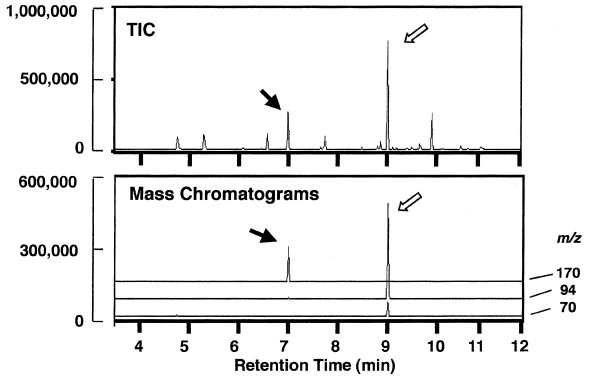

Fig. 2. Total-ion chromatogram (TIC) (upper panel) and mass chromatograms (lower panel) of pethidine and I.S. extracted from spiked human whole blood (12.5 ng of pethidine and 25 ng of I.S.) in the SI mode. In both panels, filled and open arrows show the peaks of pethidine and I.S., respectively.

seemed also aromatized (Fig. 1, lower panel); small peaks appeared at *m*/*z* 167, 114 and 70.

Fig. 2 shows the total ion chromatogram (TIC) and mass chromatograms of pethidine (12.5 ng/ml) and I.S. (25 ng/ml), extracted from spiked human whole blood. Pethidine and I.S. gave intense peaks in both TIC and mass chromatograms at 7.0 and 9.0 min, respectively. Background noises were quite small in mass chromatograms. In the EI mode, both peaks of pethidine and I.S. were found, but many impurity peaks interfered the peaks (data not shown).

3.2. Selected-ion monitoring (SIM)

We have found that mass chromatography would be quite sufficient for quantitating pethidine extracted from human body fluids; we have also tried to quantitate pethidine in whole blood with much higher sensitivity by selected-ion monitoring (SIM) of SIOMS. Fig. 3 shows SIM chromatograms for extracts of whole blood spiked with two different concentrations of pethidine; it shows that as little as 0.1 ng/ml pethidine could be easily determined.

3.3. Reliability of the SIOMS method

We quantitated pethidine spiked human whole blood by mass chromatography of SIOMS; the peak area ratio of *m*/*z* 170 to 94 was used for quantitation. Calibration curves for pethidine in whole blood and urine were drawn by plotting six different concentrations using 25 ng/ml of I.S.. It gave good linearity in the range of 0.625–25 ng/ml pethidine in whole blood or urine. The equations and *r* values

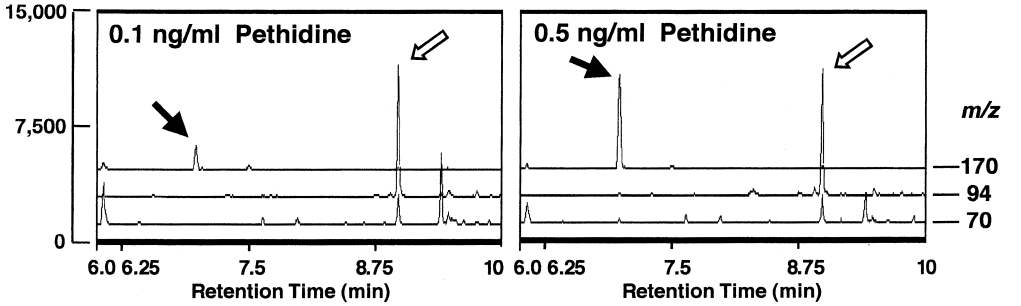

Fig. 3. Selected-ion monitoring (SIM) for two different concentrations (left, 0.1 ng/ml and right panel, 0.5 ng/ml) of pethidine in spiked whole blood. In both panels, filled and open arrows show the peaks of pethidine and I.S., respectively.

were $y = 0.0321x + 0.0049$ and $r = 0.999$ for whole blood, and $y = 0.0447x + 0.0199$ and $r = 0.997$ for urine. Its detection limit (signal-to-noise ratio = 3) was estimated to be 0.2 ng/ml (4 pg on-column) for both whole blood and urine.

The calibration curve was obtained using SIM for pethidine in whole blood in the range of 0.05–2 ng/ml with six plots; the equation and r value were $y = 1.03x + 0.0182$ and $r = 0.997$. The detection limit was estimated to be 0.02 ng/ml (0.4 pg on-column).

Several researchers quantitated pethidine by SIM of conventional EI-MS [15–17]. Their detection limits were 25 ng/ml (plasma) [15], 0.17 ng/ml (serum) [16], and 1 ng/ml (breast milk) [17]. The sensitivity of our present method by mass chromatography is almost the same as one previous method by SIM [16] or 5–125 times higher than other methods [15,17], and that by the present SIM is 8.5–1250 times higher.

