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Journal of Chromatography B, 806 (2004) 65–73
JOURNAL OF CHROMATOGRAPHY B
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Simultaneous determination of barbiturates in human biological fluids by direct immersion solid-phase microextraction and gas chromatography–mass spectrometry

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Available online 13 April 2004

Abstract

Simultaneous determination of seven barbiturates in human whole blood and urine by combining direct immersion solid-phase microextraction (DI–SPME) with gas chromatography-mass spectrometry (GC–MS) is presented. The main parameters affecting the DI–SPME process, such as SPME fibers, salt additives, pHs, extraction temperatures and immersion times were optimized for simultaneous determination of the drugs. The extraction efficiencies were 0.0180–0.988 and 0.0156–2.76% for whole blood and urine, respectively. The regression equations of the drugs showed excellent linearity for both samples; the correlation coefficients (r^2) were 0.994–0.999. The detection limits for whole blood were 0.05–1 μg ml^{-1}, and those for urine 0.01–0.6 μg ml^{-1}. Actual quantitation could be made for pentobarbital in whole blood and urine obtained from volunteers, who had been orally administered a therapeutic dose of the drug. The DI–SPME/GC–MS procedure for barbiturates established in this study is simple and sensitive enough to be adopted in the fields of clinical and forensic toxicology.

Keyword: Barbiturates

1. Introduction

Solid-phase microextraction (SPME) was first reported by Arthur and Pawliszyn in 1990 [1], and is an organic-solvent-free extraction technique that incorporates sample extraction, concentration and introduction into a single step. Direct immersion (DI) is one of the techniques for SPME, and has been applied to the detection of various drugs and poisons in biological fluids [2–6].

Barbiturates are one of the most popular sedative hypnotic group. They sometimes cause death in suicidal and accidental cases [7–9]. However, the reports for analysis of the drugs by SPME are sporadic; Queiroz et al. [10] have reported DI–SPME for phenobarbital and primidone in plasma, and Staerk et al. [11] reported headspace SPME for phenobarbital and secobarbital in urine. Hall et al. [12] reported DI–SPME for eight barbiturates in water solution, but not in biological samples.

In this study, we have optimized various conditions of DI–SPME for simultaneous determination of seven barbiturates in human biological fluids by gas chromatography (GC)–mass spectrometry (MS); and the usefulness of the established method has been evaluated.

2. Experimental

2.1. Materials

The structures of the seven barbiturates examined are given in Table 1. Primidone was obtained from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan; amobarbital from Nippon Shinyaku Co., Ltd., Kyoto, Japan; pentobarbital

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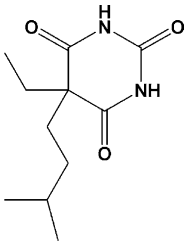
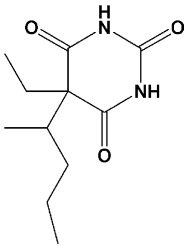
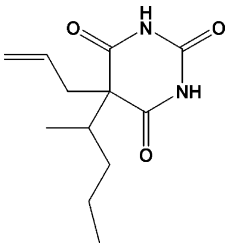
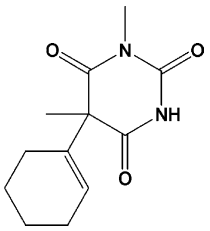
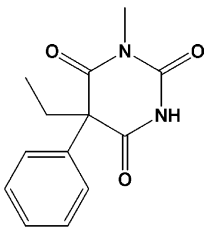
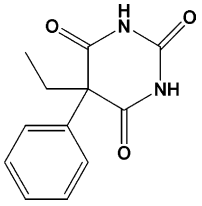
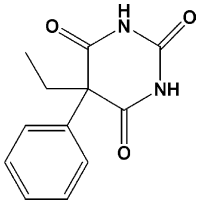
doi:10.1016/j.jchromb.2004.03.016

Table 1
Chemical structures and their major fragment ions obtained in the EI mode

Compound		MW	*m*/*z* (% intensity)
Amobarbital		226	156(100), 141(74), 55(43)
Pentobarbital		226	156(100), 141(68), 55(30)
Secobarbital		238	168(100), 167(73), 55(33)
Hexobarbital		236	221(100), 81(92), 79(66), 80(48), 157(41)
Mephobarbital		246	218(100), 117(48), 58(28)
Phenobarbital		232	204(100), 58(38), 117(33)
Primidone		218	190(100), 146(95), 117(83)

calcium from Tanabe Seiyaku Co., Ltd., Osaka, Japan; secobarbital sodium from Yoshitomi Pharmaceutical Ind. Ltd., Osaka, Japan; hexobarbital from Teikoku Chemical Ind. Co., Ltd., Osaka, Japan; mephobarbital from Bayer AG, Leverkusen–Bayerwerk, Germany; and phenobarbital from Fujinaga Pharmaceutical Co., Ltd., Tokyo, Japan. Three types of SPME fiber, 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) and 85 μm polyacrylate, and a manual SPME holder were purchased from Supelco (Bellefonte, PA, USA); an HP-1 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) from Agilent (Palo Alto, CA, USA). Other common chemicals used were of the analytical-reagent grade. Whole blood and urine were obtained from healthy subjects.

