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ORIGINAL ARTICLE

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A simple analysis of 5 thinner components in human body fluids by headspace solid-phase microextraction (SPME)

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Abstract A simple method for the extraction of 5 thinner components from human whole blood and urine, using the headspace solid-phase microextraction (SPME) method is presented. After heating a vial containing the samples with 5 compounds (toluene, benzene, *n*-butyl acetate, *n*-butanol and *n*-isoamyl acetate) at 80° C, a polydimethylsiloxane-coated SPME fiber was exposed to the headspace of the vial to allow adsorption of the compounds. The fiber needle was then injected into a capillary gas chromatography (GC) port. The headspace SPME-GC gave intense peaks for each compound and a low level of background noise was seen only for whole blood. Recovery rates of the 5 compounds by use of the headspace SPME-GC were 50–70%. Reproducibility for headspace SPME-GC data were excellent for both body fluids. The calibration curves showed linearity in the range 2–100 ng/0.5 ml whole blood or urine. The detection limits of each compound were 1.1–2.4 ng/0.5 ml sample. The present results on the analysis of 5 thinner components by headspace SPME-GC suggest its applicability to a number of other volatile compounds in forensic toxicology.

Key words Toluene · Benzene · *n*-Butyl acetate · *n*-Butanol · *n*-Isoamyl acetate · Solid-phase microextraction (SPME) · Headspace method · Capillary gas chromatography

Introduction

The inhalation of thinner vapor occasionally causes industrial accidents, and thinner abuse by the young generation is a social problem. Simple and rapid analytical techniques are required to detect thinner components from human samples in forensic toxicology. In 1990 Pawliszyn and co-workers developed a new extraction technique called solid-phase microextraction (SPME) [1] which employs a stationary phase of polydimethylsiloxane coated on to a fused silica fiber to extract a compound from aqueous or volatile samples in sealed vials. After equilibration between the headspace and the coated fiber, the fiber needle can be directly injected into a gas chromatography (GC) port for analysis. In this paper, we show that 5 thinner components can be successfully extracted from human body fluids by headspace SPME.

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Materials and methods

Materials. Toluene, benzene, *n*-butyl acetate, *n*-butanol, *n*-isoamyl acetate and ethyl benzene were obtained from Wako Pure Chemical Industries (Osaka, Japan). The SPME devices and 100 μm bonded polydimethylsiloxane fiber as assemblies for SPME were purchased from Supelco Inc. (Bellefonte, Pa.), and DB-WAX fused silica capillary columns (30 m × 0.32 mm i.d., film thickness 0.25 μm) from J & W Scientific (Folsom, Calif.). Other chemicals used were of analytical grade. Whole blood and urine were obtained from healthy subjects.

Headspace SPME procedure. The polydimethylsiloxane-coated fiber for SPME was preheated at 250° C for 1 h in an injection port of GC to remove fiber contaminants.

Stock solutions (20 μg/ml) of toluene, benzene, *n*-butyl acetate, *n*-butanol and *n*-isoamyl acetate, and internal standard (IS) solution (2 μg/ml) of ethylbenzene were prepared in methanol. Five microliters (100 ng) of the standards were added to a 7.5 ml-vial containing 0.5 ml of whole blood or urine together with 5 μl (10 ng) of IS, 1.5 ml of distilled water and a magnetic stirring bar. The vial was rapidly sealed with a silicone septum cap and heated at 80° C with stirring by use of an aluminum block heater (React-Therm heating/stirring model, Pierce, Rockford, Ill.). After heating for 15 min, the septum piercing needle of the SPME device was passed through the septum. The pretreated fiber was pushed out of the needle and exposed to the headspace of the vial for 5 min to allow adsorption of the compounds (Fig. 1). The fiber was withdrawn into the needle, pulled out from the vial, and injected into the port of a capillary gas chromatograph. The fiber was exposed in the injection port for 3 min to ensure complete desorption of all compounds.

