Detection of Tricyclic Antidepressants in Whole Blood by Headspace Solid-Phase Microextraction and Capillary Gas Chromatography

Xiao-Pen Lee, Takeshi Kumazawa*, and **Keizo Sato**

Department of Legal Medicine, Showa University School of Medicine, 1–5–8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan

Osamu Suzuki

Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431–31, Japan

Abstract

A simple method for the extraction of four tricyclic antidepressants from whole blood by headspace solid-phase microextraction (SPME) is presented. The whole blood samples contain four drugs (amitriptyline, chlorimipramine, imipramine, and trimipramine) and are heated at 100°C in a septum-capped vial in the presence of distilled water and NaOH solution; a polydimethylsiloxane-coated SPME fiber is exposed to the headspace of the vial to allow adsorption of the drugs before capillary gas chromatography (GC) with flame-ionization detection. The headspace SPME–GC produces intense peaks for each drug with very little background noise. Recoveries of the four drugs by the present method are 5.3–12.9%. The calibration curves for the drugs show linearity in the range of 31–1000 ng/0.5 mL. The detection limits of each drug are 16–25 ng/0.5 mL. Imipramine is detectable from rat blood 5 h after oral administration of imipramine (500 mg/kg body weight); the concentration is 1.44 ± 0.209 µg/mL.

Introduction

Solid-phase microextraction (SPME) is a new technique first introduced by Arthur and Pawliszyn in 1990 (1). This procedure employs a stationary phase of polydimethylsiloxane coated on a fused-silica fiber to extract compounds from aqueous or volatile samples in a sealed vial. In the headspace SPME method, the fiber can be directly injected into the port of a gas chromatography (GC) unit for analysis after equilibration between the headspace and the coated fiber. We have tried headspace or direct immersion SPME for thinner components (2), ethanol (3), organophosphate pesticides (4), carbamate pesticides (5), meperidine (6), cocaine (7), local anaesthetics (8,9), and phenothiazines (10) in human body fluids. In this paper, we showed that four tricyclic antidepressants could be successfully extracted from human whole blood by headspace SPME–GC.

* Author to whom correspondence should be addressed.

Experimental

Materials

Four tricyclic antidepressants were used in the present study; their chemical structures are shown in Table I. Amitriptyline–HCl was obtained from Yamanouchi Pharmaceutical (Tokyo, Japan), chlorimipramine–HCl and imipramine–HCl were obtained from Ciba-Geigy (Basel, Switzerland), and trimipramine maleate was obtained from Shionogi (Osaka, Japan). SPME devices and 100-µm bonded polydimethylsiloxane fiber assemblies were purchased from Supelco (Bellefonte, PA), and DB-1 fused-silica capillary columns (30 m × 0.32-mm i.d., 0.25-µm film thickness) were purchased from J&W Scientific (Folsom, CA). Other common chemicals used were of analytical grade. Whole blood was obtained from healthy subjects.

Headspace SPME procedure

In preliminary experiments, we tested various temperatures for headspace vials; higher temperatures gave higher recoveries for the present nonvolatile tricyclic antidepressants. However, because of the high pressure and damage to the septum, 100°C was the maximum temperture. The effects of pH and various salts, such as NaCl and K_2CO_3, were also checked; alkalinization using only NaOH resulted in the best recoveries for each drug. The desorption times (1, 3, and 5 min) for the present SPME fiber in the injection port at 280°C were checked, but there were no differences between peak areas on the gas chromatograms among the three periods for the four drugs. Thus, 1 min of exposure was sufficient for desorption in the injection port.

The polydimethylsiloxane-coated fiber for SPME was pretreated in a GC injection port at 250°C for 1 h to remove fiber contaminants. Stock solutions (50 µg/mL each) of the tricyclic antidepressants were prepared in methanol. Whole blood (0.5 mL), four tricyclic antidepressants (500 ng each), 0.5 mL of distilled water, and 100 µL of a 5M NaOH solution were added to a 7.5-mL vial containing a magnetic stirring bar. The vials were rapidly sealed with silicone septum caps and heated at 100°C with stirring through the use of an aluminum block heater (React-Therm Heating/Stirring Model, Pierce, Rockford,

IL). After heating for 30 min, the septum-piercing needle of the SPME device was passed through the septum. The fiber was pushed out from the needle and exposed to the headspace of the vial for 60 min at 100°C to allow adsorption of the drugs (Figure 1). The fiber was withdrawn into the needle, pulled out from the vial, injected into the port of a capillary GC, and exposed in the injection port for 1 min for complete desorption of all drugs.