2.2. DI–SPME procedure

Prior to the experiments, new PDMS, PDMS/DVB and polyacrylate fibers were pretreated at 250 °C for 60 min, at 260 °C for 30 min and at 300 °C for 120 min, respectively.

To 0.5 ml of whole blood containing barbiturates was added 1.5 ml of 0.5 M perchloric acid for deproteinization. After stirring vigorously with a vortex mixer for 1 min, the mixture was centrifuged at 1630 × *g* for 10 min; 1.5 ml of clear supernatant was decanted into a 2 ml micro reaction vials (Supelco) containing a magnetic stirring bar and 0.5 g of sodium sulfate. The pH of the supernatant was adjusted to 6.0–7.0 with 0.65 ml of 1.0 M sodium hydroxide, and the vial was sealed with a silicone septum cap. It was heated on an aluminum block heater (Reacti-Therm™ Heating–Stirring Model; Pierce, Rockford, IL, USA) at 60 °C for 15 min with stirring, and then the SPME syringe needle was penetrated through the vial septum. The fiber was pushed out and directly immersed in the sample solution at 60 °C with stirring. After 60 min of immersion, the fiber was retracted to the SPME syringe needle and the SPME holder was pulled out of the vial for GC injection. The fiber was exposed in the injection port for 10 min to ensure complete desorption of the compounds. After each injection, the SPME fiber was washed with distilled water to prevent salt accumulation on the surface.

For urine, 0.5 ml of the sample was placed in the 2 ml micro reaction vial containing 1.5 ml of distilled water and 0.5 g of sodium sulfate without any process of deproteinization and pH adjustment. The following procedure was exactly the same as that for whole blood.

2.3. GC–MS conditions

GC–MS analysis was performed on a Shimadzu GCMS-QP5050A instrument (Shimadzu Corp., Ltd, Kyoto, Japan) equipped with the HP-1 capillary column. The mass spectrometer was operated in the electron impact (EI) mode at an ionization energy of 70 eV. The temperature of the injection port was set at 270 °C, and that of the interface at 240 °C. The column temperature was held at 100 °C for 1 min and then raised to 280 °C at 20 °C min^{-1}. Helium was used as carrier gas at a flow rate of 1.1 ml min^{-1}. The samples were injected in the splitless mode and the splitter was opened after 1 min. Quantitative analysis was carried out in the selected ion monitoring (SIM) mode. Major fragment ions of the drugs were listed also in Table 1. The monitored ions for quantitation were as follows; *m/z* 156 for amobarbital and pentobarbital; *m/z* 168 for secobarbital; *m/z* 221 for hexobarbital; *m/z* 218 for mephobarbital; *m/z* 204 for phenobarbital; *m/z* 190 for primidone.

3. Results and discussion

3.1. Optimization of conditions for DI–SPME

To select the optimal conditions for simultaneous analysis of barbiturates, SPME fibers, salt additives, pHs of the sample solution, extraction temperatures and immersion times were examined for 0.5 ml of whole blood, to which 10 μg each of phenobarbital and primidone and 0.5 μg of other drugs had been added.

At the first step, three types of fibers, such as PDMS, PDMS/DVB and polyacrylate, were tested under the conditions of pH at 6.0–7.0 in the presence of sodium sulfate. Polyacrylate fiber gave the highest extraction efficiencies for all drugs except hexobarbital (Fig. 1). Therefore, polyacrylate fiber was selected for simultaneous extraction of barbiturates.

At the second step, the influence of pHs in the mixture was tested. It was adjusted to pH 5.0, 6.0, 7.0 and 8.0 with 1.0 M sodium hydroxide in the presence of 0.5 g of sodium chloride. The best pH for most compounds was 6.0–7.0 (Fig. 2). As an exception, primidone could be extracted at pH 8.0 with a high efficiency.

At the third step, the effect of various salt additives was examined. The pH of the sample solution was adjusted to 6.0–7.0 in the presence of 0.5 g each of ammonium citrate dibasic, ammonium acetate, ammonium chloride, calcium chloride dihydrate, sodium chloride and sodium sulfate. The highest extraction efficiencies for all compounds were obtained with sodium sulfate (Fig. 3).

At the fourth step, various extraction temperatures were examined. The pH was also adjusted to 6.0–7.0 in the presence of 0.5 g of sodium sulfate. The fiber was exposed to the solution for 60 min at 40, 50, 60 and 70 °C. The highest extraction efficiencies for most compounds were obtained at 60 °C (Fig. 4).

At the fifth step, various immersion times were then tested at pH 6.0–7.0 in the presence of 0.5 g of sodium sulfate and the fiber was exposed to the solution at 60 °C for various intervals. Equilibria for all compounds were not attained even at 90 min (Fig. 5). Thus, we immersed the fiber into the sample for 60 min as a reasonable compromise between an acceptable extraction time and reliability of the method.