GC conditions. GC analyses were carried out on a Shimadzu GC-14B gas chromatograph equipped with flame ionization detection (Shimadzu Co., Kyoto, Japan). The GC conditions were: column

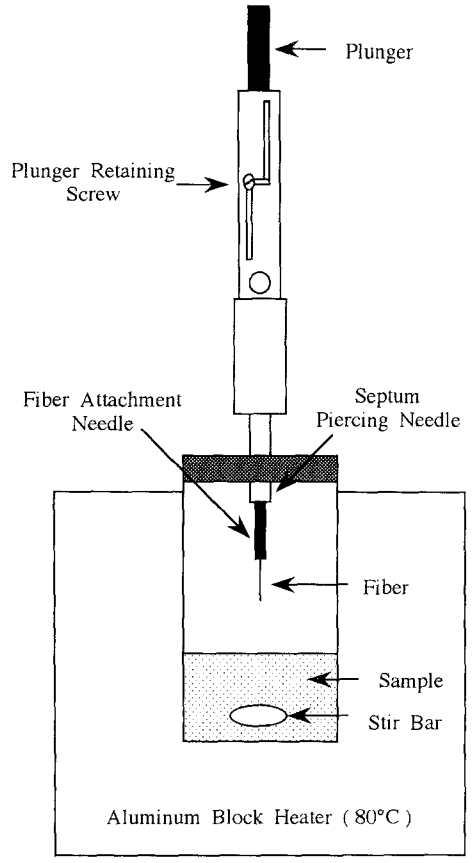

Fig. 1 Schematic illustration of the headspace SPME method

temperature 35–230° C (1 min hold at 35° C, 20° C/min); injection temperature 200° C, and helium flow rate 4 ml/min. In the case of the authentic samples dissolved in methanol, a 1-μl aliquot was subjected to GC analysis. 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 headspace SPME extracts from whole blood and urine samples (0.5 ml), to which 100 ng each of 5 thinner components had been added, in the presence of ethylbenzene as IS (10 ng). The headspace SPME-GC for whole blood and urine gave intense peaks for each compound. Separation of all compounds from each other and from impurities was generally satisfactory. To check background noise, whole blood and urine were treated as above in the absence of the components and IS. The background for whole blood gave small impurity peaks over a wide range of temperatures (Fig. 2, upper panels) but no interfering peaks appeared around the compounds. The background for urine samples was very clean.

The recovery rates of the 5 thinner components were calculated by comparing the peak area obtained from non-extracted authentic compounds dissolved in methanol (100 ng each on column) with that obtained from the headspace SPME extracts of whole blood and urine samples, and were in the range 50–70% for whole blood and urine.

