Simple Analysis of Diphenylmethane Antihistaminics and Their Analogues in Bodily Fluids by Headspace Solid-Phase Microextraction–Capillary Gas Chromatography

Masanobu Nishikawa*, **Hiroshi Seno**, **Akira Ishii**, and **Osamu Suzuki**†

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

Takeshi Kumazawa

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

Kanako Watanabe and **Hideki Hattori**

Department of Legal Medicine, Aichi Medical University, Nagakute-cho, Aichi 480-11, Japan

Abstract

Thirteen antihistaminic drugs and their analogues are tested for their extraction by headspace solid-phase microextraction from human whole blood and urine. Their determination is made by using capillary gas chromatography with flame ionization detection. Relatively high recoveries are obtained for terodiline, diphenhydramine, diphenylpyraline, and orphenadrine in urine; but the recoveries in blood extracts are 4–51 times lower than those in urine extracts for all drugs. Benactyzine and piperilate are not suited for the extraction method. The calibration curves are drawn for four drugs spiked to whole blood and for eleven drugs spiked to urine; excellent linearity is confirmed for the drugs. The detection limits for the drugs are 76–473 ng/mL in blood and 13–186 ng/mL in urine. Diphenhydramine is determined for whole blood obtained from a male subject who had received oral administration of 30 mg diphenhydramine–HCl 150 min before the sampling; the concentrations of the drug are 0.12 and 1.22 µg/mL for blood and urine, respectively.

Introduction

Diphenylmethane antihistaminics are one of the most commonly used drug groups for the treatment of colds, asthma, and other allergic diseases; they are easily obtainable at drug stores and easily abused. Fatal cases involving their ingestion have been reported (1,2).

Solid-phase microextraction (SPME) is a technique for extracting organic compounds that was first introduced by Pawliszyn's group in 1990 (3). This procedure employs a stationary phase of polydimethylsiloxane coated on a fused-silica fiber to extract compounds from aqueous or volatile samples in sealed vials (Figure 1). After equilibration between the headspace and the coated fiber, the fiber needle can be directly injected into a gas chromatography (GC) port for analysis. SPME has been applied to drugs and toxic substances in biological samples; we have tried this method for tricyclic antidepressants (4), local anaesthetics (5), cocaine (6), phenothiazines (7), and organophosphate pesticides (8).

In this study, we successfully extracted and detected some of the diphenylmethane antihistaminics and their analogues from human whole blood and urine by headspace SPME–GC.

Experimental

Materials

Thirteen antihistaminic drugs were examined in this study. Diphenhydramine–HCl, doxylamine succinate, orphenadrine–

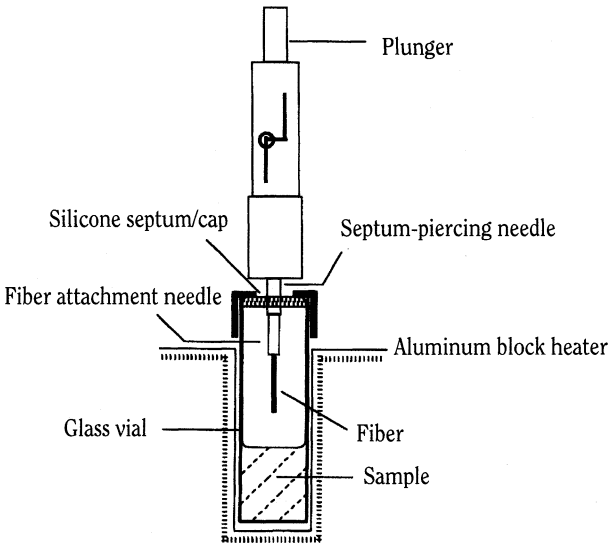

Figure 1. Schematic illustration of the headspace SPME method.

*Present address: Research Institute for Biological Sciences Okayama, 7549-1 Yoshikawa, Kayoh-cho, Okayama 716-12, Japan.
†Author to whom correspondence should be addressed.