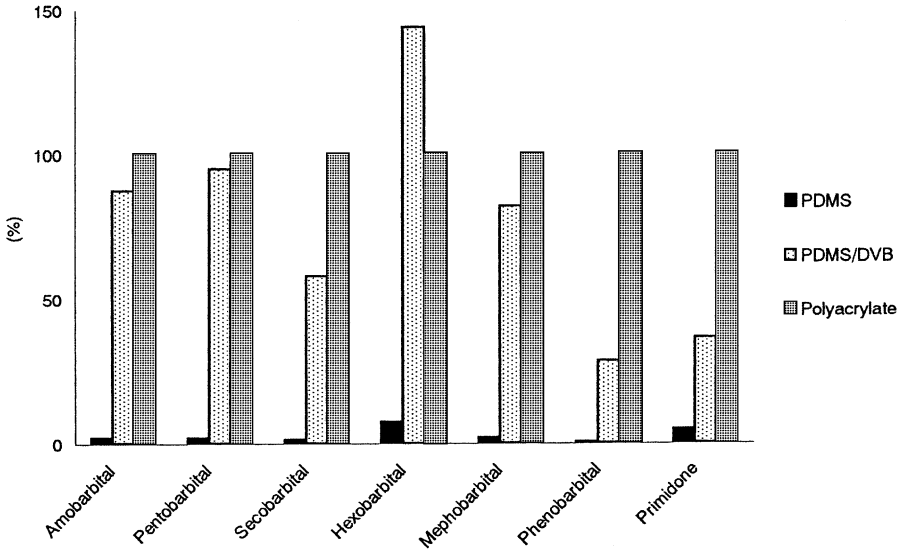


Fig. 1. Effect of different SPME fibers on extraction efficiency by DI–SPME for seven barbiturates in human whole blood. The amount of each drug extracted with the polyacrylate fiber was set at 100%. Each results represents the mean of triplicate experiments.

3.2. Reliability of the method

Fig. 6 shows SIM chromatograms obtained after injection of the methanol standard solution (0.1 μl), that contained 20 ng of phenobarbital and primidone, 1 ng of other drugs, and the chromatograms obtained from 0.5 ml whole blood or urine spiked with 10 μg each of phenobarbital and primidone and 0.5 μg of other drugs. All compounds could be separated well under our GC–MS conditions.

The extraction efficiencies were calculated by comparing peak areas obtained from extracts of spiked whole blood and urine with those obtained by direct GC–MS injection of the standard compounds dissolved in methanol. For whole blood, the efficiencies for amobarbital, pentobarbital, secobarbital, hexobarbital, mephobarbital and phenobarbital were 0.213–0.988%; those for primidone 0.0180–0.0915% (Table 2). For urine, those for amobarbital, pentobarbital, secobarbital, hexobarbital, mephobarbital and phenobarbital were 0.613–2.76%; those for primidone 0.0156–0.181% (Table 3).

Tables 4 and 5 show regression equations for the drugs extracted from human whole blood and urine, respectively. The equations for amobarbital, secobarbital, mephobarbital, phenobarbital and primidone were constructed by plotting peak area ratios with hexobarbital (2 μg in 0.5 ml of whole blood and 0.15 μg in 0.5 ml of urine) as internal standard (IS); those for hexobarbital and pentobarbital were constructed with mephobarbital (2 μg in 0.5 ml of whole blood and 0.15 μg in 0.5 ml of urine) as IS. The equations showed good linearity in the ranges as shown in Tables 4 and 5. The detection limits for whole blood were 0.05–1 $\mu g\,ml^{-1}$; those for urine 0.01–0.6 $\mu g\,ml^{-1}$. Therapeutic blood

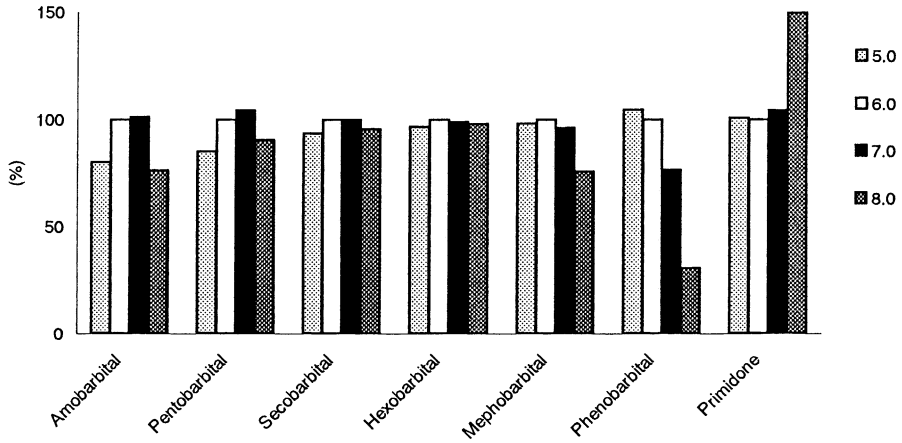


Fig. 2. Effect of different pHs on extraction efficiency by DI–SPME for seven barbiturates in human whole blood. The amount of each drug extracted at pH 6.0 was set at 100%. Each results represents the mean of triplicate experiments.

