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Application of solid-phase microextraction and gas chromatography–mass spectrometry for the determination of chlorophenols in urine

Maw-Rong Lee*, Yao-Chia Yeh, Wei-Shin Hsiang, Chih-Cheng Chen

Department of Chemistry, National Chung-Hsing University, Taichung 40227, Taiwan

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Abstract

This study investigated the feasibility of applying solid-phase microextraction (SPME) combined with gas chromatography–mass spectrometry to analyze chlorophenols in urine. The SPME experimental procedures to extract chlorophenols in urine were optimized with a polar polyacrylate coated fiber at pH 1, extraction time for 50 min and desorption in GC injector at 290°C for 2 min. The linearity was obtained with a precision below 10% R.S.D. for the studied chlorophenols in a wide range from 0.1 to 100 μg/l. In addition, sample extraction by SPME was used to estimate the detection limits of chlorophenols in urine, with selected ion monitoring of GC–MS operated in the electron impact mode and negative chemical ionization mode. Detection limits were obtained at the low ng/l levels. The application of the methods to the determination of chlorophenols in real samples was tested by analyzing urine samples of sawmill workers. The chlorophenols were found in workers, the urinary concentration ranging from 0.02 μg/l (PCP) to 1.56 μg/l (2,4-DCP) depending on chlorophenols. The results show that trace chlorophenols have been detected with SPME–GC–MS in the workers of sawmill where chlorophenol-containing anti-stain agents had been previously used.

Keywords: Chlorophenols

1. Introduction

In addition to their use as intermediates in industry and in the production of dyes, plastics and pharmaceuticals, chlorophenols have been extensively utilized as preservative agents, pesticides, antiseptics and disinfectants [1]. The US Environmental Protection Agency (EPA) lists some chlorophenols as priority pollutants owing to their carcinogenicity and considerable persistence. The monitoring of chlorophenols in human urine and other biological samples are normally used as an indication of occupational exposure [2] or exposure to environmental contamination [3]. Therefore, a rapid, accurate and sensitive analytical means of identifying and determining trace chlorophenols in urine samples needs to be developed. The extraction efficiency from the complex matrix (particularly in a urine sample) affects the detection level. Moreover, selecting the optimum means of sample

*Corresponding author.

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preparation is essential for trace analysis of chlorophenols in urine.

Many methods based on chromatographic techniques have been proposed to determine chlorophenols in urine, including GC–ECD [4,5], GC–MS [6] and LC–EC [7]. The conventional extraction methods for chlorophenols in urine are solvent extraction [4] and solid-phase extraction (SPE) [7]. The liquid–liquid extraction, although the most frequently method among conventional techniques, produces emulsions and different extraction efficiencies for various compounds. In addition, it requires large amounts of solvent; it is slow and laborious as well. SPE is extensively used for the trace enrichment of retained organic compounds which can be carried out by elution with an adequate solvent [8]. However, SPE can be expensive with the cartridges usually disposed of after one extraction. The entire analysis can be lengthy with a series of stages including washing, conditioning, elution, and slow drying. In addition the extraction methods use organic solvents that pose a threat to the environment and human health. In addition, solvent disposal is rather expensive. Therefore, a simple, fast and solvent-free extraction method must be developed. Solid-phase microextraction (SPME) does not require the use of an organic solvent. The mechanism of SPME is based on an equilibrium of analyte concentration between the sample and that in the solid-phase fiber coating. Pawliszyn et al. first described this sampling technique [9–15]. Zhang and Pawliszyn thoroughly described the merits and fundamentals of how to extract trace organic compounds from a complex matrix [16]. The widespread application of SPME to extract organic compounds from aqueous samples is attributed to its solvent-free methodology, simplicity, rapidity and relatively low cost [17–22]. The extraction of analytes in SPME method depends on the absorbent coating of fiber, as well as on variables, e.g. extraction time or equilibrium time, conditions of matrix, desorption time and temperature. In this study, we systematically evaluate the optimum procedures for analyzing chlorophenols in urine by using SPME–GC–MS. The feasibility of applying this technique is also evaluated by investigating the detection limits, linear dynamic ranges and reproducibility for several chlorophenols.

