DETECTION OF COCAINE IN HUMAN URINE BY SOLID-PHASE MICROEXTRACTION AND CAPILLARY GAS CHROMATOGRAPHY WITH NITROGEN-PHOSPHORUS DETECTION

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原　　報

DETECTION OF COCAINE IN HUMAN URINE BY SOLID-PHASE MICROEXTRACTION AND CAPILLARY GAS CHROMATOGRAPHY WITH NITROGEN-PHOSPHORUS DETECTION

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固相ミクロ抽出/窒素燐検出キャピラリーガスクロマトグラフィーによるヒト尿中コカインの検出

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Summary

A method for extraction of cocaine from human urine with solid-phase microextraction (SPME) is presented. A polydimethylsiloxane-coated SPME fiber was immersed directly into a human urine sample containing cocaine and cocapropylene as internal standard (IS) in a vial to allow adsorption of the compounds. The fiber needle was then injected into the port of a capillary gas chromatography (GC) instrument with nitrogen-phosphorus detection. The SPME-GC gave big peaks for cocaine and IS. Recovery of cocaine and IS with use of the SPME-GC were 20 and 30 %, respectively. The detection limit of cocaine was about 6 ng/0.5 ml of urine.

Key words: Solid-phase microextraction (SPME); Cocaine; Cocapropylene; Gas chromatography; Nitrogen-phosphorus detection

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Introduction

Solid-phase microextraction (SPME) is a recent technique for extracting organic compounds from an aqueous matrix into a stationary phase of polydimethylsiloxane immobilized on a fused-silica fiber [1]. The analytes are thermally desorbed from the fiber in the injector of a gas chromatograph. Recently, reports have appeared on the headspace SPME for some drug groups [2, 3]. In this study, we present that cocaine and cocapropylene as internal standard (IS) can be successfully extracted from human urine by SPME.

Experimental

Materials

Pure powder of cocaine-HCl was purchased from Shionogi & Co., Ltd., Osaka. Cocapropylene was synthesized with benzoylecgonine and iodopropane by the method of Roy *et al.* [4]. SPME devices and 100 μm bonded polydimethylsiloxane fiber assemblies for SPME were purchased from Supelco, Inc. (Bellefonte, PA, USA); and DB-1 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 analytical grade. Urine was obtained from healthy subjects.

SPME procedure

The polydimethylsiloxane-coated fiber for SPME was pretreated in an injection port of a gas chromatograph at 250 ℃ for 1 h to remove fiber contaminants; a severely contaminated fiber could be cleaned by thermal desorption in the injection port at 280 ℃ for 1–2 h. To a 1 ml-conical vial containing 20 μl of 2.5 % NaF solution and a small triangle-shaped magnetic stirring bar, were added 0.5 ml of urine, cocaine and IS (250 ng each). The vial was sealed with a silicone septum cap, and the contents were mixed for 10 s. The syringe needle of the SPME device was passed through the septum. The pretreated fiber was push out from the needle and immersed directly into the sample solution in the vial for 30 min to allow adsorption of the compounds. The fiber was withdrawn into the needle and pulled out from the vial. It was then injected into the port of the gas chromatograph; the fiber was exposed in the injection port for 3 min to ensure desorption of the compounds from it.

GC conditions

GC analyses were carried out on an HP-5890 Series II gas chromatograph equipped with nitrogen-phosphorus detection (Hewlett-Packard, Palo Alto, CA, USA). The GC conditions were: column temperature 120 to 280 ℃ at 10 ℃/min; injection temperature 240 ℃; detector

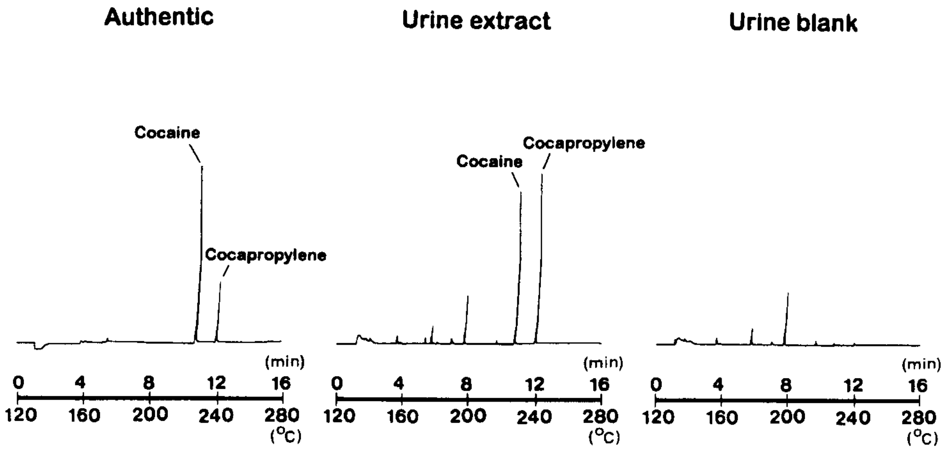

Fig. 1. Capillary GC–NPD for cocaine and cocapropylene extracted from human urine by use of the direct-immersion SPME. The mixture of cocaine and cocapropylene (250 ng each) was added to 0.5 ml of human urine. The vertical scale of the authentic chromatogram is not the same as that of the urine extract and urine blank chromatograms.

temperature 280 ℃; and helium flow rate 3 ml/min. In the case of the authentic samples dissolved in methanol, a 1-μl aliquot of it was subjected to GC analysis. The samples were injected in the splitless mode at a column temperature of 120 ℃ and the splitter was opened after 1 min.

Results and discussion

We tried headspace SPME for cocaine with both polydimethylsiloxane and polyacrylate fibers, but it was unsuccessful because of low recoveries and low reproducibility (data not shown); this is the reason why we have adopted the direct-immersion SPME.

Figure 1 shows gas chromatograms for the authentic compounds (20 ng each on column) dissolved in methanol and for direct-immersion SPME extracts from 0.5 ml of urine samples, to which 250 ng each of the compounds had been added. Cocaine and IS were detected with high sensitivity and separated well from each other on the gas chromatograms under our GC conditions (Fig. 1, left panel). Their retention times were 10.8 and 12.1 min, respectively. The SPME extract also gave big peaks for both compounds (Fig. 1, middle panel). To check background noises, urine was treated as above in the absence of both compounds; only a few background noises appeared at column temperatures around 130–200 ℃ (Fig. 1, right panel), and gave no problems.

The recoveries of cocaine and IS (250 ng each for 0.5-ml samples) were calculated by comparing the peak areas obtained from the SPME extracts of the spiked urine samples with those obtained from the non-extracted authentic compounds (20 ng each on column) dissolved in methanol; the values for cocaine and IS were 20 and 30 %, respectively. The calibration curve for cocaine extracted from urine samples showed linearity in the range of 30−250 ng/0.5 ml with cocapropylene as IS. The detection limit of cocaine was about 6 ng/0.5 ml of urine.

In the present study, we have been able to extract and detect cocaine in human urine by use of SPME-GC; this is first report to use SPME for extraction of cocaine from human samples. The merits to use the SPME are that the analytical procedure is much simpler and faster than those by liquid-liquid and solid-phase extractions and that much cleaner extracts can be obtained. Extensive studies to measure cocaine and cocaethylene in human urine and whole blood by SPME-GC are now in progress in our laboratories.

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