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原　　報

DETERMINATION OF SOME CARBAMATE PESTICIDES IN HUMAN BODY FLUIDS BY HEADSPACE SOLID PHASE MICRO EXTRACTION 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体液中カーバメート系農薬の定量

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Summary

Headspace solid phase micro extraction (SPME) has been applied to six carbamate pesticides, *viz*. xylylcarb, XMC, isoprocarb, fenobucarb, propoxur and carbofuran, in human whole blood and urine. Sample solution, after mixing with sodium chloride, was heated in a vial at 70 ℃, and an SPME fiber was exposed to the headspace of the vial. Immediately after the fiber was pulled out, the fiber needle was injected into the port of a gas chromatograph with a flame ionization detector. Recoveries were 0.582−1.87 % for whole blood, and 2.97−20.6 % for urine; it was highest for fenobucarb and lowest for carbofuran. The detection limits were about 0.1−0.5 μg/ml of whole blood, and 0.01−0.05 μg/ml of urine.

Key words: Solid phase micro extraction (SPME); Headspace method; Gas chromatography; Carbamate pesticides; Isoprocarb; XMC; Fenobucarb; Xylylcarb; Propoxur; Carbofuran

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Introduction

Carbamates are widely used pesticides; accidental or suicidal cases due to carbamate poisoning are sometimes encountered [1−6]. Solid phase micro extraction (SPME) was first introduced by Arthur and Pawliszyn in 1990 [7], and recently reports have appeared for SPME of compounds of forensic interest [8−15]. In this study, we present that headspace SPME can be used for six carbamate pesticides in whole blood and urine before their capillary gas chromatography (GC).

Experimental

Materials

Chemical structures of six carbamate pesticides used in this study are listed in Fig. 1. Isoprocarb, XMC, fenobucarb, xylylcarb and propoxur were purchased from Wako Pure Chemical Ind., Ltd., Osaka; carbofuran was donated by Nissan Chemical Ind., Ltd., Funahashi. SPME devices and their 100 μm bonded polydimethylsiloxane fiber assemblies were purchased from Supelco Inc., Bellefonte, PA, USA; an RTX-35 fused silica capillary column (30 m × 0.32 mm ID, film thickness 0.25 μm) from Restek Corp., Bellefonte, PA, USA. Other common chemicals used were of the highest purity commercially available. Whole blood and urine were obtained from healthy subjects.

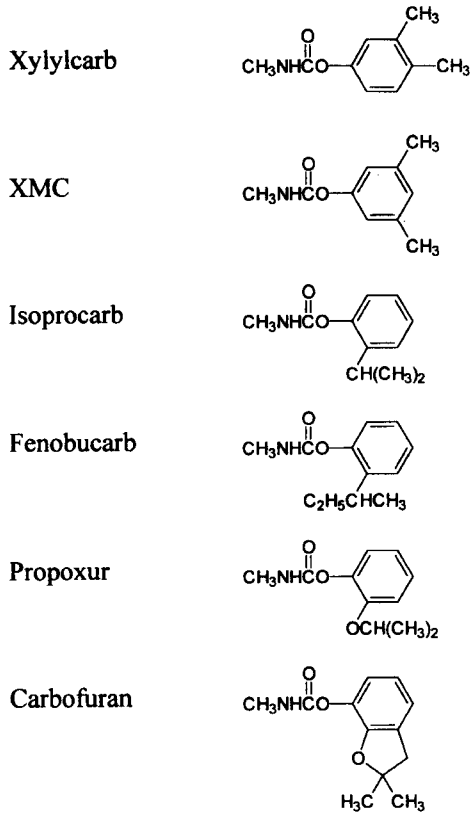

Fig. 1. Chemical structures of six carbamate pesticides used in this study.

Extraction procedure

To 100 μl of whole blood placed in a 7.5-ml vial were added 900 μl of distilled water and 0.5 g of sodium chloride. In the case of urine, 1 ml of the sample was put into the 7.5-ml vial containing 0.5 g of sodium chloride. For addition tests, 1 μg each of the carbamate pesticides was added to 100 μl of whole blood and 1 ml of urine. The vial was sealed with a silicone septum cap, and shaken gently for 10 s. The septum piercing needle of the SPME fiber holder was passed through the septum after 10-min of pre-heating of the vial at 70 ℃ on an aluminum block heater. The fiber was exposed to the headspace of the vial at 70 ℃. After 30-min

exposure, the fiber was retracted into the needle, pulled out and then immediately inserted into the GC port for desorption of the pesticides at 185 ℃ for 2 min in the injector.