The recoveries of pethidine and I.S. were determined by mass chromatography of SIOMS; the peak areas in whole blood spiked with known amounts (2.5 or 12.5 ng/ml for pethidine and 25 ng/ml for I.S.) of the compounds were compared with the peak areas of authentic samples. The recoveries of pethidine from whole blood were 97.9±17.2% (mean±SD, $n = 5$) at 2.5 ng/ml and 113±14.6% at 12.5 ng/ml; that for I.S. was 96.1±8.95% ($n = 10$) at 25 ng/ml.

To check the reproducibility of our present method, we quantitated 2.5 and 12.5 ng/ml of pethidine spiked to whole blood using each calibration curve by mass chromatography. The coefficients of intra-day variations were 8.9% at 2.5 ng/ml and 8.8% at 12.5 ng/ml, respectively ($n = 5$).

3.4. Quantitative determination of pethidine in human whole blood and urine

To validate our method, we quantitate pethidine in whole blood and urine of two healthy volunteers after oral administration of pethidine (10 mg). The whole blood and urine samples were obtained 2 and 6 h after its administration. The TIC and mass chromatograms of whole blood and urine samples are shown in Fig. 4 (2 h after the administration). To quantitate the concentrations of pethidine, we used 0.5 ml of whole blood, and 50 μl (2 h after administration) or 25 μl (6 h after administration) of

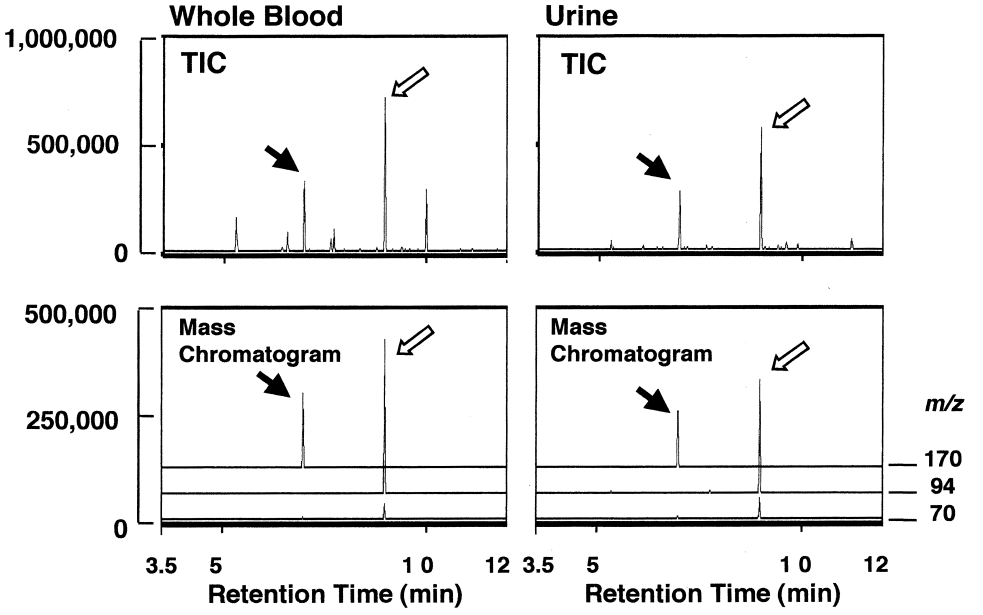

Fig. 4. The TIC and mass chromatograms for pethidine (filled arrows) and I.S. (open arrows) in whole blood (left panels) and urine (right panels) obtained 2 h after oral administration of 10 mg pethidine from subject 1.

Table 1
Levels of pethidine in whole blood and urine samples obtained from two volunteers after its oral administration (10 mg)

Subject	Sample	Concentration of pethidine (ng/ml)	
		2 h after administration	6 h after administration
1	Whole blood	26.9	15.0
	Urine	270	333
2	Whole blood	33.7	18.8
	Urine	497	810

urine were used because the concentrations were above the calibration curves. The results are summarized in Table 1. Usually, a single dose of pethidine is 50–100 mg [1,2], and its therapeutic concentration is 0.2–0.8 μg/ml [18]. Thus, our present method can be sufficiently applicable for monitoring of pethidine concentration in serum or blood samples.

4. Conclusion

This is the first report describing the determination of pethidine in human body fluids by SIOMS. The present method is highly sensitive for detecting and identifying pethidine. SIOMS seems useful in the field of clinical and forensic toxicology.

Acknowledgements

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

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