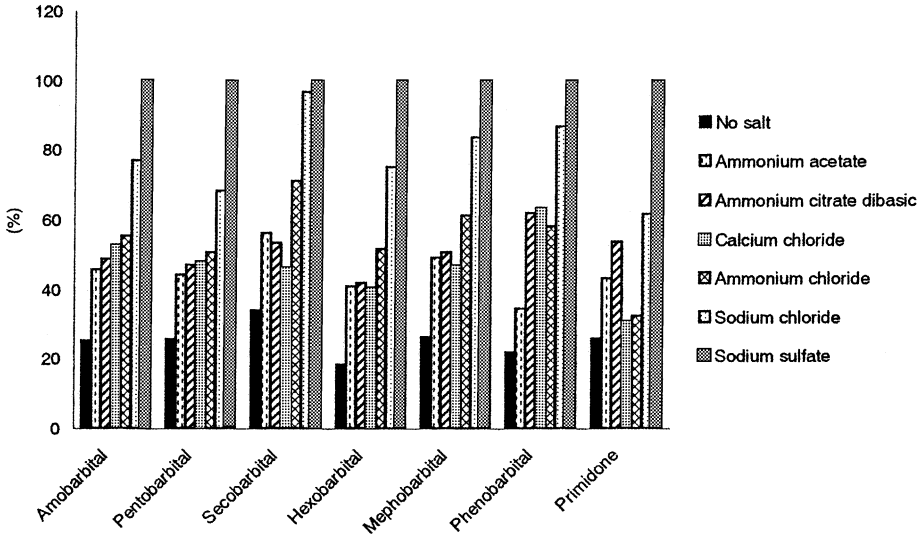


Fig. 3. Effect of different salts (0.5 g each) on the extraction efficiency by DI–SPME for seven barbiturates in human whole blood. The amount of each drug extracted with sodium sulfate was set at 100%. Each results represents the mean of triplicate experiments.

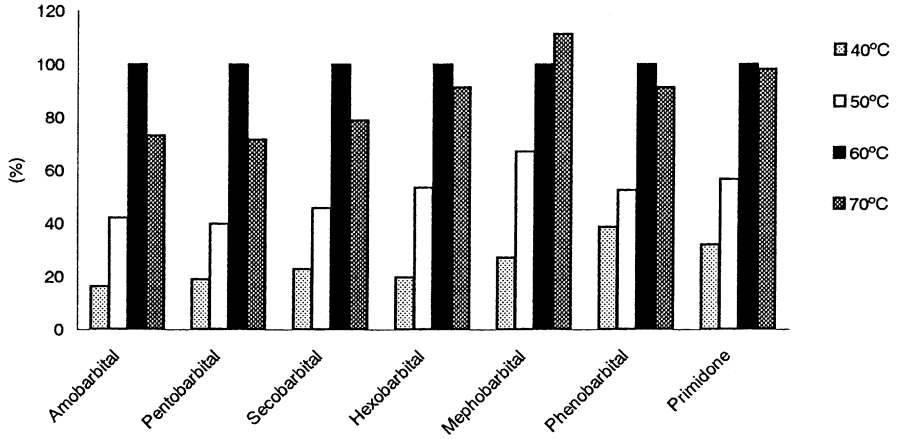


Fig. 4. Effect of different extraction temperatures on extraction efficiency by DI–SPME for seven barbiturates in human whole blood. The amount of each drug extracted at 60 °C was set at 100%. Each results represents the mean of triplicate experiments.

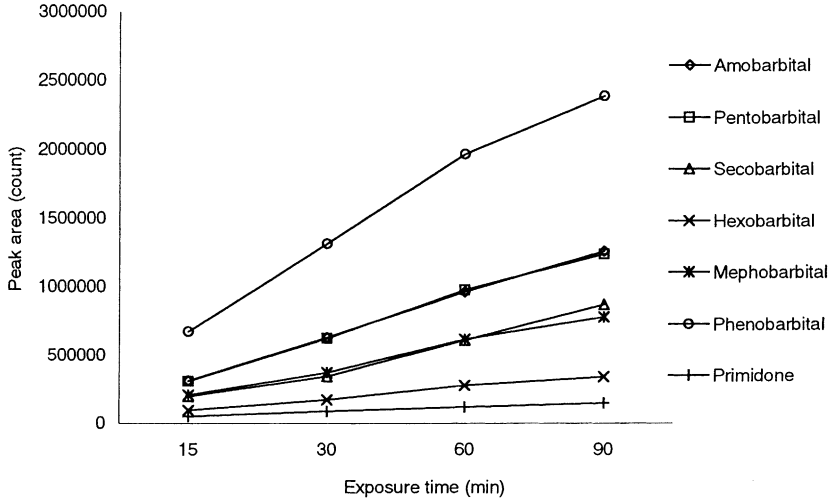


Fig. 5. Effect of different immersion times on extraction efficiency by DI–SPME for seven barbiturates from human whole blood. Each results represents the mean of triplicate experiments.