GC conditions

GC was performed on an HP-6890 gas chromatograph equipped with flame ionization detection (Hewlett-Packard, Palo Alto, CA, USA) and an RTX-35 fused silica capillary column. Column temperature was set at 100 ℃ for 1 min, and then programmed from 100 to 200 ℃ at 5 ℃/min. Injector and detector temperatures were 185 and 200 ℃, 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 2 shows gas chromatograms for the authentic compounds (10 ng each on column) dissolved in methanol, headspace SPME extracts and their backgrounds. The retention times

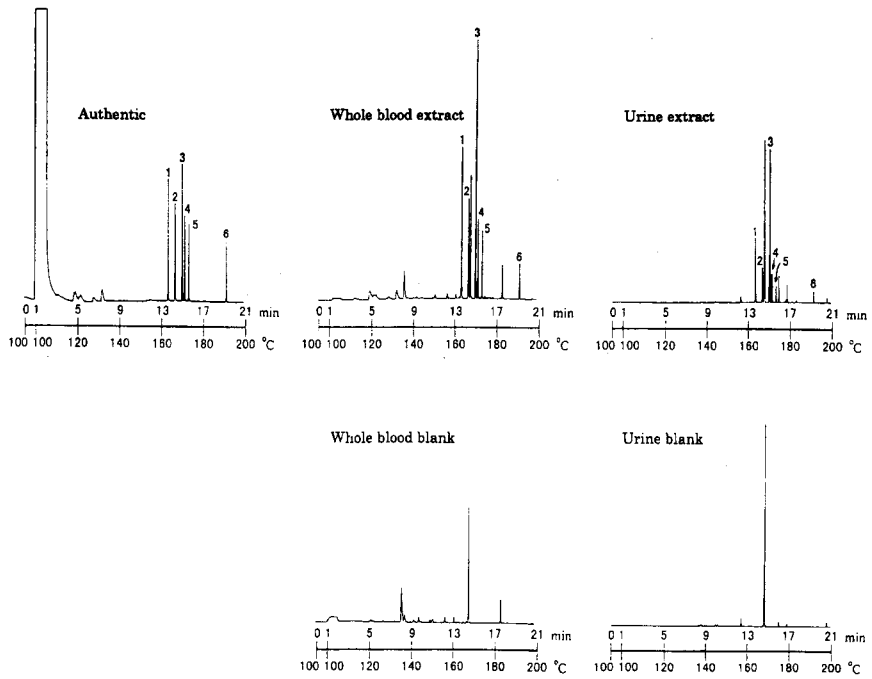

Fig. 2. Gas chromatograms for non-extracted authentic compounds dissolved in methanol (10 ng on column), extracts from human whole blood (10 μg/ml for each compound) and urine (1 μg/ml for each compound) using headspace SPME, and their backgrounds. The magnification rate of the vertical axis of chromatograms for urine extract and blank (right panels) was eight-fold lower than that of other chromatograms. 1: isoprocarb; 2: XMC; 3: fenobucarb; 4: xylylcarb; 5: propoxur; 6: carbofuran.

were 13.8, 14.4, 15.1, 15.4, 15.7 and 19.3 min for isoprocarb, XMC, fenobucarb, xylylcarb, propoxur and carbofuran, respectively. No overlapping impurity peaks appeared for both whole blood and urine samples. The recoveries were calculated by measuring the peak areas for the SPME extracts against those for the authentic compounds in methanol; those for isoprocarb, XMC, fenobucarb, xylylcarb, propoxur and carbofuran extracted from whole blood were 1.20, 1.04, 1.87, 1.71, 0.891 and 0.582 %, respectively; those from urine were 9.00, 6.32, 20.6, 5.13, 3.72 and 2.97 %, respectively.

A few kinds of salts were used for SPME to increase recoveries of some compounds in the previous reports [10,12,15]. In this study, sodium chloride was added to the sample solution without any pH changes. The recoveries were more than three times better than those without the salt. Neither acidification nor alkalinization of the test solutions gave better results (unpublished observation).

The calibration curves for the six carbamate pesticides extracted from whole blood were linear in the range of 0.4−10 μg/ml for isoprocarb and fenobucarb, 2−10 μg/ml for XMC, xylylcarb, propoxur and carbofuran. The detection limits were about 0.1−0.5 μg/ml for whole blood, and about 0.01−0.05 μg/ml for urine. Picotte *et al.* [2] reported a suicide case with carbofuran. In their report the concentration of carbofuran was found to be 17 μg/ml of cardiac blood, which can sufficiently be measured by our method.

This is the first report to extract carbamate pesticides from biological fluids by SPME. It seems useful in forensic, clinical and environmental toxicology.

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