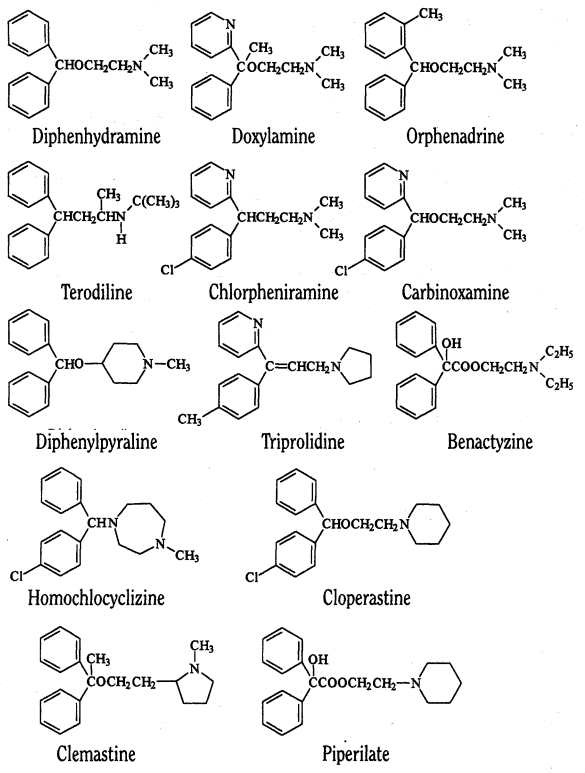

Figure 2. Chemical structures of antihistaminic drugs and their analogues used in this study.

HCl, (±)-chlorpheniramine maleate, carbinoxamine maleate, diphenylpyraline–HCl, triprolidine–HCl, benactyzine–HCl, homochlorcyclizine–2HCl, and clemastine fumarate were purchased from Sigma Chemical (St. Louis, MO). Terodiline–HCl, cloperastine–HCl and piperilate–HCl were provided by Kissei Pharmaceutical (Matsumoto, Japan), Yoshitomi Pharmaceutical (Osaka, Japan), and Nippon Shinyaku (Kyoto, Japan), respectively. The chemical structures of these drugs are shown in Figure 2. SPME devices fitted with 100-µm bonded polydimethylsiloxane-coated fibers were purchased from Supelco (Bellefonte, PA). Human whole blood and urine were obtained from a healthy 31-year-old subject.

SPME procedure

We established the procedure by making preliminary experiments for the present compounds and also by partly adopting the data in our previous study on tricyclic antidepressants (4); earlier experiments showed that acrylate fibers were unsuitable due to degradation and poor reproducibility. Thus, polydimethylsiloxane fibers were chosen for SPME. The headspace temperature of 98°C was found to be optimum; lower temperatures had shown lower recoveries.

The polydimethylsiloxane-coated fiber for SPME was pretreated in a GC injection port at 250°C for 1 h to remove fiber contaminants. A 0.5-mL volume of whole blood containing the drugs was diluted with the same volume of distilled water in a 5-mL glass vial. After adding 0.1 mL of 10N NaOH solution, the vial was sealed tightly with a silicone septum cap and then heated at 98°C for 10 min on an aluminum block heater. Following the preheating, the septum-piercing needle of the SPME device was passed through the septum (Figure 1). The pretreated fiber was extruded from the needle and kept in the headspace of the vial at 98°C for 10 min to allow adsorption of the compounds. The fiber was retracted into the needle and then immediately injected into the GC injection port; the fiber was exposed for 5 min to ensure complete desorption of the compounds. In the case of the urine sample, the SPME procedure was essentially the same as that for the whole blood samples except that the dilution with water was omitted.

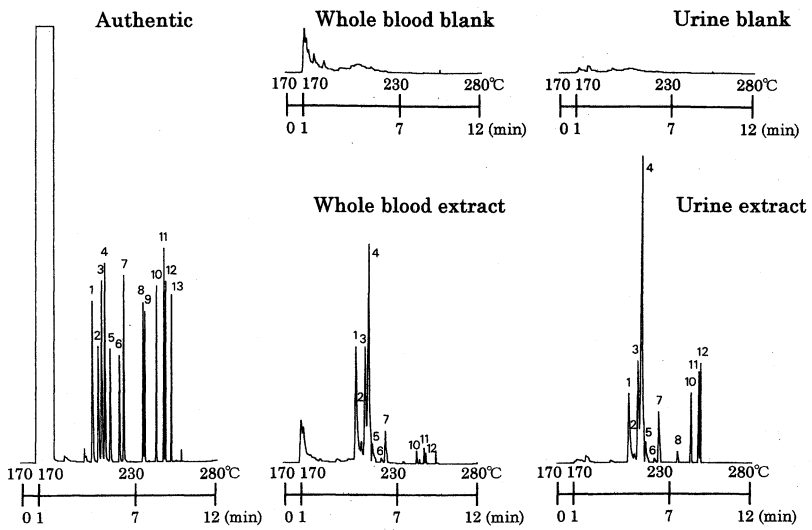

Figure 3. Gas chromatograms for nonextracted authentic drugs in methanol (0.1 µg of each drug in the acid salt form on column), extracts from human whole blood and urine (2 µg of each drug in the acid salt form for 0.5 mL of blood or 1 mL of urine) using SPME, and their backgrounds. Peaks: 1, diphenhydramine; 2, doxylamine; 3, orphenadrine; 4, terodiline; 5, chlorpheniramine; 6, carbinoxamine; 7, diphenylpyraline; 8, triprolidine; 9, benactyzine; 10, homochlocyclizine; 11, cloperastine; 12, clemastine; 13, piperilate. Chromatograms for blood and urine samples were drawn at vertical scales of 2 and 0.5 to that for the authentic drugs, respectively.