2. Experimental

2.1. Materials

The analytical standards 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP) and 2,4,6-trichlorophenol (2,4,6-TCP) from Environmental Solution (NC, USA) were dissolved in isopropanol at 5 mg/ml as stock solutions. Pentachlorophenol (PCP) was obtained from Supelco (PA, USA). The standard 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP) from TCI (Tokyo, Japan) was purified by recrystallization from ether. The internal standard 2,4,6-tribromophenol was purchased from TCI and used as received. All chemicals were of analytical reagent grade, and purified water was obtained from a Milli-Q purification system. The real urine samples were collected from the workers of a sawmill at Taichung Hsien (Taiwan) where a sodium chlorophenolate product had been used. The sawmill employs 25 workers. Ten urine samples (four men, six women) were obtained at the morning break time. The samples were immediately frozen and stored in a refrigerator until further treatment. All glassware was silanized prior to use by soaking the glassware overnight in toluene solution at a concentration of 10% dichlorodimethylsilane. The glassware was rinsed in toluene and methanol and then thoroughly dried for 4 h. Urine samples were prepared by spiking with stock standard chlorophenols solution and the internal standard into urine that was pooled from four individuals. All analyses were performed with 40-ml vials containing 25 ml of solution equipped with a 1-inch stir bar and a stirring plate. SPME fiber coated with an 85-μm film thickness polyacrylate was obtained from Supelco. Next, the fibers were conditioned under helium in the hot injection port of the gas chromatograph at 300°C for 2–3 h prior to use. Optimization of the SPME was then studied with respect to extraction efficiency for chlorophenols used to spike a 25-ml water sample. Finally, the needle on the SPME manual holder was set at its

maximum length 4 cm in the GC injector, a desorption temperature of 290°C for 2 min was used to produce the highest sensitivity for detection of chlorophenols.

2.2. Instrumentation

The concentrations of the chlorophenols in the urine were determined using SPME sorption and GC–MS with a Hewlett-Packard MS Engine GC–MS (Palo Alto, CA, USA). A split/splitless injector was used in the splitless mode. GC separations were performed with a 30-m fused-silica capillary column DB-5.625 with an internal diameter of 0.25 mm and a stationary phase thickness of 0.5 μm (J&W Scientific, Folsom, CA, USA). Helium carrier gas was held at a rate of 1 ml/min using electronic pressure control. For fiber injection, the injector was held isothermally at 290°C. The transfer line was held at 310°C. The ion source of mass spectrometer was maintained at 250°C and 150°C for electron impact ionization (EI) and chemical ionization (CI), respectively. The column was initially set at 60°C, ramped at 30°C/min to 190°C and from 190°C to 310°C at a rate of 10°C/min. Next, EI ionization with electron energy of 70 eV, positive and negative chemical ionization (NCI) with methane as reagent gas were used to trace the optimum ionization mode for chlorophenols analysis. GC–MS of the chlorophenols was then determined using selected ion monitoring (SIM) at the nominal molecular mass and isotopic molecular mass at a constant ratio of chlorophenols (Table 1). The ions used for quantitation were the molecular ions of chlorophenols.

3. Results and discussion

3.1. Coating evaluation

As an equilibrium or partition technique, SPME does not exhaustively extract organic compounds from an aqueous sample. A linear relationship arises between the amount of analyte absorbed by the fiber and sample concentration [22]. The distribution constant K and the volume of the stationary phase on the fiber determine the linear range and the sensitivity of SPME sampling technique. The distribution constant is used as a measure of that coating's affinity for the target analyte. A coating with a high K value of an analyte has a high affinity and adsorbs large amounts of analyte. The high affinity results in a good sensitivity for extraction and wide linear range. To determine the K value for chlorophenols of fiber coated with a 85-μm film thickness polyacrylate, the fiber is exposed to a standard water solution spiked with the analytes at the optimum extraction conditions previously established in our laboratory [23]. The K values are calculated according to the equation described by Loueh and Pawliszyn [9]. For the studied chlorophenols, the K values are given as well as the solubility data and K_{ow} values [24]. The solubility of chlorophenols in water increases with a decreasing K value. Therefore, chlorophenol with a high K value is efficiently extracted from water into the fiber's coating, thereby improving the sensitivity detection in SPME–GC–MS analysis.