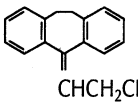
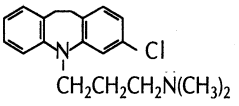
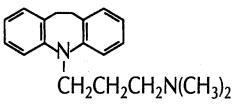
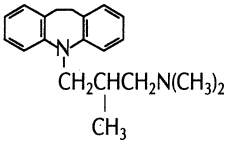
GC conditions

GC analyses were carried out on a Shimadzu GC-14B GC equipped with flame-ionization detection (FID) (Shimadzu, Kyoto, Japan). The column temperature was 100–300°C (1 min hold at 100°C, 15°C/min increase), the injection temperature was 280°C, and the helium flow rate was 4 mL/min. In the case of the authentic samples dissolved in methanol, a 1-µL aliquot (50 ng each on the column) was subjected to GC analysis. The samples were injected in the splitless mode with a column temperature of 100°C, and the splitter was opened after 1 min.

Animal experiments

Male Wistar rats weighing 300 g ± 10 were obtained from a commercial supplier (Saitama Experimental Animal Supply, Saitama, Japan). Three rats orally received imipramine–HCl (500 mg/kg body weight) dissolved in saline with a catheter. The cardiac blood samples were collected with syringes under nembutal anesthesia 5 h after administration and stored frozen at –40°C until analysis. Before SPME extraction, 0.2 mL of the rat blood after drug administration was diluted with rat blood without drugs to produce a 0.5-mL volume and placed in a 7.5-mL vial. The subsequent SPME procedure was the same as described above.

Table I. Chemical Structures of Four Tricyclic Antidepressants

Drug	Structure
Amitriptyline	
Chlorimipramine	
Imipramine	
Trimipramine	

Results and Discussion

Figure 2 shows the effects of exposure time on extraction of four tricyclic antidepressants (500 ng each) from human whole blood in the presence of distilled water and NaOH with the use of a 100-µm bond polydimethylsiloxane-coated SPME fiber. The equilibria were attained after 50–120 min of exposure. We thus exposed the fiber to the headspace for 60 min to get good reproducibility.

Figure 3 shows gas chromatograms for nonextracted authentic drugs (50 ng each on the column) dissolved in methanol and for headspace SPME extracts from 0.5-mL human whole blood samples, to which 500 ng each of the drugs had been added, in the presence of distilled water only or distilled water plus NaOH. Amitriptyline and chlorimipramine were separated from each other on the chromatograms, but imipramine and trimipramine appeared to overlap under these conditions (Figure 3A). In the presence of distilled water only, the headspace SPME–GC of four drugs produced very small peaks for amitriptyline, imipramine, and trimipramine (Figure 3B) together with many impurity peaks at column temperatures of 210–250°C (Figure 3C). In the presence of 0.5 mL distilled water and 100 µL 5M NaOH solution, the headspace SPME–GC

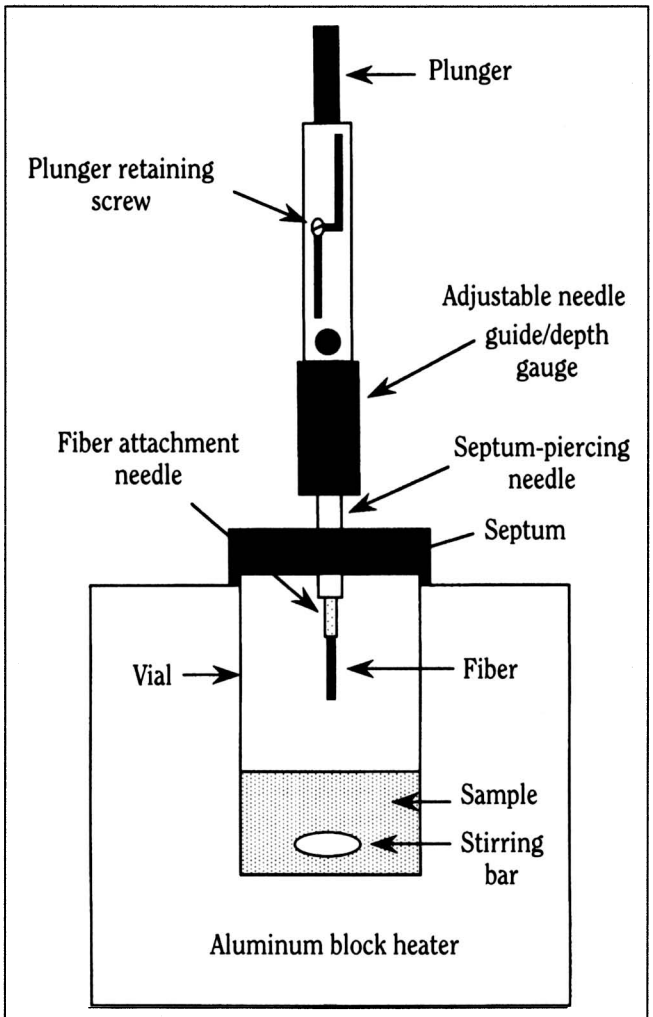


Figure 1. Schematic illustration of the headspace SPME method.

produced intense peaks for each drug (Figure 3D) with only a few small impurity peaks around 150–250°C (Figure 3E).