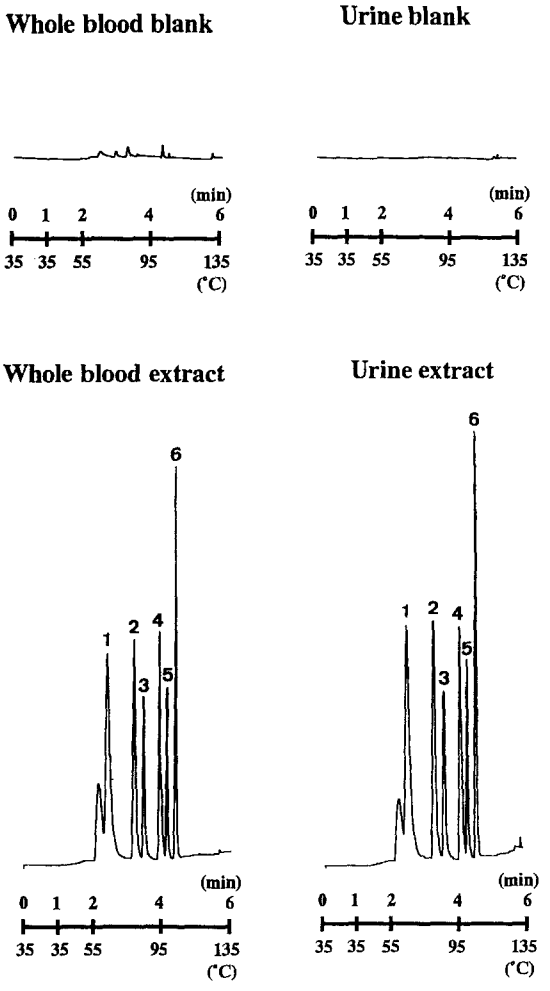

Fig. 2 Capillary GC with FID for 5 thinner components from human whole blood and urine by use of headspace SPME. Key: 1, benzene; 2, toluene; 3, *n*-butyl acetate; 4, ethylbenzene (IS); 5, *n*-butanol; 6, *n*-isoamyl acetate

Table 1 shows the reproducibility of the headspace SPME-GC data for the 5 compounds in human whole blood and urine. The CV values were not more than 5.98% for both whole blood and urine samples.

Figure 3 shows calibration curves for 5 thinner components with different amounts of the 5 components after extraction from whole blood by use of the headspace SPME. They showed linearity in the range 2–100 ng/0.5 ml (2–100 ng/7.5 ml vial). The equations for the curves were: $y = 0.015x + 0.0027$ for toluene, $y = 0.019x + 0.0021$ for benzene, $y = 0.009x + 0.007$ for *n*-butyl acetate, $y = 0.007x + 0.005$ for *n*-butanol and $y = 0.015x + 0.017$ for *n*-isoamyl acetate. The correlation coefficients (r value) of each calibration curve were 0.999 for benzene and *n*-isoamyl acetate, 0.986 for toluene, *n*-butyl acetate and *n*-butanol. The calibration curves for the 5 compounds added to urine were also linear in the same range. The equations for the curves were: $y = 0.015x + 0.001$ for toluene, $y = 0.020x + 0.007$ for benzene, $y = 0.010x + 0.009$ for *n*-butyl acetate, $y = 0.008x + 0.001$ for *n*-butanol and $y = 0.016x + 0.011$ for *n*-isoamyl acetate. The correla-

Table 1 Reproducibilities for headspace SPME-GC data of 5 compounds of thinner from whole blood and urine

Compound	Percent reproducibility[a]				
	Benzene	Toluene	*n*-Butyl acetate	*n*-Butanol	*n*-Isoamyl acetate
Whole blood					
Mean ± SD	90 ± 4.6	98 ± 3.6	95 ± 4.0	87 ± 5.2	96 ± 2.4
CV	5.05	3.67	4.21	5.98	2.50
Urine					
Mean ± SD	105 ± 4.2	101 ± 3.0	97 ± 3.8	89 ± 4.4	99 ± 2.0
CV	4.00	2.97	3.92	4.94	2.02

[a] The values were calculated by comparing the peak area on gas chromatograms obtained from distilled water extracts of the 5 thinner components with that obtained from extracts of whole blood and urine samples. Each compound (100 ng each) was added to 0.5 ml of samples. The values are means ± SD (standard deviation) and CV (coefficient of variation) from 6 experiments.

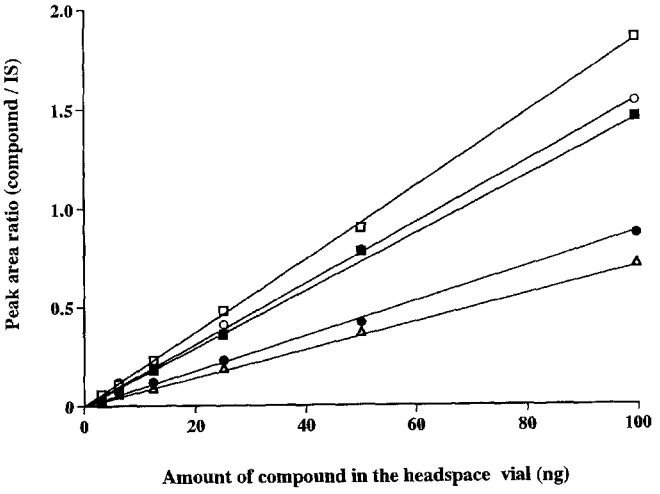

Fig. 3 Calibration curves of benzene (□), *n*-isoamyl acetate (○), toluene (■), *n*-butyl acetate (●) and *n*-butanol (△) for whole blood. Five compounds were measured against ethylbenzene (10 ng) as IS. Each point represents the mean of duplicate determinations