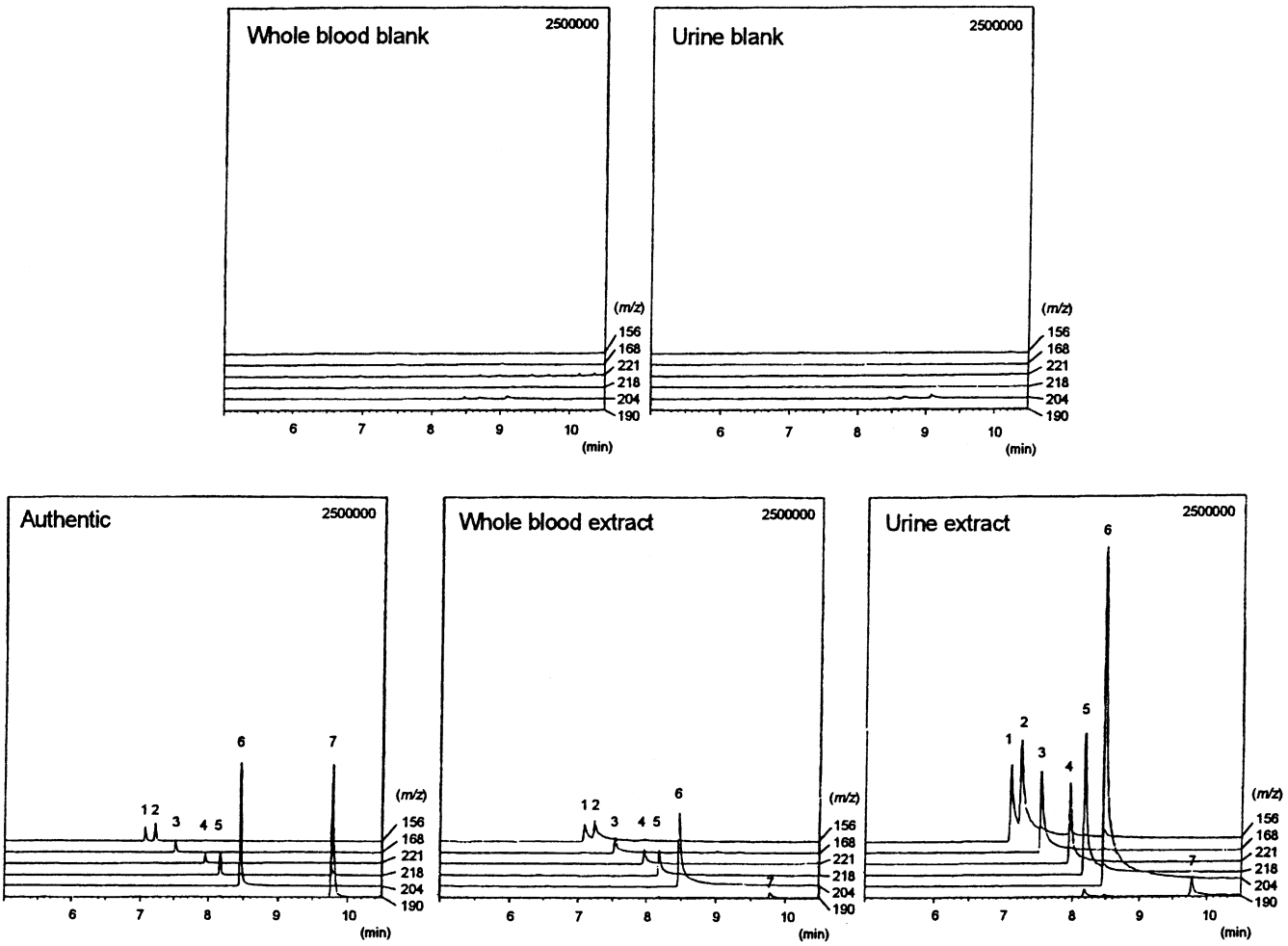


Fig. 6. SIM chromatograms for the authentic drugs without extraction, for extracts from human whole blood and urine by DI–SPME. Peaks: 1, amobarbital; 2, pentobarbital; 3, secobarbital; 4, hexobarbital; 5, mephobarbital; 6, phenobarbital; 7, primidone.

Table 2
Extraction efficiencies of seven barbiturates in human whole blood obtained by DI–SPME/GC–MS

Compound	Amount added ($\mu g\,ml^{-1}$)	Intra-day ($n = 5$)		Inter-day ($n = 5$)	
		Amount extracted[a] ($\mu g\,ml^{-1}$)	Extraction efficiency (%)	Amount extracted[a] ($\mu g\,ml^{-1}$)	Extraction efficiency (%)
Amobarbital	0.4	0.00228 ± 0.00053	0.570	0.00144 ± 0.00017	0.360
	4	0.0173 ± 0.0044	0.433	0.0166 ± 0.0029	0.415
Pentobarbital	0.4	0.00223 ± 0.00062	0.558	0.00138 ± 0.00014	0.345
	4	0.0178 ± 0.0051	0.445	0.0192 ± 0.0023	0.480
Secobarbital	0.4	0.00395 ± 0.00041	0.988	0.00175 ± 0.00032	0.438
	4	0.0283 ± 0.0032	0.708	0.0277 ± 0.0055	0.693
Hexobarbital	0.4	0.00248 ± 0.00022	0.620	0.00157 ± 0.00015	0.393
	4	0.0170 ± 0.0016	0.425	0.0237 ± 0.0026	0.593
Mephobarbital	0.4	0.00324 ± 0.00031	0.810	0.00151 ± 0.00048	0.378
	4	0.0246 ± 0.0022	0.615	0.0333 ± 0.0077	0.833
Phenobarbital	2	0.00968 ± 0.00285	0.484	0.00571 ± 0.00119	0.286
	20	0.0706 ± 0.0108	0.353	0.0782 ± 0.0135	0.391
Primidone	8	0.00144 ± 0.00034	0.0180	0.00181 ± 0.00031	0.0226
	80	0.0303 ± 0.0063	0.0379	0.0393 ± 0.0072	0.0491

[a] The values are means ± S.D.