The recoveries and coefficients of within-day variation (CV) measured by the method for four tricyclic antidepressants (100 or 500 ng each) in human whole blood samples are presented in Table II. The recoveries were 5.3–12.9% in the presence of distilled water and NaOH. The CV values of within-day measurements for the four drugs were not greater than 7.4%. The day-to-day variation was measured by adding 500 ng each to 0.5 mL blood for all compounds on three different days; the CV values were not greater than 8.4%.

Figure 4 shows calibration curves for the drugs extracted from human whole blood in the presence of distilled water and NaOH solution by the present method. All drugs showed linearity in the range of 31–1000 ng/0.5 mL for whole blood. The equations for the curves were: $y = 0.0039x + 0.0776$ for amitriptyline, $y = 0.0032x + 0.0060$ for imipramine, $y = 0.0032x + 0.0195$ for trimipramine, and $y = 0.0006x + 0.0051$ for chlorimipramine. The correlation coefficients (r) of each calibration curve were 0.9998 for chlorimipramine, 0.9996 for imipramine, and 0.9992 for amitriptyline and trimipramine. The detection limits (signal-to-noise ratio = 3) of amitriptyline, imipramine, and trimipramine were 16 ng/0.5 mL; the detection limit of chlorimipramine was 25 ng/0.5 mL.

Figure 5 shows gas chromatograms for nonextracted authentic drugs (50 ng each on column) dissolved in methanol and for a headspace SPME extract from a 0.2-mL rat blood sample 5 h after oral administration of imipramine–HCl (500 mg/kg body weight) to which 500 ng of chlorimipramine was added as the internal standard. Imipramine could be detected on gas chromatograms with use of the present method (Figure 5B). The background for control rat blood had small impurity peaks over a wide range of temperatures, but no interfering peaks appeared around the imipramine and internal standard (Figure 5C). The concentration of imipramine after administration was 1.44 µg/mL rat blood ± 0.209 (mean plus or minus standard deviation, three replicates).

In this study, we were able to extract and detect four tricyclic antidepressants in human whole blood through the use of headspace SPME–GC. To our knowledge, this is the first trial to use headspace SPME for extraction of tricyclic antidepressants from human blood samples. The merits of headspace SPME are that the analytical procedure is simpler than those by liquid–liquid and solid-phase extractions and that much cleaner extracts can be obtained, as evidenced in Figures 3D and 3E.

We carefully examined recoveries of four tricyclic antidepressants from human whole blood samples for the

