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Original

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固相ミクロ抽出ーガスクロマトグラフィー／質量分析法によるヒト体液中バルビタールの定量

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Summary

The determination of barbital 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 drug was extracted from the matrices by DI-SPME with 85-μm polyacrylate-coated fibers and detected by GC/MS. Extraction efficiencies were 0.42 and 0.47% for 2,000 and 400 ng/ml of barbital in whole blood, 4.21 and 3.43% for 100 and 20 ng/ml of the drug in urine, respectively. Regression equations were linear in the range of 125–4,000 ng/ml for whole blood and in the range of 6.25–200 ng/ml for urine; the detection limits were 60 and 3 ng/ml, respectively. The DI-SPME plus GC/MS procedure for barbital established in this study is simple and sensitive enough to be adopted in the fields of therapeutic drug monitoring, clinical toxicology and forensic toxicology.

Key words : Solid-phase microextraction (SPME); Barbital; Metharbital; Gas chromatography/mass spectrometry (GC/MS)

Introduction

Solid-phase microextraction (SPME) was first introduced by Arthur and Pawliszyn in 1990 [1]. Since then, our group applied the technique to analyses of various types drugs and poisons [2-8]. Recently, we reported simultaneous determination of seven barbiturates (amobarbital, pentobarbital, secobarbital, hexobarbital, mephobarbital, phenobarbital and primidone) in human body fluids by direct immersion (DI)-SPME and gas chromatography/mass spectrometry (GC/MS) [8]. In the above report, barbital, being occasionally used as a long-lasting hypnotic, was not included, because the peak of barbital was too broad and

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the sensitivity was insufficient under the previous GC/MS conditions. In this study, we have re-optimized the conditions for analysis of barbital in human body fluids by DI-SPME and GC/MS, and have been able to detect the drug with high sensitivity.

Experimental

Materials

Barbital was purchased from Sigma (St. Louis, MO, USA) and metharbital (1-methylbarbital) (internal standard, IS) was kindly donated from Dainippon Pharmaceutical Co., Ltd. (Osaka). An SPME device and 85-μm polyacrylate-coated SPME fibers were purchased from Supelco Inc. (Bellefonte, PA, USA); a DB-1 MS fused silica capillary column (30 m x 0.32 mm ID, film thickness 0.25 μm) from J & W Scientific (Folsom, CA, USA). Other common chemicals used were of the analytical grade. Whole blood and urine were obtained from healthy subjects.

Extraction procedure

DI-SPME was performed essentially according to our previous paper [8]. To 0.5 ml of whole blood containing barbital and IS, 1.5 ml of 0.5 M perchloric acid solution was added for deproteinization. After stirring vigorously with a Vortex mixer for 1 min, the mixture was centrifuged at 3,000 rpm for 10 min; 1.5 ml of the clear supernatant was decanted into a 2-ml micro-reaction vial containing a magnetic stirring bar. The pH was adjusted to 6–7 with 70 μl of 5 M and 180 μl of 1 M sodium hydroxide solutions, and then 0.5 g of sodium sulfate was added to it. After the vial was sealed with a silicone septum cap and stirred, the SPME syringe needle was passed through the septum. The SPME fiber was pushed out and immersed into the sample solution; then the vial was heated on an aluminum block heater at 60°C with stirring. After 60 min of the immersion, the fiber was withdrawn into the needle and pulled out of the vial. It was immediately inserted into the GC injection port. The fiber was exposed in the port for 1 min to ensure desorption of the drugs.

For urine, 1 ml of the sample was placed in the 2-ml micro-reaction vial containing the stirring bar and 0.5 g of sodium sulfate without any process of deproteinization and the pH adjustment. The vial was sealed with a silicone septum cap and the following procedure was exactly the same as that for whole blood.

Table 1. Major fragment ions obtained from mass spectra of barbital and IS in the EI mode

Compound	MW	*m/z* (% intensity)
Barbital	184	141 (100), 156 (90), 112 (20)
Metharbital	198	155 (100), 170 (83), 112 (28)

GC/MS conditions

GC/MS analysis was performed on a Shimadzu GCMS-QP2010 instrument (Kyoto) equipped with the DB-1 MS capillary column. Column temperature was set at 40°C and held for 1 min; then the temperature was programmed up to 250°C at 30°C/min. Injection, interface and ion source temperatures were set at 310, 310 and 240°C, respectively. Helium was used as carrier gas at a flow rate of 2 ml/min. The injection was made in the splitless mode, and the splitter was opened after 1 min. The mass spectrometer was operated in the positive electron impact ionization mode at an ionization energy of 70 eV. Full scan data were obtained with a mass range of *m/z* 50–300. Major fragment ions of the drugs are listed in Table 1. Quantitative analysis was carried out in the selected ion monitoring (SIM) mode. The ions monitored for quantitation were *m/z* 141 for barbital and *m/z* 155 for IS.