GC conditions

For GC, an HP-6890 series GC equipped with a flame ionization detector (FID) (Hewlett-Packard, Palo Alto, CA) was used with a fused-silica capillary column DB-1 (30 m × 0.32-mm i.d., 0.25-µm film thickness) (J &W Scientific, Folsom, CA) and a computing integrator C-R6A (Shimadzu, Kyoto, Japan). The column temperature was 170–280°C (1 min hold at 170°C and 10°C/min), the injection and detector temperature was 250°C, and the flow rate of the helium carrier gas was 3 mL/min. Injection was made in the splitless mode at a column

Table I. Recovery of 13 Diphenylmethane Antihistaminics and Their Analogues from Bodily Fluids by Headspace SPME

Drug*	Mean percent recovery ± SD (CV)†	
	Whole blood	Urine
1. Diphenhydramine	4.97 ± 0.48 (9.7)	23.9 ± 1.36 (5.7)
2. Doxylamine	0.98 ± 0.10 (10.2)	3.92 ± 0.36 (9.1)
3. Orphenadrine	3.57 ± 0.44 (12.2)	26.0 ± 1.71 (6.6)
4. Terodiline	7.21 ± 0.96 (13.3)	75.4 ± 6.00 (7.9)
5. Chlorpheniramine	0.835 ± 0.128 (15.3)	7.10 ± 0.48 (6.7)
6. Carbinoxamine	ND	1.92 ± 0.22 (11.6)
7. Diphenylpyraline	1.04 ± 0.121 (11.6)	12.1 ± 0.67 (5.5)
8. Triprolidine	ND	2.93 ± 0.32 (10.9)
9. Benactyzine	ND	ND
10. Homochlorcyclizine	0.327 ± 0.053 (15.9)	14.4 ± 1.41 (9.8)
11. Cloperastine	0.321 ± 0.037 (11.6)	15.3 ± 1.13 (7.4)
12. Clemastine	0.300 ± 0.026 (8.7)	15.4 ± 1.49 (9.7)
13. Piperilate	ND	ND

* Drugs are numbered in the order of retention times (see Figure 3). Each acid salt (2 µg) of the drugs was added to 1 mL of human urine or 0.5 mL of human blood.
† Recoveries of antihistaminics were calculated by comparing the peak areas obtained from the extracts of the spiked human bodily fluid samples with those obtained from nonextracted authentic drugs (20 ng of each drug in acid salt form on column). Average of five replicate determinations. SD = standard deviation. CV = coefficient of variation. ND = not detected.

Table II. Calibration Curves for Diphenylmethane Antihistaminics and Their Analogues Extracted from Human Whole Blood by Headspace SPME

Drug*	Equation†	Correlation coefficient	Concentration range (µg/mL)	Detection limit (ng/mL)
1. Diphenhydramine	$y = 0.600x + 0.135$	0.998	0.35–5.6	100
3. Orphenadrine	$y = 0.446x + 0.078$	1.000	0.35–5.6	136
4. Terodiline	$y = 0.999x + 0.357$	0.997	0.18–5.7	76
7. Diphenylpyraline	$y = 0.150x + 0.015$	1.000	1.4–5.7	473

* For drug numbering, see Table I.
† Peak area ratio (y) versus spiked compound concentration (x).