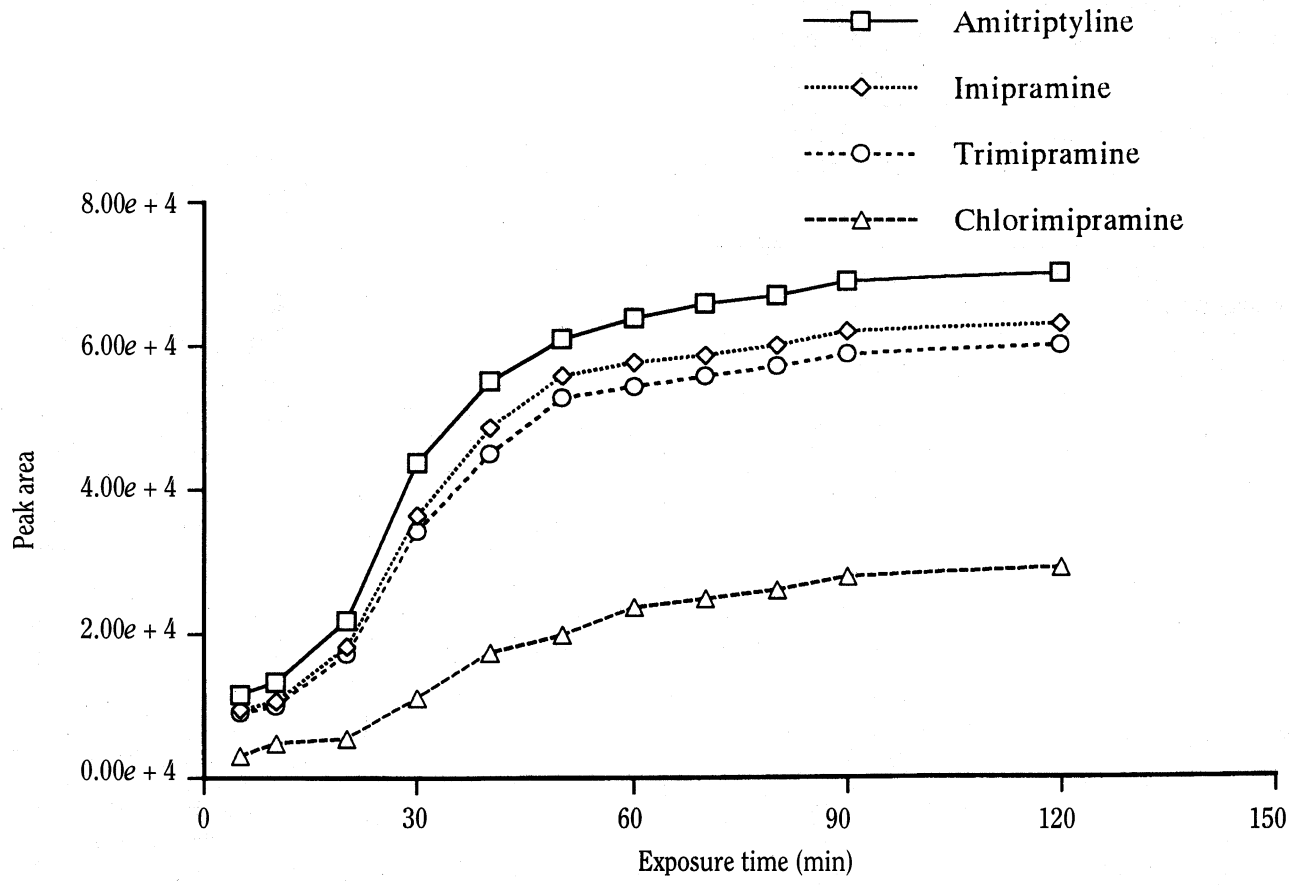


Figure 2. The effects of exposure time on extraction of four tricyclic antidepressants from whole blood samples with the use of a 100-µm bond polydimethylsiloxane-coated SPME fiber. Each drug (500 ng each in the acid salt form) was added to a 7.5-mL vial containing 0.5 mL of whole blood, 0.5 mL distilled water, and 100 µL 5M NaOH. After heating for 30 min at 100°C with stirring, the SPME fiber was exposed to the headspace of the vial. Each point represents the mean of duplicate determinations.

headspace SPME and found that only 5.3–12.9% of the drugs dissolved in whole blood was adsorbed to the SPME fiber. Such relatively low recoveries are common phenomena for SPME (3–10); in an early paper on SPME by Louch et al. (11), volatiles such as xylene, toluene, and benzene were reported to give recovery rates of approximately 1–20%. Therefore, our present method is in concert with the previous findings because excellent linearity was obtained by the present headspace SPME (Figure 4).

Entire amounts of a compound adsorbed to an SPME fiber can be introduced into GC; thus sensitivity becomes higher than that of the conventional liquid–liquid or solid-phase extraction, especially when the recovery by SPME exceeds 10%. The detection limit of imipramine extracted by the present SPME was 32 ng/mL blood; the detection limit of the same compound extracted with Extrelut columns was reported to be 2000 ng/mL (12).

In previous reports (13–16), various salts were added to human body fluids to improve recovery of volatiles, nonvolatiles, and stimulants for headspace SPME and also for the