Table 3
Extraction efficiencies of seven barbiturates in human urine obtained by DI–SPME/GC–MS

Compound	Amount added ($\mu g\,ml^{-1}$)	Intra-day ($n = 5$)		Inter-day ($n = 5$)	
		Amount extracted[a] ($\mu g\,ml^{-1}$)	Extraction efficiency (%)	Amount extracted[a] ($\mu g\,ml^{-1}$)	Extraction efficiency (%)
Amobarbital	0.08	0.00101 ± 0.00021	1.26	0.000946 ± 0.000192	1.18
	0.8	0.0134 ± 0.0024	1.68	0.00856 ± 0.00119	1.07
Pentobarbital	0.08	0.00111 ± 0.00018	1.39	0.00181 ± 0.00035	2.26
	0.8	0.0169 ± 0.0026	2.11	0.0153 ± 0.0019	1.91
Secobarbital	0.08	0.00177 ± 0.00021	2.21	0.00147 ± 0.00030	1.84
	0.8	0.0221 ± 0.0030	2.76	0.0214 ± 0.0012	2.67
Hexobarbital	0.08	0.00144 ± 0.00023	1.80	0.000878 ± 0.000195	1.10
	0.8	0.0167 ± 0.0028	2.09	0.0134 ± 0.0019	1.68
Mephobarbital	0.08	0.00135 ± 0.00017	1.69	0.00100 ± 0.00001	1.25
	0.8	0.0185 ± 0.0036	2.31	0.0113 ± 0.0019	1.41
Phenobarbital	0.8	0.00711 ± 0.00065	0.889	0.00525 ± 0.00065	0.656
	8	0.0535 ± 0.0041	0.668	0.0491 ± 0.0103	0.613
Primidone	4	0.00155 ± 0.00037	0.0388	0.000624 ± 0.000136	0.0156
	40	0.0327 ± 0.0017	0.0817	0.0303 ± 0.0006	0.0758

[a] The values are means ± S.D.

Table 4
Regression equations, quantitation ranges and detection limits for seven barbiturates extracted from human whole blood obtained by DI–SPME/GC–MS

Compound	$y = ax + b$[a]			Quantitation range ($\mu g\,ml^{-1}$)	Detection limit ($\mu g\,ml^{-1}$)
	a	b	r^2		
Amobarbital	3.94×10^{-1}	-6.97×10^{-2}	0.998	0.2–40	0.05
Pentobarbital	4.39×10^{-1}	-2.91×10^{-2}	0.998	0.2–40	0.05
Secobarbital	3.69×10^{-1}	-1.53×10^{-1}	0.998	0.2–40	0.05
Hexobarbital	2.40×10^{-1}	-2.02×10^{-2}	0.998	0.2–40	0.05
Mephobarbital	5.44×10^{-1}	-5.87×10^{-2}	0.996	0.2–40	0.05
Phenobarbital	8.77×10^{-2}	-1.86×10^{-2}	0.995	0.5–50	0.3
Primidone	4.84×10^{-3}	-9.76×10^{-3}	0.994	2–200	1

[a] y is the ratio of analyte peak area to that of the IS, and x is the concentration of each barbiturate. The slope a and intercept b values calculated by regression are means obtained from three experiments. Each equation was obtained from plots at five or seven concentrations.

Table 5
Regression equations, quantitation ranges and detection limits for seven barbiturates extracted from human urine obtained by DI–SPME/GC–MS

Compound	$y = ax + b$[a]			Quantitation range ($\mu g\,ml^{-1}$)	Detection limit ($\mu g\,ml^{-1}$)
	a	b	r^2		
Amobarbital	4.77×10^{-1}	-9.01×10^{-3}	0.998	0.05–5	0.01
Pentobarbital	5.42	-1.24×10^{-2}	0.999	0.05–5	0.01
Secobarbital	5.31×10^{-1}	-8.77×10^{-3}	0.997	0.05–5	0.01
Hexobarbital	3.60	-4.42×10^{-2}	0.998	0.05–5	0.01
Mephobarbital	7.50×10^{-1}	-2.23×10^{-2}	0.998	0.05–5	0.01
Phenobarbital	1.11×10^{-1}	-1.22×10^{-2}	0.995	0.25–25	0.15
Primidone	3.36×10^{-3}	1.20×10^{-2}	0.996	1–100	0.6

[a] The explanations for y, x, a, and b are the same as specified in the legend of Table 4.

levels of the barbiturates were reported to be several $\mu g\,ml^{-1}$ to several 10 $\mu g\,ml^{-1}$ [13]. Therefore, our present method is sensitive enough to analyze their therapeutic levels.