Table III. Calibration Curves for Diphenylmethane Antihistaminics and Their Analogues Extracted from Human Urine by Headspace SPME

Drug*	Equation†	Correlation coefficient	Concentration range (µg/mL)	Detection limit (ng/mL)
1. Diphenhydramine	$y = 0.495x + 0.020$	0.997	0.18–2.8	62
2. Doxylamine	$y = 0.073x - 0.008$	0.996	0.28–4.6	186
3. Orphenadrine	$y = 0.651x + 0.140$	0.999	0.18–5.6	43
4. Terodiline	$y = 1.580x + 0.328$	0.998	0.18–2.8	13
5. Chlorpheniramine	$y = 0.100x + 0.003$	0.994	0.14–2.2	130
6. Carbinoxamine	$y = 0.031x + 0.000$	0.985	0.29–4.6	180
7. Diphenylpyraline	$y = 0.246x + 0.014$	0.998	0.18–2.8	97
8. Triprolidine	$y = 0.059x - 0.014$	0.997	0.35–5.7	113
10. Homochlorcyclizine	$y = 0.171x + 0.024$	0.999	0.16–2.6	61
11. Cloperastine	$y = 0.141x + 0.079$	0.983	0.18–2.9	41
12. Clemastine	$y = 0.118x + 0.049$	0.983	0.15–2.4	38

* For drug numbering, see Table I.
† Peak area ratio (y) versus spiked compound concentration (x).

temperature of 170°C, and the splitter was opened after 1 min.

Administration of diphenhydramine

A healthy 31-year-old male subject (56 kg in weight) volunteered to receive 30 mg of powdery diphenhydramine–HCl orally. Whole blood (heparinized) and urine samples were collected 150 min after the administration.

Results and Discussion

Typical chromatograms of extracts for diphenylmethane antihistaminics and their analogues obtained by the SPME procedure are shown in Figure 3. All authentic drugs were detected and separated from each other under our GC conditions. Nine drugs could be recovered from whole blood samples (see also Table I); their recoveries ranged only from 0.3 to 7.2%. For the urine extract chromatogram, 11 drugs could be detected with recoveries ranging from 1.9 to 75.4%. Terodiline was recovered at the highest efficiency for both bodily fluids (Figure 3, peak 4). Diphenhydramine and orphenadrine, which are structurally very similar, were recovered at almost the same efficiency. Benactyzine and piperilate were not detected, probably because these compounds have a hydroxyl group and an ester bond (Figure 2). Urine samples gave much higher recoveries than blood samples (Table I). Homochlorcyclizine, cloperastine, and clemastine were recovered from urine with 44–51 times higher efficiency than from blood.

Tables II and III show calibration curves for diphenylmethane antihistaminics and their analogues from whole blood and urine by headspace SPME. They were drawn according to the peak–area ratios with orphenadrine (1.76 µg in a vial) as the internal standard (IS). For the curves of orphenadrine, diphenhydramine (1.75 µg in a vial) was used as the IS. Diphenhydramine, orphenadrine, terodiline, and diphenylpyraline in whole blood showed excellent linearity; correlation coefficients were 0.99–1.0 despite the low recoveries (Table II). This was also true for the 11 drugs in urine (Table III). The detection limits (signal-to-noise ratio = 3) of the four drugs in whole blood were 76–473 ng/mL; those for urine were 13–186 ng/mL.

Figure 4 shows GC chromatograms after headspace SPME for whole blood and urine of a male subject who had received oral administration of 30 mg diphenhydramine–HCl

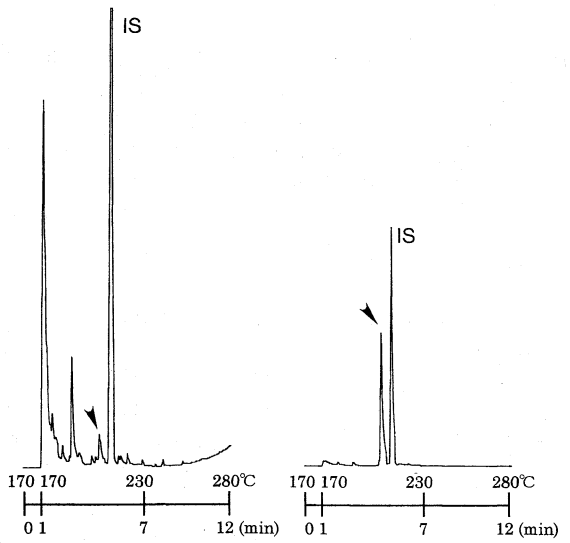

Figure 4. Gas chromatograms for the headspace SPME extracts of whole blood and urine samples obtained 150 min after oral administration of 30 mg diphenhydramine–HCl. In each vial, 1.8 µg of orphenadrine was spiked as the internal standard. The arrows show the peaks of diphenhydramine. The vertical scale of the whole blood extract is 32 times higher than that of the urine extract.

150 min before sampling. The concentrations were 0.12 µg/mL for blood and 1.22 µg/mL for urine.

In this study, we tried using headspace SPME for the extraction of diphenylmethane antihistaminics and their analogues in human whole blood and urine. In previous reports, they were extracted from biological samples by liquid–liquid and solid-phase extraction (9–11). The merits of using the headspace SPME are that the procedure is much simpler and more rapid than those by liquid–liquid and solid-phase extractions and that much cleaner extracts can be obtained, as evidenced in Figure 3.