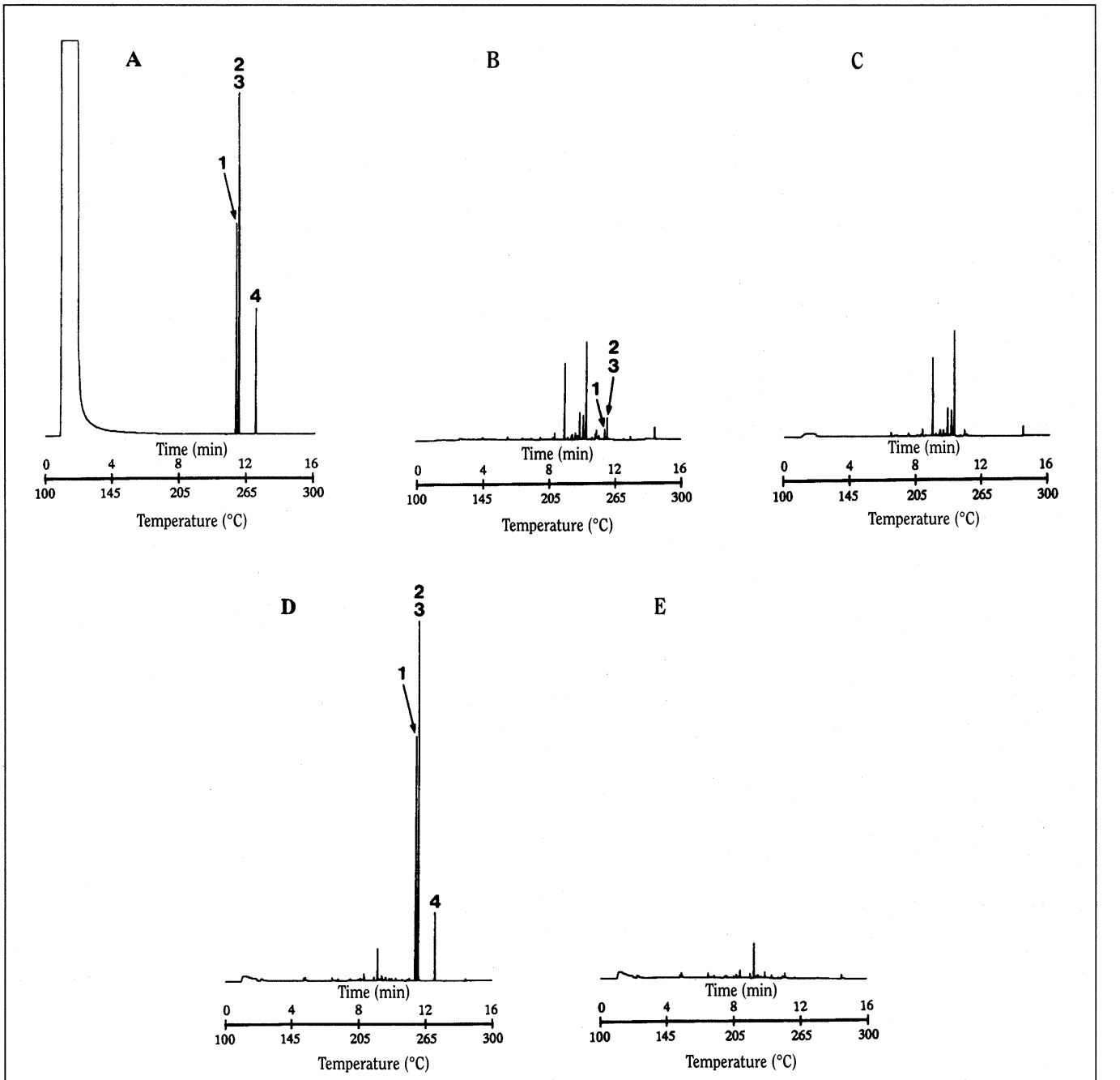


Figure 3. Capillary GC with FID for four tricyclic antidepressants extracted from human whole blood by the use of headspace SPME. (A) Authentic drugs (50 ng each in the acid salt form on column) without extraction. (B) An extract in the presence of 0.5 mL distilled water only. (C) A blank extract in the presence of 0.5 mL distilled water only. (D) An extract in the presence of 0.5 mL distilled water and 100 µL 5M NaOH solution. (E) A blank extract in the presence of 0.5 mL distilled water and 100 µL 5M NaOH solution. The mixture of four drugs (500 ng each) was added to 0.5 mL whole blood. Peaks: 1, amitriptyline; 2, imipramine; 3, trimipramine; and 4, chlorimipramine.

Table II. Recoveries and Coefficients of Within-Day Variation of Four Tricyclic Antidepressants from Human Whole Blood with the Use of Headspace SPME*

Drug	Amount added to 0.5 mL blood (ng)	Amount found in 0.5 mL blood (ng)	Recovery (%)	Coefficient of variation (%)
Amitriptyline	500	64.5 ± 2.55	12.9 ± 0.51	4.0
	100	12.5 ± 0.59	12.5 ± 0.59	4.7
Imipramine	500	57.5 ± 2.35	11.5 ± 0.47	4.1
	100	11.7 ± 0.38	11.7 ± 0.38	3.2
Trimipramine	500	55.5 ± 2.05	11.1 ± 0.41	3.7
	100	10.8 ± 0.50	10.8 ± 0.50	4.6
Chlorimipramine	500	29.0 ± 1.35	5.8 ± 0.27	4.7
	100	5.3 ± 0.39	5.3 ± 0.39	7.4

* Values are mean plus or minus standard deviation of three experiments. Recoveries were calculated by comparing the peak areas obtained from the extracts of the spiked whole blood sample in the presence of 0.5 mL distilled water and 100 µL 5M NaOH with that obtained from nonextracted authentic drugs (50 ng each on column) dissolved in methanol.

