SENSITIVE DETERMINATION OF PENTAZOCINE IN WHOLE BLOOD AND URINE BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION

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原　　報

SENSITIVE DETERMINATION OF PENTAZOCINE IN WHOLE BLOOD AND URINE BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION

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表面電離検出ガスクロマトグラフィーによる全血ならびに尿中ペンタゾシンの高感度定量

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Summary

Pentazocine, a narcotic analgesic drug, could be measured by gas chromatography (GC) with surface ionization detection (SID), with very high sensitivity. The calibration curve showed satisfactory linearity in the range of 100−1000 pg on column. The detection limit of pentazocine was about 50 pg on column (2.5 ng/ml of a sample). After the establishment of a detailed procedure of extraction of the drug from human samples with Sep-Pak C_{18} cartridges, it could be actually quantitated for whole blood and urine obtained from a volunteer who had received i.m. injection of the 15 mg drug 3 h before the sampling; the levels were 65 ng/ml and 1.84 μg/ml, respectively.

Key words: Pentazocine; Narcotic analgesics; Surface ionization detection; Gas chromatography; Sep-Pak C_{18} cartridges

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Introduction

Pentazocine is a narcotic analgesic drug, which is frequently abused and controlled in many countries. For analysis of pentazocine in biological samples, gas chromatography (GC) with nitrogen-phosphorus detection (NPD) [1−3] and high-performance liquid chromatography [4−6] are being used.

In 1985, Fujii and Arimoto [7] reported surface ionization detection (SID) for GC, a new type of detection system, which was very sensitive and specific especially to tributylamine and triethylamine, suggesting that tertiary amino groups with straight side chain structures give the highest response to this detector. However, during the progress of our studies on GC-SID [8−12], it has become more obvious that tertiary amino compounds with ring structures, such as dextromethorphan, dimemorphan [11] and meperidine [12], also show high sensitivity by this method.

In the present paper, we report that pentazocine also gives high response to SID. A detailed procedure for rapid extraction of the drug with Sep-Pak C_{18} cartridges from whole blood and urine is also presented.

Experimental

Materials

The chemical structure of pentazocine is shown in Fig.1. The 15 mg-vials of the drug for injection were purchased from Yamanouchi Pharmaceutical Co. Ltd., Tokyo. For use of the drug as the authentic standard, the drug was extracted from the injection solution with 3 ml of chloroform under alkaline conditions. The organic layer was appropriately diluted with methanol for preparing the standard. Sep-Pak C_{18} cartridges were purchased from Waters Associates, Milford, MA, USA., and a DB-17 fused silica capillary column (30 m x 0.32 mm i.d., film thickness 0.25 μm) from J&W Scientific, Folsom, CA, USA. Other common chemicals used were of the highest purity commercially available. Whole blood and urine were obtained from healthy subjects.

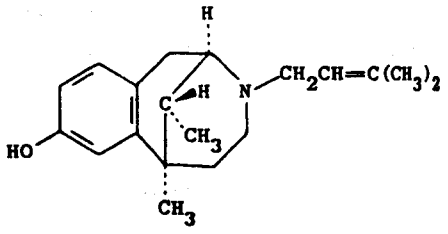

Fig. 1 . Chemical structure of pentazocine.

Administration of pentazocine

A male healthy subject of 31 years of age volunteered to take part in this study; informed consent was obtained from this subject. He received an i.m. injection of 15 mg pentazocine;

and blood (heparinized) and urine were obtained 3 h after the injection.

Extraction with Sep-Pak C_{18} cartridges

For pretreatment of a Sep-Pak C_{18} cartridge, 10 ml of methanol and 10 ml of distilled water were passed through it.

To 1 ml of whole blood or urine, in the presence and absence of pentazocine, 6 ml of distilled water and 3 ml of 1 M Na_2CO_3 were added. The sample solution was then loaded on the pretreated Sep-Pak cartridge at a flow rate not greater than 5 ml/min. It was washed with 10 ml of distilled water twice, and finally 3 ml of chloroform/ethanol (9:1) was passed through it to elute the drug. The minor aqueous layer (upper phase) was discarded by aspiration with a Pasteur pipette. The organic layer was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 100 μl of methanol and a 2-μl aliquot of it was injected into the GC port.