tion coefficients (*r* value) of each calibration curve were 0.999 for benzene and *n*-isoamyl acetate, 0.987 for toluene, and 0.986 for *n*-butyl acetate and *n*-butanol. The detection limit of each compound was about 1.1–2.4 ng/0.5 ml of whole blood and urine.

This is the first report to extract and detect 5 thinner components from human body fluids by use of the headspace SPME-GC method. Data on SPME are limited at present because it was developed only in 1990 [1] and is not yet in widespread use. The SPME method has been applied to analysis of organic compounds in water or groundwater, caffeine in beverages, and halogenated volatiles in food [2–7]. In the previous reports, the thinner compounds were separated from human body fluids by use of the conventional headspace method [8–14]. For the analysis of volatiles by the conventional method, 0.1–1.0 ml of headspace vapor should be injected into GC ports. Thus middle-bore capillary columns with splitless injection cannot be used for such a headspace method. The combination of SPME with the headspace method has enabled the use of middle-bore capillary GC, which gives sharp peaks and much higher sensitivity (Fig. 2).

The present results on the analysis of the thinner components by the headspace SPME-GC show its applicability to a number of other volatile compounds in forensic toxicology, although ethanol and methanol cannot be concentrated by the SPME (unpublished observation).

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BOOK REVIEW

Sellier KG, Kneubuehl BP (1994) Wound Ballistics and the Scientific Background. Elsevier, Amsterdam New York, 479 pages, Dfl. 260.00. ISBN 0-444-81511-2

Several textbooks on ballistics are available, predominantly dealing with interior and exterior ballistics. There are also numerous publications on the forensic aspects of wounds caused by firearms. Wound ballistics form the basis on which gunshot wounds are examined from a forensic point of view. Despite the fact that many original articles on wound ballistics have been published, there are relatively few comprehensive books on the subject. This gap is now filled by Sellier and Kneubuehl's book.

A strong point of this book is the understanding of physical processes leading to biological effects. In the chapter "Physical Basics of Wound Ballistics", the principles of mechanics and fluid dynamics as well as measurement techniques are described and explained. Even scientists who have been concerned with wound ballistics for a long time are likely to benefit from this chapter. After outlining the history of weapons and ammunition and a short presentation of interior and exterior ballistics, the authors describe the main aspects of general wound ballistics and the use of tissue simulants. The special wound ballistics of short handguns and shoulder weapons are explained in separate chapters, emphasizing their special properties. A large chapter contains numerous tables on material properties, caliber and projectile designations, ballistics data of ammunition, twists, shotgun calibers and shot pellets as well as firing tables. The Anglo-American and the metric systems are considered together with conversion tables. The collection of tables and an English, German and French glossary of ballistic terms may be of great help in the daily routine. The bibliography and references contain more than 350 literature sources.

The authors explain and evaluate the different concepts in wound ballistics. Not everybody will agree with every evaluation. For example, the authors seem to favour the Kinetic Energy Deposit Concept for measuring the effectiveness of handgun ammunition. This method has been rejected by FBI workshops and Martin Fackler among others.

Nevertheless the authors have succeeded in writing a comprehensive and precise textbook on wound ballistics which is easy to read despite the fundamental physical background. The book will be of great value to forensic scientists, pathologists, criminal lawyers and trauma surgeons. It can be used as an introduction to the subject as well as a reference book.

The book is available in North America from Elsevier Science Inc., P.O. Box 945, Madison Square Station, New York, NY 10160-0757, USA and in the rest of the world from Elsevier Science B.V., Sara Burgerhartstraat 25, 1055 KV Amsterdam, The Netherlands.

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