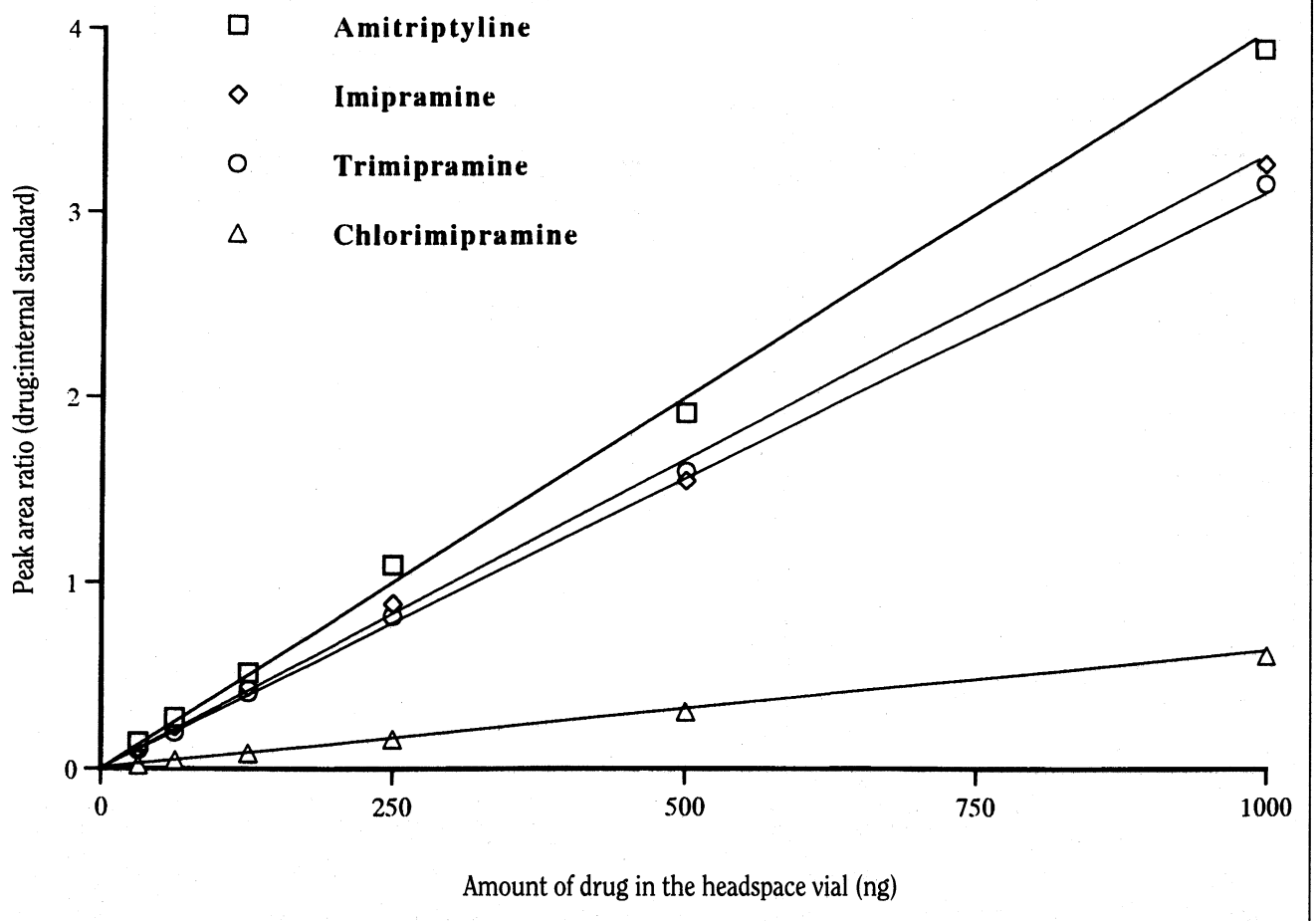


Figure 4. Calibration curves of four tricyclic antidepressants for human whole blood. The amounts of drugs were expressed as those in the salt form (see standards in Materials section). Amitriptyline, imipramine, and trimipramine were measured against chlorimipramine (500 ng) as an internal standard; chlorimipramine was measured against amitriptyline (500 ng) as an internal standard. Each point represents the mean of duplicate determinations.