GC conditions

GC was carried out on a Shimadzu GC-15A gas chromatograph equipped with an SID system. A DB-17 fused silica capillary column and a spilt-splitless injector were used. The GC conditions were: column temperature, 150−280 ℃ (1 min hold at 150 ℃); injection temperature, 280 ℃; and helium flow rate, 3 ml/min. The SID conditions were: detector temperature, 280 ℃; heating current through platinum emitter, 2.2A; emitter temperature, *ca.*600 ℃; and ring electrode bias voltage, +200 V with respect to the collector electrode. The sample was injected in the splitless mode at a column temperature of 150 ℃ and the splitter was opened after 1 min.

Results

Figure 2 shows gas chromatograms by GC-SID for whole blood and urine samples, spiked and not spiked with 40 ng pentazocine. Although many impurity peaks appeared in the backgrounds especially for urine samples, the drug peaks did not overlapped any impurity peaks. The recovery was 90.8 % for the whole blood sample and 102 % for the urine sample.

The calibration curve for pentazocine is shown in Fig.3. It showed excellent linearity in the range of 100−1000 pg on column. The equation and the r value for the curve were: $y=8.65x+40.17$, $r=0.9990$. The detection limit was 50 pg on column (2.5 ng/ml of a sample).

The gas chromatograms for whole blood and urine samples obtained 3 h after i.m. injection of pentazocine are shown in Fig.4. The drug appeared as big peaks for both samples. The levels were 65 ng/ml for the blood sample and 1.84 μg/ml for urine sample.

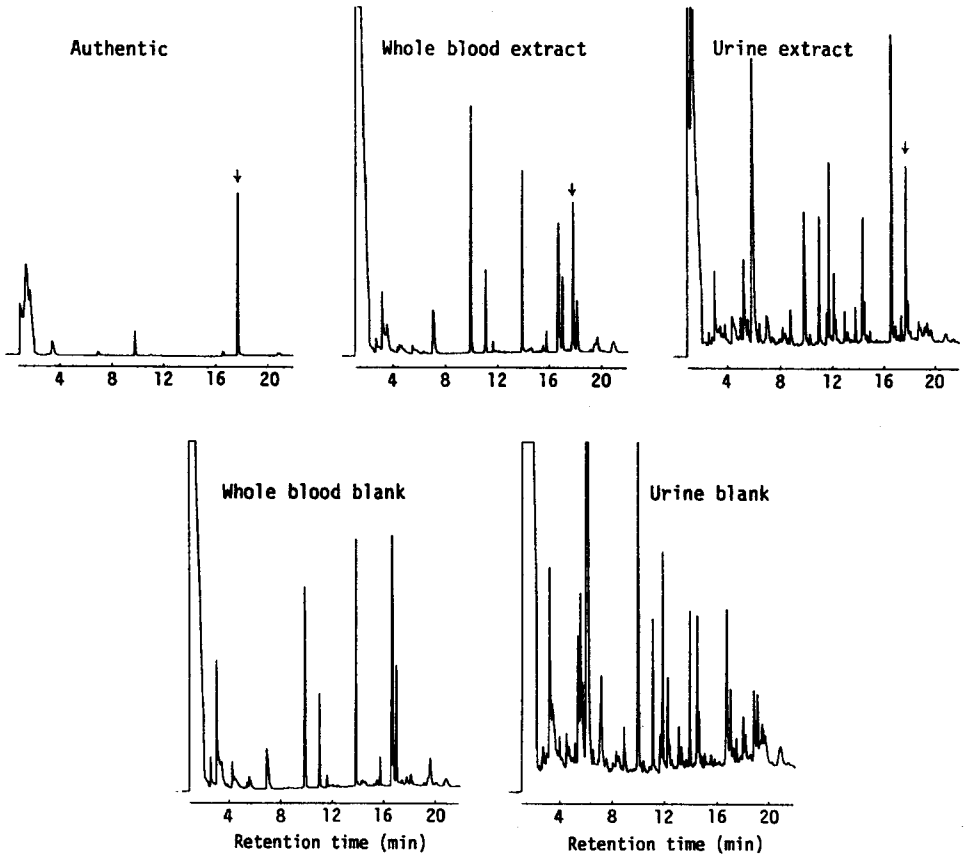

Fig. 2. Capillary GC-SID for extracts of 1 ml human whole blood and urine samples, spiked and not spiked with 40 ng pentazocine. The extraction was made with Sep-Pak C_{18} cartridges. For GC conditions, see text. The arrows show the peaks of pentazocine.