In previous reports, various salts were added to human bodily fluids to improve recoveries of the test compounds for headspace SPME (5,8). In our preliminary experiments, however, the addition of NaCl or K_2CO_3 did not improve their recoveries. For whole blood, the use of clear supernatant fractions after precipitation of blood proteins followed by centrifugation resulted in no improvement of recoveries of the drugs (unpublished observations). Thus the drugs were extracted by headspace SPME only after alkalinization of both samples. Much lower recoveries of the drugs found in whole blood are probably due to binding of the drugs to proteins or membrane lipids of the blood.

We have found that only 0.30–7.21% and 1.92–75.4% of the diphenylmethane antihistaminics and their analogues dissolved in whole blood and urine, respectively, were adsorbed by the SPME fiber exposed to the headspace at 98°C (Table I). Such low recoveries are common phenomena for SPME (3–8). Despite the low recoveries, excellent quantitative results (Tables II and III) and relatively low coefficients of variation (CV) (Table I) were obtained using headspace SPME. In view of recovery rates, terodiline, orphenadrine, diphenhydramine, and diphenylpyraline are the most recommendable for extraction by headspace SPME. Benactyzine and piperilate are not suitable for headspace SPME.

In this study, we used a conventional FID for GC analysis. If a nitrogen–phosphorus or surface ionization detector is used (9,12), much higher sensitivity can be expected. The therapeutic concentrations of diphenhydramine and orphenadrine in human plasma were reported to be 0.1–1.0 µg/mL and 0.1–0.2 µg/mL, respectively (13); for terodiline and diphenylpyraline, such information is not available, to our knowledge. This means that both therapeutic and toxic levels of diphenhydramine and orphenadrine can be measured even by the present GC with an FID after headspace SPME.

Conclusion

To our knowledge, this is the first trial to use headspace SPME for the extraction of diphenylmethane antihistaminics and their analogues from biological samples. Because of simplicity, low background noises, and excellent quantitative results, this headspace SPME method is recommendable for analysis of diphenhydramine, diphenylpyraline, terodiline, and orphenadrine in forensic and clinical toxicology.

References

1. B. Levine and Y.H. Caplan. A fatality involving bromodiphenhydramine, codeine and glutehimide. *Bull. Int. Assoc. Forensic Toxicol.* **19(3):** 26–28 (1987).
2. P. Kintz, A. Tracqui, and P. Mangin. Toxicological findings after fatal methaqualone and diphenhydramine (Mandrax) self poisoning. *Bull. Int. Assoc. Forensic Toxicol.* **21(3):** 38–40 (1991).
3. C.L. Arthur and J. Pawliszyn. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **62:** 2145–48 (1990).
4. T. Kumazawa, X.-P. Lee, M.-C. Tsai, H. Seno, A. Ishii, and K. Sato. Simple extraction of tricyclic antidepressants in human urine by headspace solid-phase microextraction (SPME). *Jpn. J. Forensic Toxicol.* **13:** 25–30 (1995).
5. 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).
6. 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).
7. 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).
8. 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).
9. S.D. Yoo, J.E. Axelson, and D.W. Rurak. Determination of diphenhydramine in biological fluids by capillary gas chromatography using nitrogen–phosphorus detection. *J. Chromatogr.* **378:** 385–93 (1986).

10. H. Maurer and K. Pfleger. Screening procedure for the detection of alkanolamine antihistamines and their metabolites in urine using computerized gas chromatography–mass spectrometry. *J. Chromatogr.* **428:** 43–60 (1988).
11. H. Seno, H. Hattori, T. Kumazawa, and O. Suzuki. Positive- and negative-ion mass spectrometry of diphenylmethane antihistaminics and their analogues and rapid clean-up of them from biological samples. *Forensic Sci. Int.* **62:** 187–208 (1993).
12. H. Hattori, S. Yamamoto, M. Iwata, E. Takashima, T. Yamada, and O. Suzuki. Determination of diphenylmethane antihistaminic drugs and their analogues in body fluids by gas chromatography with surface ionization detection. *J. Chromatogr.* **581:** 213–18 (1992).
13. A.C. Moffat, J.V. Jackson, M.S. Moss, and B. Widdop. *Clarke's Isolation and Identification of Drugs,* 2nd ed. Pharmaceutical Press, London, 1986, pp. 557–58, 833–34.

Manuscript accepted December 3, 1996.