3.2. Selection of optimum conditions for SPME

The amount of an analyte extracted heavily relies on the mass transfer of an analyte through the

Table 1
Analytical conditions of chlorophenols, as determined by GC–MS with various ionization modes

No.	Compound	t_R	Selected ion/confirmed ion (isotope ratio)		
		(min)	EI	PCI	NCI
1	2-Chlorophenol (2-CP)	3.35	128/130 (3:1)	129/131 (3:1)	128/130 (3:1)
2	2,4-Dichlorophenol (2,4-DCP)	4.34	162/164 (3:2)	163/165 (3:2)	162/164 (3:2)
3	2,4,6-Trichlorophenol (2,4,6-TCP)	5.34	196/198 (1:1)	197/199 (3:1)	196/198 (1:1)
4	2,3,4,6-Tetrachlorophenol (2,3,4,6-TeCP)	6.64	232/230 (4:3)	233/231 (4:3)	232/230 (4:3)
5	Pentachlorophenol (PCP)	8.17	266/264,268 (15:9:10)	267/269 (3:2)	230/232 (3:2)

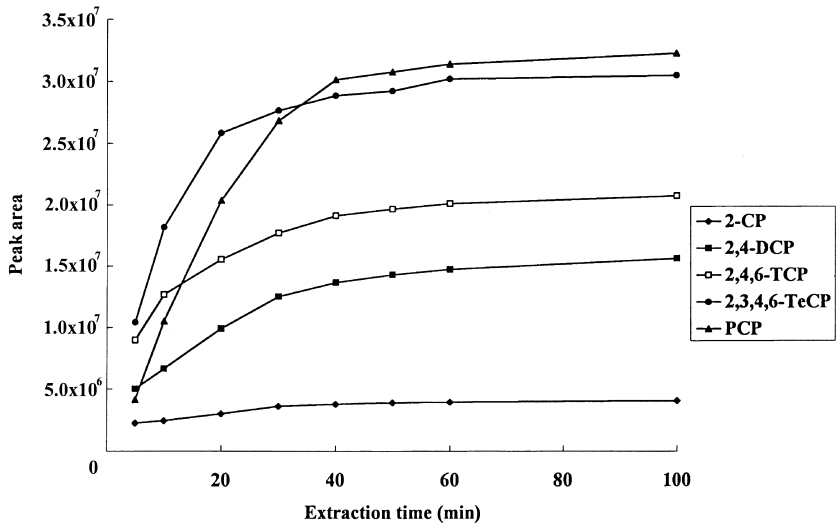

Fig. 1. Effect of extraction time on peak areas of chlorophenols, produced by SPME–GC–MS.

aqueous phase [8] and the time of extraction. During extraction, the urine samples were continuously agitated with a magnetic stir bar on a stir plate revolving at around 1000 r.p.m. Under these conditions, the amount of analyte transferred into the adsorbed phases is increased, thereby decreasing the length of time required to reach equilibrium. To determine the effect of extraction time, extractions were performed from 5 to 100 min. Fresh samples were used for each extraction time tested. The exposure time profiles were constructed by plotting the amount of analytes eluted versus the time that the fiber was exposed to the urine samples. All chlorophenols reached equilibrium in over 50 min (Fig. 1). Therefore, extraction for chlorophenols in urine samples with SPME was carried out at 50 min.

Decreasing the solubility of chlorophenols in urine enhances the amount of chlorophenols extracted. To achieve this purpose, salt is added to the urine, the matrix pH is varied, or derivatives are formed. In this case, acid and various salts are added (singularly and in combination) to investigate the effect of extraction by the fiber. Table 2 reveals the factor increase obtained for all extracting conditions using a 50-min extraction time, which was compared to the extraction for the original urine samples (pH 6.2) of the same chlorophenol concentration (25 μg/l). The extraction enhancement is increased with decreasing the pH of the urine solution from 7 to 1 (Fig. 2). The enhancement is related to the *K* values of chlorophenols. A higher increased factor is obtained with the high *K* value compounds. The extraction for PCP at pH 1 is better by a factor of approximately 9 than that obtained in the original solution. The salting out

Table 2
Matrix effect enhancement of the extraction of chlorophenols in urine with SPME

Compound	pK_a	*K*	Factor increase					
			pH 1	NaCl	KCl	pH 1 with NaCl	pH 1 with KCl	pH 10 with KCl+NaCl
2-CP	8.48	8	1.2	2.5	1.9	2.2	1.8	2.0
2,4-DCP	7.25	84	1.3	1.2	1.3	0.8	1.0	0.9
2,4,6-TCP	7.42	110	2.8	2.2	1.6	1.0	1.3	1.2
2,3,4,6-TeCP	–	131	5.2	1.8	2.1	1.0	1.8	1.4
PCP	4.74	212	9.2	3.2	3.8	1.1	2.5	1.8