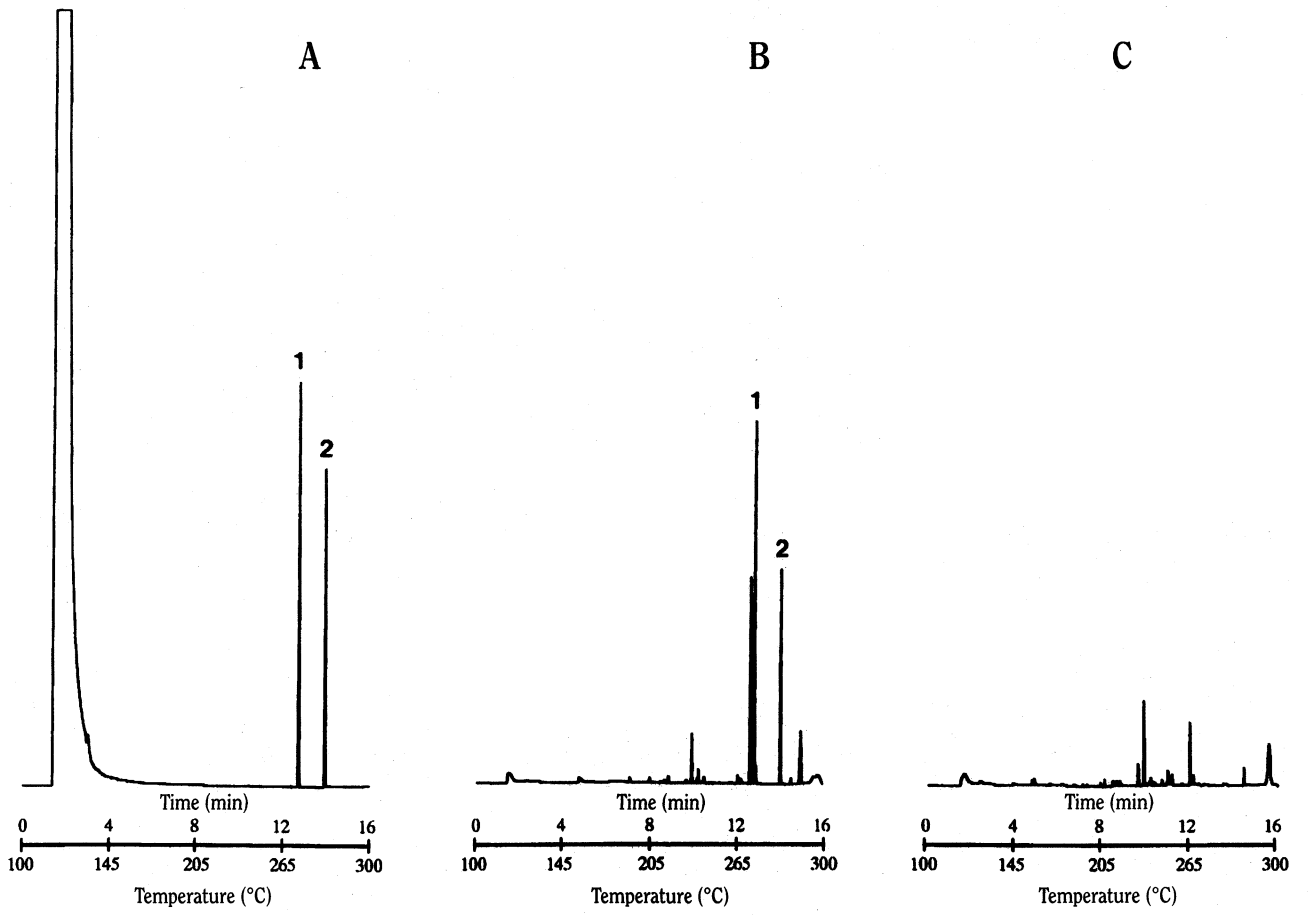


Figure 5. Capillary GC with FID for extracts of rat blood (0.2 mL) 5 h after oral administration of imipramine (500 mg/kg body weight) by the use of headspace SPME. Five hundred nanograms of chlorimipramine was added to the vial as an internal standard. (A) Authentic drugs (50 ng each in the acid salt form on column) without extraction. (B) An extract of rat blood after oral administration of imipramine. (C) A blank extract for control rat blood. Peaks: 1, imipramine; 2, chlorimipramine.

conventional headspace GC. Prior to the present study, the effects of various salts, such as NaCl and K_2CO_3, were preliminarily checked for the headspace SPME of four tricyclic antidepressants in whole blood samples. The addition of the salts was not useful; the alkalinization using only NaOH gave the best recoveries for each drug (unpublished data).

We have demonstrated the headspace SPME–GC for four tricyclic antidepressants (Figure 3D). The coexistence of many antidepressants in a sample is, of course, rare, but our results on the headspace SPME–GC chromatograms can be used for selection of an appropriate internal standard against the drug to be analyzed.

Therapeutic plasma concentrations for the tricyclic antidepressants used in the present study were reported to be less than 0.24 µg/mL, and toxic effects occur with blood concentrations greater than approximately 0.3 µg/mL (17). At toxic levels (500 mg/kg body weight), imipramine could actually be detected from rat blood (Figure 5B).

We had tried to measure therapeutic concentration of imipramine in blood 4 h after oral administration of 50 mg of imipramine–HCl to a 31-year-old male volunteer weighing 74 kg by the present headspace SPME and GC–FID. As a result, it was difficult to detect the drug by GC–FID, but a sharp peak could be detected by GC with a much more sensitive detector, a surface ionization detector (18–20). The imipramine concentration was 23.9 ng/mL whole blood (unpublished result).

Conclusion

Headspace SPME seems very useful for the detection of both therapeutic and toxic levels of tricyclic antidepressant drugs in body fluids in combination with various types of GC detectors because of simplicity, low background noise, and no requirement of organic solvent.

References

1. C.L. Arthur and J. Pawliszyn. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **62:** 2145–48 (1990).
2. X.-P. Lee, T. Kumazawa, and K. Sato. A simple analysis of 5 thinner components in human body fluids by headspace solid-phase microextraction (SPME). *Int. J. Legal Med.* **107:** 310–13 (1995).

