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原　　報

DETECTION OF MEPERIDINE (PETHIDINE) IN HUMAN BLOOD AND URINE BY HEADSPACE SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY

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ヘッドスペース固相ミクロ抽出／ガスクロマトグラフィーによるヒト血中及び尿中メペリジン（ペチジン）の検出

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Summary

A simple extraction method is presented for meperidine (pethidine) in human whole blood and urine using headspace solid phase microextraction (SPME) with diphenylpyraline as internal standard (IS). Clear supernatant of whole blood after deproteinization, and untreated urine, containing meperidine and IS, were heated at 100 ℃ in the presence of NaOH and NaCl in a vial with a silicone septum cap; and then an SPME fiber was exposed in the headspace of a vial to allow adsorption of the drugs before gas chromatography. Recoveries of meperidine were 3 % for whole blood and 18 % for urine. Calibration curve for whole blood samples was linear in the range of 0.125–1 μg/ml. The equation and r value 0for the curve were: $y=0.375x+0.0378$ and $r=0.993$. The detection limit was about 0.1 and 0.02 μg/ml for whole blood and urine, respectively.

Key words: Solid phase microextraction (SPME); Headspace method; Gas chromatography; Meperidine; Pethidine; Diphenylpyraline

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Introduction

Meperidine is a narcotic analgesic drug, which is widely used in therapeutic practice. It is usually extracted from biological samples by liquid-liquid or solid-phase extraction [1]. Recently, solid phase microextraction (SPME), which was first introduced by Arthur and Pawliszyn in 1990 [2], has been employed for analysis of compounds of forensic interest [3-5]. In the present study, we demonstrate that the headspace SPME can be used for analysis of meperidine in human whole blood and urine.

Experimental

Materials

Meperidine hydrochloride was donated by Tanabe Seiyaku Co., Ltd., Osaka; diphenylpyraline hydrochloride (internal standard, IS) was purchased from Sigma Chemical Co., St. Louis, MO, USA. SPME devices and their 100 μm bonded polydimethylsiloxane fiber assemblies were purchased from Supelco Inc., Bellefonte, PA, USA; DB-17 fused silica capillary columns (30 m × 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.

Extraction procedure

One milliliter of whole blood, spiked or not spiked with meperidine and IS, was mixed with 1 ml of 1 M perchloric acid solution for deproteinization. After shaking vigorously on a Vortex-type mixer, it was centrifuged at 3,000 rpm for 5 min. The supernatant was decanted to a 7.5-ml vial, and 100 μl of 10 M sodium hydroxide solution and 0.5 g of sodium chloride were added. The vial was sealed with a silicone septum cap, and shaken for 10 s. In the case of urine, the procedure of deproteinization and centrifugation was omitted; 1 ml of urine, spiked with the drugs, was directly put into the 7.5-ml vial containing 100 μl of 0.1 M sodium hydroxide solution and 0.5 g of sodium chloride. The septum piercing needle of the SPME fiber holder was passed through the septum after heating the vial at 100℃ for 10 min on an aluminum block heater. The fiber was exposed in the headspace of the vial at 100℃ for 30 min. The fiber was retracted into the needle, pulled out and then immediately analyzed by gas chromatography (GC).

GC conditions

GC was performed on an HP-5890 Series II gas chromatograph equipped with flame ionization detection (Hewlett-Packard, Palo Alto, CA, USA) and a DB-17 fused silica capillary column.