The intra- and inter-day precision (coefficient of variation) and accuracy values from one individual are presented in Tables 6 and 7. The precision values at all concentrations for intra-assay study varied from 1.2 to 13.4% for whole blood and from 1.3 to 14.1% for urine, while those for inter-assay study varied from 2.5 to 13.8% for whole blood and from 1.7 to 13.1% for urine. Furthermore, we investigated intra-day precision data obtained from four different individuals. The

Table 6
Accuracy and precision data for seven barbiturates extracted from human whole blood

Compound	Amount added ($\mu g\,ml^{-1}$)	Samples from one individual						Samples from different individuals Intra-day ($n = 4$)
		Intra-day ($n = 4$)			Inter-day ($n = 4$)			
		Amount detected[a] ($\mu g\,ml^{-1}$)	Accuracy (%)	Precision (%)	Amount detected[a] ($\mu g\,ml^{-1}$)	Accuracy (%)	Precision (%)	Precision (%)
Amobarbital	1	1.07 ± 0.06	107.0	5.6	1.02 ± 0.07	102.4	6.6	4.5
	20	19.8 ± 1.4	99.2	6.9	19.3 ± 1.2	96.6	6.4	5.8
Pentobarbital	1	1.08 ± 0.10	108.0	9.4	1.04 ± 0.13	104.1	12.4	10.1
	20	20.5 ± 0.5	102.3	2.2	20.7 ± 0.5	103.5	2.5	5.3
Secobarbital	1	1.04 ± 0.07	104.0	7.0	1.04 ± 0.08	103.9	7.3	11.5
	20	19.2 ± 0.8	96.1	4.1	18.9 ± 0.9	94.3	4.7	14.2
Hexobarbital	1	1.04 ± 0.12	104.0	11.2	1.01 ± 0.14	101.3	13.8	7.6
	20	21.2 ± 0.2	106.1	1.2	20.5 ± 0.9	102.3	4.4	5.3
Mephobarbital	1	1.09 ± 0.04	109.0	3.3	0.96 ± 0.07	96.3	7.6	6.3
	20	21.1 ± 0.7	105.3	3.4	19.8 ± 1.6	99.2	7.9	6.6
Phenobarbital	2.5	2.45 ± 0.16	98.0	6.6	2.48 ± 0.17	99.4	7.0	20.9
	25	25.1 ± 1.3	100.3	5.1	23.1 ± 2.3	92.5	9.8	14.5
Primidone	10	9.62 ± 1.29	96.2	13.4	11.0 ± 1.5	109.6	13.7	21.0
	100	92.7 ± 3.6	92.7	3.9	97.4 ± 5.5	97.4	5.6	8.7

[a] The values are means ± S.D.

values were from 4.5 to 21.0% for whole blood and from 3.1 to 14.9% for urine (Tables 6 and 7).

The accuracy values for intra-assay and inter-assay study were in the ranges of 92.5–119.6% for whole blood and 93.0–110.0% for urine. Although equilibria were not attained under the present conditions as shown in Fig. 5, accuracy and precision values were satisfactory.

3.3. Actual determination of pentobarbital in whole blood and urine after its oral administration to volunteers

The established method in this study was actually applied to the samples of human whole blood and urine after oral administration of pentobarbital to volunteers. A therapeutic dose of pentobarbital calcium (50 mg) was administered

Table 7
Accuracy and precision data for seven barbiturates extracted from human urine

Compound	Amount added ($\mu g\,ml^{-1}$)	Samples from one individual						Samples from different individuals Intra-day ($n = 4$)
		Intra-day ($n = 4$)			Inter-day ($n = 4$)			
		Amount detected[a] ($\mu g\,ml^{-1}$)	Accuracy (%)	Precision (%)	Amount detected[a] ($\mu g\,ml^{-1}$)	Accuracy (%)	Precision (%)	Precision (%)
Amobarbital	0.1	0.093 ± 0.004	93.0	4.3	0.096 ± 0.007	96.0	7.3	12.2
	2	2.17 ± 0.10	108.6	4.6	2.06 ± 0.09	102.9	4.6	4.7
Pentobarbital	0.1	0.102 ± 0.003	102.0	2.9	0.109 ± 0.007	109.0	6.4	4.3
	2.5	2.42 ± 0.03	96.9	1.3	2.47 ± 0.04	98.8	1.7	5.0
Secobarbital	0.1	0.096 ± 0.006	96.0	6.3	0.101 ± 0.007	101.0	6.9	10.4
	2	2.02 ± 0.14	100.8	7.0	2.00 ± 0.15	100.2	7.4	5.9
Hexobarbital	0.1	0.108 ± 0.002	108.0	1.9	0.110 ± 0.005	110.0	4.5	12.4
	2.5	2.46 ± 0.04	98.4	1.6	2.44 ± 0.15	97.4	6.0	6.1
Mephobarbital	0.1	0.106 ± 0.006	106.0	5.7	0.107 ± 0.006	107.0	5.6	6.6
	2	1.94 ± 0.11	96.9	5.6	1.92 ± 0.10	95.8	5.0	3.1
Phenobarbital	0.5	0.473 ± 0.020	94.6	4.2	0.466 ± 0.021	93.2	4.5	14.9
	10	10.9 ± 0.8	109.2	7.7	9.81 ± 0.98	98.1	10.0	12.2
Primidone	2	2.02 ± 0.29	101.2	14.1	2.17 ± 0.29	108.6	13.1	10.5
	40	39.1 ± 2.5	97.8	6.3	43.2 ± 2.8	108.0	6.5	10.4

[a] The values are means ± S.D.