3. T. Kumazawa, H. Seno, X.-P. Lee, A. Ishii, O. Suzuki, and K. Sato. Detection of ethanol in human body fluids by headspace solid-phase microextraction (SPME)/capillary gas chromatography. *Chromatographia* **43:** 393–97 (1996).
4. X.-P. Lee, T. Kumazawa, K. Sato, and O. Suzuki. Detection of organophosphate pesticides in human body fluids by headspace solid-phase microextraction (SPME) and capillary gas chromatography with nitrogen–phosphorus detection. *Chromatographia* **42:** 135–40 (1996).
5. H. Seno, T. Kumazawa, A. Ishii, M. Nishikawa, K. Watanabe, H. Hattori, and O. Suzuki. Determination of some carbamate pesticides in human body fluids by headspace solid phase microextraction and gas chromatography. *Jpn. J. Forensic Toxicol.* **14:** 199–203 (1996).
6. H. Seno, T. Kumazawa, A. Ishii, M. Nishikawa, H. Hattori, and O. Suzuki. Detection of meperidine (pethidine) in human blood and urine by headspace solid phase microextraction and gas chromatography. *Jpn. J. Forensic Toxicol.* **13:** 211–15 (1995).
7. T. Kumazawa, K. Watanabe, K. Sato, H. Seno, A. Ishii, and O. Suzuki. Detection of cocaine in human urine by solid-phase microextraction and capillary gas chromatography with nitrogen–phosphorus detection. *Jpn. J. Forensic Toxicol.* **13:** 207–10 (1995).
8. T. Kumazawa, X.-P. Lee, K. Sato, H. Seno, A. Ishii, and O. Suzuki. Detection of ten local anaesthetics in human blood using solid-phase microextraction (SPME) and capillary gas chromatography. *Jpn. J. Forensic Toxicol.* **13:** 182–88 (1995).
9. T. Kumazawa, K. Sato, H. Seno, A. Ishii, and O. Suzuki. Extraction of local anaesthetics in human blood by direct immersion–solid phase micro extraction (SPME). *Chromatographia* **43:** 59–62 (1996).
10. H. Seno, T. Kumazawa, A. Ishii, M. Nishikawa, K. Watanabe, H. Hattori, and O. Suzuki. Detection of some phenothiazines by headspace solid phase microextraction and gas chromatography. *Jpn. J. Forensic Toxicol.* **14:** 30–34 (1996).
11. D. Louch, S. Mortlaugh, and J. Pawliszyn. Dynamics of organic compound extraction from water using liquid-coated fused silica fibers. *Anal. Chem.* **64:** 1187–99 (1992).
12. O. Suzuki, H. Hattori, M. Asano, and H. Brandenberger. Positive and negative ion mass spectrometry of tricyclic antidepressants. *Z. Rechtsmed.* **97:** 239–50 (1986).
13. D.S. Christmore, R.C. Kelly, and L.A. Doshier. Improved recovery and stability of ethanol in automated headspace analysis. *J. Forensic Sci.* **29:** 1038–44 (1984).
14. K. Kato, T. Nagata, K. Kimura, K. Kudo, T. Imamura, and M. Noda. Components of paint thinner in body fluids clearly detected using the salting-out technique. *Forensic. Sci. Int.* **44:** 55–60 (1990).
15. H. Tsuchihashi, K. Nakajima, M. Nishikawa, S. Suzuki, Y. Oka, and K. Otsuka. Screening test of stimulants in human urine utilizing headspace gas chromatography for field test. *Forensic. Sci. Int.* **45:** 181–89 (1990).
16. B.D. Page and G. Lacroix. Application of solid-phase microextraction to the headspace gas chromatographic analysis of halogenated volatiles in selected foods. *J. Chromatogr.* **648:** 199–211 (1993).
17. *Clark's Isolation and Identification of Drugs.* A.C. Moffat, J.V. Jackson, M.S. Moss, and B. Widdop, Eds. Pharmaceutical Press, London, England, 1986, pp. 307–1069.
18. T. Fujii and H. Arimoto. New sensitive and selective detector for gas chromatography: Surface ionization detector with a hot platinum emitter. *Anal. Chem.* **57:** 2625–28 (1985).
19. H. Hattori, S. Yamamoto, M. Iwata, E. Takashima, T. Yamada, and O. Suzuki. Sensitive determination of phenothiazines in body fluids by gas chromatography with surface ionization detection. *J. Chromatogr.* **579:** 247–52 (1992).
20. H. Seno, H. Hattori, T. Iizumi, T. Kumazawa, and O. Suzuki. Determination of dextromethorphan and dimemorphan in body fluids by gas chromatography with surface ionization detection. *Jpn. J. Forensic Toxicol.* **10:** 236–40 (1992).

Manuscript accepted February 18, 1997.