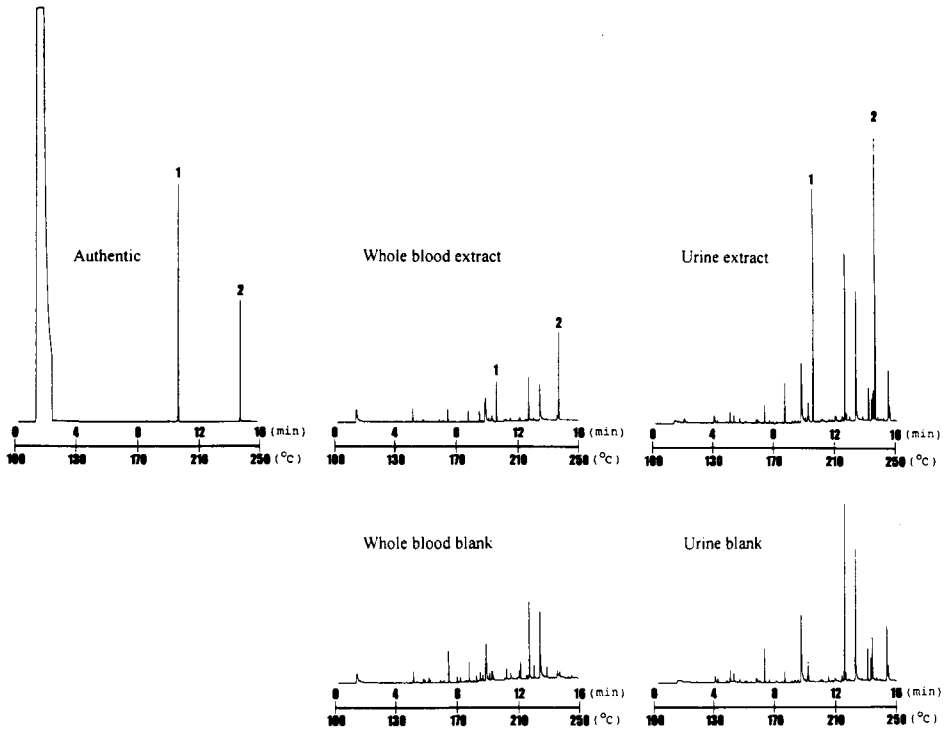

Fig. 1. Gas chromatograms for non-extracted authentic drugs in methanol (0.4 μg of meperidine and 0.2 μg of IS on column), extracts from human whole blood and urine using headspace SPME, and their backgrounds. For the addition tests, 1 μg of meperidine and 0.5 μg of IS were added to 1 ml of a sample. The vertical scale of the authentic chromatogram (upper left panel) is not the same as that of other chromatograms. 1: meperidine; 2: diphenylpyraline (IS).

Column temperature was set at 100℃ for 1 min, and then programmed from 100℃ to 250℃ at 10℃/min. Injection and detector temperatures were 240 and 280℃, respectively, and helium flow rate 3 ml/min. The samples were injected in the splitless mode, and the splitter was opened after 1 min.

Results and discussion

Figure 1 shows gas chromatograms for the authentic compounds (0.4 μg of meperidine and 0.2 μg of IS on column) dissolved in methanol, headspace SPME extracts (1 μg of meperidine and 0.5 μg of IS in 1 ml of a sample) and their backgrounds. The retention times for meperidine and IS were 10.7 and 14.8 min, respectively. The peaks of meperidine were separated from impurity peaks for both samples; a small impurity peak overlapped the peak of IS, but it gave almost no problem. The recoveries were calculated by measuring the peak area

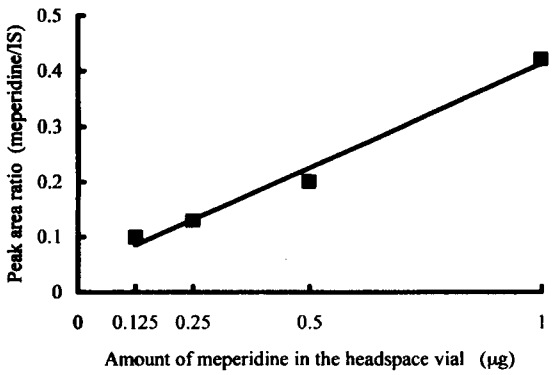

Fig. 2. Calibration curve for meperidine with diphenylpyraline as IS. The vertical axis shows the peak area ratio of meperidine to IS (0.5 μg/ml of whole blood).

for the SPME extracts against that for the authentic compounds in methanol. Those of meperidine were 3 and 18 % for whole blood and urine samples, respectively, those of IS were 14 and 49 %.

Sodium chloride was used for SPME to increase recoveries in a report [6]. We also added the salt to the samples in this study, and recoveries of meperidine and IS were approximately 4.5 times and 8 times better, respectively, than those without sodium chloride.

The calibration curve for a whole blood sample, with diphenylpyraline as IS, is shown in Fig. 2. It was linear in the range of 0.125–1 μg/ml. The equation and r value were: $y=0.375x+0.0378$ and $r=0.993$. The detection limits of meperidine were about 0.1 μg/ml of whole blood and about 0.02 μg/ml of urine. Therapeutic concentration of meperidine in human plasma was reported to be 0.2–0.8 μg/ml, and toxic effects are usually associated with blood concentrations greater than 2 μg/ml [7]. These mean that the present SPME method is applicable to measurements of the drug at both therapeutic and toxic levels.

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