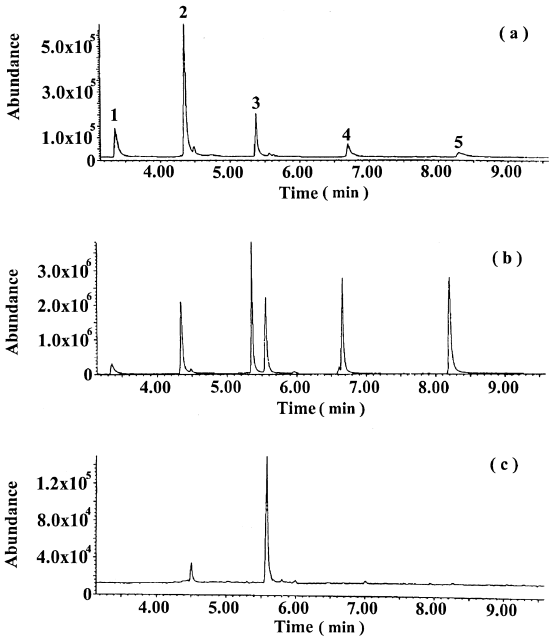

Fig. 2. Mass ion chromatogram of (a) 25 μg/l chlorophenols in urine at pH 7, (b) at pH 1 and (c) blank urine, produced by SPME–GC–MS.

effect by adding salt into the aqueous matrix is conventionally used to increase the amount of organics extracted in water. Saturated sodium chloride (5 *M*) or potassium chloride (5 *M*) was added to the urine samples for examining the effect of salting out. Three extractions were performed for every condition. Fresh samples were used for each time tested. A lower pK_a value implies a greater improvement in the amount extracted, regardless of the salt added (Table 2). This improvement is attributed to the decreases in the solubility of the chlorophenols caused by the salt, forcing these analytes into the fiber. Under the combined acid and salt conditions (Table 2), the improvement of the extraction is not better than that obtained with either one alone. This is because at acidic condition, the presence of the saturated salt hinders the shift of the ionized form of chlorophenols towards the neutral form, which has a higher affinity for the fiber. Therefore, for extracting chlorophenols in urine by SPME, the urine is adjusted only at pH 1.

3.3. Analytical data

The detection limit of SPME to determine chlorophenols in urine heavily relies on the amount of the analytes adsorbed by coating the fiber and the sensitivity of the GC–MS. The linear range experiments provide the necessary information to estimate the detection limits, based on the lowest detectable peak that has signal-to-noise ratio of 3. The SPME and capillary column procedures were performed at the optimum conditions in this study. The EI ionization and NCI of MS were used to determine the detection limits of chlorophenols in urine. In addition, the SIM mode is performed by MS in quantitative analysis to increase sensitivity. In general, the most abundant ion is used for the ion of monitoring; the specific ion is used as the confirmed ion (Table 1). The signal is obtained by measuring the peak area over the scans during elution of chlorophenols in the GC. Table 3 summarizes the LOD results for all analytes obtained using SPME–GC–MS. The detection limits for the determination of chlorophenols except 2-CP (41 ng/l in EI, 98 ng/l in NCI) can be

Table 3
Comparison of detection limits of MS coupled with SPME for chlorophenols analysis in urine with EPA method for water analysis

Compound	SPME (ng/l)		EPA method (ng/l)[a] (L–L extraction)	
	EI	NCI	Method 604 (FID)	Method 625 (GC–MS)
2-CP	41	98	310	3300
2,4-DCP	6	2	390	2700
2,4,6-TCP	9	0.03	640	2700
2,3,4,6-TeCP	1	6	–	–
PCP	9	8	7400	3600

[a] For analysis of chlorophenols in water [11].
Method 625 analysis with full scan mode of MS.

down to as low as the ng/l range in urine. Compared with the results in water samples, the NCI technique does not offer much better sensitivity for those chlorophenols in which the number of chlorine molecules is higher than three. This outcome can be attributed to the complex matrix in urine which hampers the chlorophenol adsorption to the fiber. Peak tailing is the major disadvantage of using NCI mode. In addition, SPME offers a higher sensitivity for trace chlorophenols analysis in urine than the conventional liquid–liquid extraction method, which was used in the US EPA methods 604 (FID) and 625 (GC–MS with full scan) to determine chlorophenols in water.