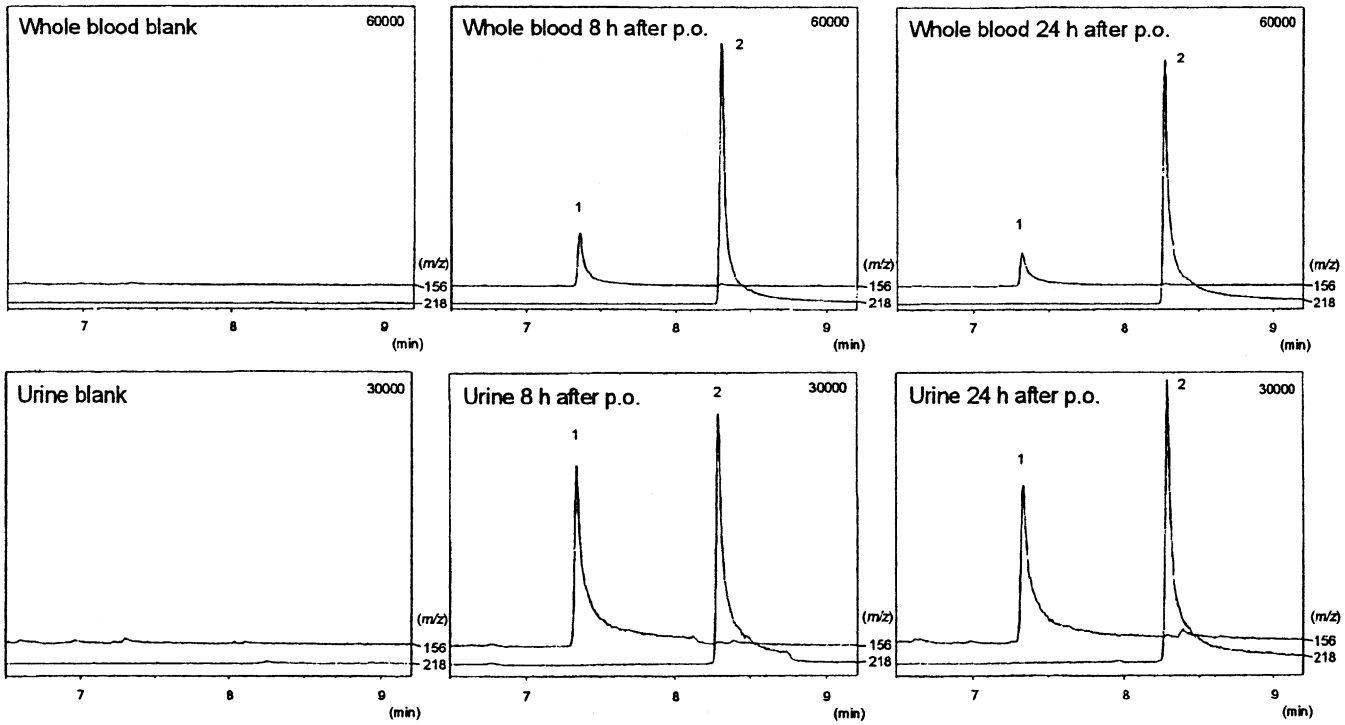


Fig. 7. SIM chromatograms obtained by DI–SPME/GC–MS from extracts of whole blood and urine of a female volunteer at 8 and 24 h after oral administration of the pentobarbital. The amount of mephobarbital used as IS was 2 μg for 0.5 ml of whole blood and 0.15 μg for 0.5 ml of urine. Peaks: 1, pentobarbital; 2, mephobarbital (IS).

orally to a 41-year-old male and a 30-year-old female volunteers. Blood and urine were collected at 8 and 24 h after the administration. The SIM chromatograms obtained from the female volunteer are shown in Fig. 7. The drug concentrations in whole blood of the male volunteer were 0.502 and 0.292 $\mu g\,ml^{-1}$ at 8 and 24 h after administration, and those of the female volunteer were 0.794 and 0.454 $\mu g\,ml^{-1}$, respectively; those in urine were 0.145 and 0.115 $\mu g\,ml^{-1}$ for the male volunteer, and 0.422 and 0.192 $\mu g\,ml^{-1}$ for the female volunteer, respectively.

4. Conclusion

To our knowledge, this is the first report dealing with DI–SPME/GC–MS for simultaneous determination of seven barbiturates from human whole blood and urine. They could be rapidly and simultaneously determined even at the therapeutic levels. The DI–SPME/GC–MS established in this study is recommendable in the fields of the therapeutic drug monitoring, clinical toxicology and forensic toxicology.

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