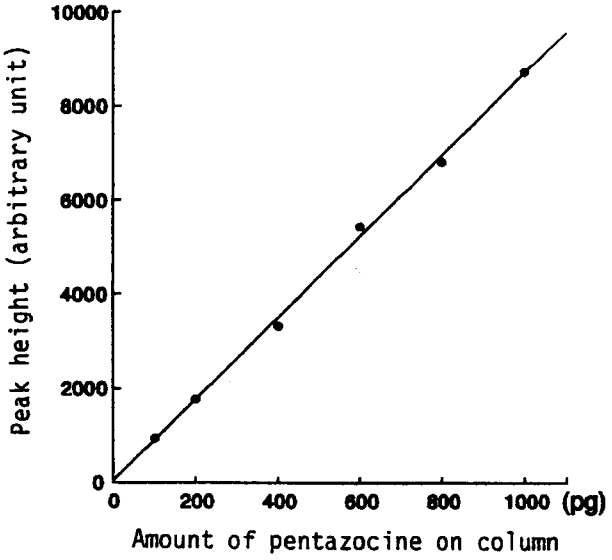

Fig. 3. Calibration curve for pentazocine.

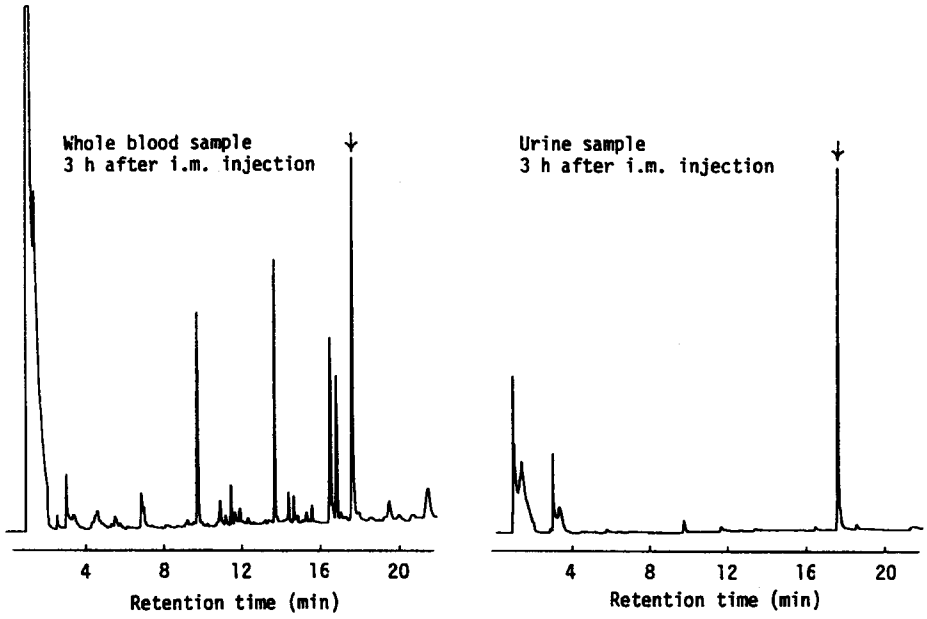

Fig. 4 . Capillary GC-SID for extracts of whole blood and urine samples obtained 3 h after i.m. injection of 15 mg pentazocine. The arrows show the peaks due to pentazocine.

Discussion

This is the first trial to demonstrated that pentazocine, a narcotic analgesic drug, can be detected with high sensitivity by GC-SID, on the basis of addition tests (Fig.2) and actual measurements after i.m. injection of this drug (Fig.4) for whole blood and urine samples.

The sensitivity of the present GC-SID for pentazocine should be compared with that of GC-NPD. Kintz et al [3] reported that the detection limit of capillary GC-NPD for pentazocine was 10 ng/ml of a sample; Mackell and Poklis [1] reported the limit to be 0.5 μg/ml of a sample using the conventional packed-column GC-NPD.

The therapeutic concentration of this drug was reported to be 50—200 ng/ml of plasma; the fatal concentration may be more than 3.3 μg/ml of blood [13]. Since the detection limit of the present GC-SID for pentazocine (2.5 ng/ml) is much below the therapeutic and toxic levels, this method seems useful for detecting its abuse many hours after administration and for drug monitoring in clinical pharmacology, together with its use for autopsy samples in forensic science.

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