The linear ranges were also examined by extracting the spiked urine samples ranging from 0.1 μg/l to 100 μg/l for each compound with 0.1 μg/l 2,4,6-tribromophenol used as internal standard under the optimum conditions. The studied chlorophenols in urine were analyzed, in which SPME is linear over approximately three orders of the amounts, with linear correlation coefficients exceeding 0.999 in all cases.

The precision of the method was investigated under optimum conditions, by performing eight extraction from urine which contained all studied chlorophenols at a concentration of 25 μg/l. The precision expressed as R.S.D.s of the fiber ranged between 5 and 10%, which is deemed satisfactory for determining chlorophenols in urine.

3.4. Application

The applicability of the method to the determination of chlorophenols in real samples was tested by analyzing urine samples of the workers in a sawmill. The aim of this study was to establish whether workers at a sawmill where chlorophenol-containing anti-stain agents had been previously used were still exposed to chlorophenols. Occupational exposures to chlorophenols have been monitored by measuring urinary excretion or concentrations in blood. Since 1989, the chlorophenol-containing anti-stain agents have been prohibited in Taiwan, and nowadays products without chlorophenol are used. Nevertheless, workers can still be exposed to chlorophenols via a contaminated environment. The chromatograms of a real urine sample and a urine blank sample are shown in Fig. 3. From the results (Table 4), chlorophenols were found in all urine samples except one. The concentration of chlorophenols ranged from 0.02 μg/l (PCP) to 1.56 μg/l (2,4-DCP).

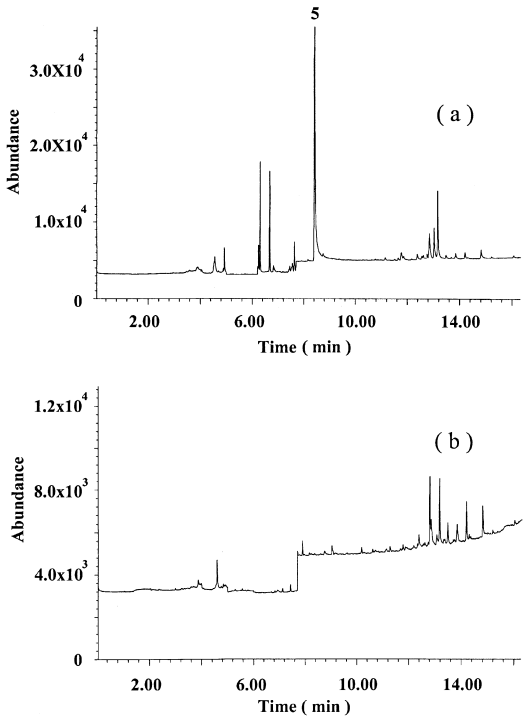

Fig. 3. Mass ion chromatograms of (a) urine sample S_1, (b) blank urine, produced by SPME–GC–MS.

4. Conclusion

This study demonstrates that SPME is a precise, reproducible means of analyzing trace chlorophenols from urine. Linearity is verified over a wide range. The optimum conditions for extraction to obtain a high recovery are at urine sample pH 1, equilibrium time 50 min and desorption in GC injector at 290°C for 2 min. The application of SPME–GC–MS system to the determination of chlorophenols in real samples was tested by analyzing the urine samples of workers in a sawmill. Chlorophenols were detected ranging from 0.02 μg/l (PCP) to 1.56 μg/l (2,4-DCP). The technique offers a low level ng/l sensitivity to determine trace amounts of chlorophenols

Table 4
Chlorophenol concentrations in the urine of sawmill workers

Subject	2-CP (μg/l)	2,4-DCP (μg/l)	2,4-TCP (μg/l)	2,4,6-TeCP (μg/l)	PCP (μg/l)
S_1	ND	ND	ND	ND	0.05
S_2	ND	0.10	0.07	0.05	0.03
S_3	ND	ND	0.02	ND	ND
S_4	0.6	0.22	0.24	0.08	0.08
S_5	ND	0.08	0.10	0.04	ND
S_6	ND	0.18	0.12	0.07	0.07
S_7	0.20	1.56	0.15	0.05	0.02
S_8	ND	0.55	0.14	0.12	0.02
S_9	ND	0.82	0.08	0.13	0.04
S_{10}	ND	ND	ND	ND	ND

ND: below limit of detection.

in urine samples which contain high levels of interference.

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