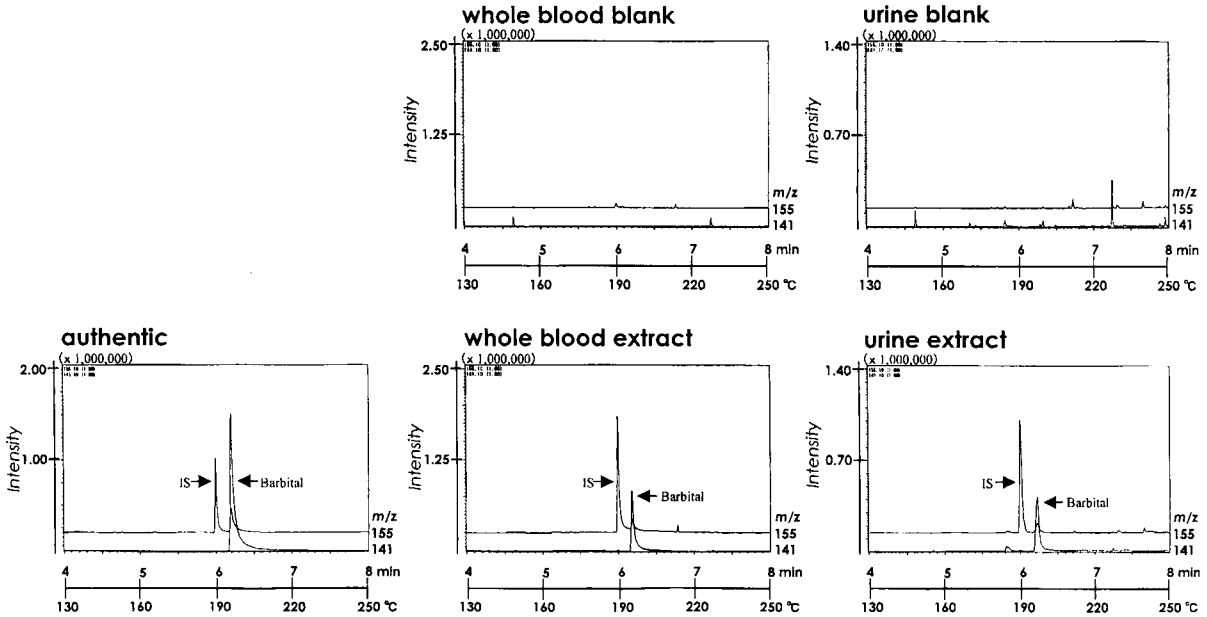

Fig. 1. SIM chromatograms for the authentic drugs dissolved in methanol and for extracts from human whole blood and urine in the presence and absence of the drugs obtained by DI-SPME. For direct injection, 15 and 2 ng on-column of barbital and IS, respectively, were used. Amounts of barbital and IS spiked were: 1,000 and 500 ng into 0.5 ml whole blood; 100 and 20 ng into 1 ml of urine, respectively.

Results and discussion

Figure 1 shows SIM chromatograms for the non-extracted authentic barbital and IS (15 and 2 ng on column, respectively) dissolved in methanol and for whole blood and urine extracts in the presence and absence of the drugs. To obtain the extracts, 1,000 and 500 ng of barbital and IS, respectively, were spiked to 0.5 ml of whole blood; 100 and 20 ng were spiked to 1 ml of urine. The retention times of barbital and IS were 6.2 and 6.0 min, respectively. The extraction efficiencies, which were calculated by comparing the peak areas for the SPME extracts with those for the authentic compounds dissolved in methanol, and their within-day variations (CV values) are listed in Table 2. The efficiencies were 0.42 and 0.47% for 2,000 and 400 ng/ml of barbital in whole blood, 4.21 and 3.43% for 100 and 20 ng/ml of the drug in urine, respectively; within-day CV values were less than 14% for both samples.

Table 3 shows regression equations for barbital in whole blood and urine samples. The equations were constructed by plotting peak area ratios of barbital to IS (500 ng in 0.5 ml of whole blood and 25 ng in 1 ml of urine), and were linear in the range of 125–4,000 ng/ml for whole blood and in the range of 6.25–200 ng/ml for urine; the detection limits (signal-to-noise ratio = 3) were about 60 ng/ml for whole blood and 3 ng/ml for urine. Therapeutic, toxic and fatal blood levels were reported to be 2–20, 20–50 and >50 μg/ml [9]; and 10–26, 60–80 and >100 μg/ml [10], respectively. Therefore, our method is sufficiently applicable to the analysis of barbital even at the therapeutic levels.

Table 2. Extraction efficiencies and their within-day CV values for barbital in human whole blood and urine obtained by DI-SPME plus GC/MS

Sample	Concentration added (ng/ml)	Extraction efficiency (%) (n=3)	CV (%) (n=3)
Whole blood	2,000	0.42	8.58
	400	0.47	10.7
Urine	100	4.21	3.49
	20	3.43	5.31

The factors for the success in detecting barbital peaks without any derivatization in the present study seem to be the rapid elevation of over temperature from 40°C and also the use of metharbital as IS, a compound with a very close structure acting as a good carrier for barbital.

SPME is an organic-solvent-free extraction technique, which incorporates sample extraction, concentration and introduction into a single step; this technique is now widespread in the world. The present SPME method for barbital seems useful for therapeutic drug monitoring, clinical toxicology and forensic toxicology, because of its simplicity and sensitivity.

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Table 3. Regression equations, quantitation ranges and detection limits for barbital in whole blood and urine obtained by DI-SPME plus GC/MS

Sample	Equation[a]	r^2	Quantitation range (ng/ml)	Detection limit (ng/ml)
Whole blood	$y = 0.000952x + 0.0396$	0.999	125 – 4,000	60
Urine	$y = 0.00787x + 0.0268$	0.999	6.25 – 200	3

[a] y is the peak area ratio of barbital to IS, and x is the concentration of barbital. The slope and intercept values are means obtained from duplicate experiments. Each equation was plotted at six concentrations. To 0.5 ml of whole blood and 1 ml of urine, 500 and 25 ng of IS were added, respectively.

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