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Biological Monitoring of Human Exposure to Volatile Halogenated Hydrocarbons Using Urinalysis with Capillary GC-ECD

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Abstract □ For the risk assessment of human exposure to volatile halogenated hydrocarbons, a dynamic purge trap/on-column cryofocusing method using capillary gas chromatograph-^{63}Ni electron capture detector and thermal desorption unit was applied to analyze the free forms, metabolites of 1,1,2-trichloroethylene and 1,1,2,2-tetrachloroethylene. The urine sample was diluted with distilled water, hydrolyzed and sealed. Then the inert gas was infused to purge out free 1,1,2-trichloroethylene, free 1,1,2,2-tetrachloroethylene and trichloroethanol. These compounds were trapped to Tenax^{R} GC-gas trap device throughout clean up tube. Being undetectable to gas chromatograph directly, trichloroacetic acid was methyl esterificated and trapped in the manner above mentioned. The optimal incubation time to get best recovery of methyl ester was 4 hours at 60℃. The concentrations of free volatile halogenated hydrocarbons and their metabolites in urine were obtained from 5 healthy volunteers. This analytical method is expected to make the biological monitoring, more precise and convenient.

Keywords □ Biological monitoring, volatile halogenated hydrocarbons, urinalysis, GC-ECD, purge trap/on-column cryofocusing method.

Volatile halogenated hydrocarbons (VHH) are widely used in high technology industry as a degreasing agent or solvent for dry cleaning. Most of them are discharged into ambient air due to their volatilities[1,2], and into ground water. Therefore, the main invasion route of VHH to human body is thought to be inhalation by breathing[3,4], and oral intake of drinkable water. Being VHH reported to pose grave problems of environmental pollution, there are many reports about the analytical method of VHH in ambient air[5-7], and water[8-10]. For the analysis in biological sample from the industrial worker exposed to high levels of VHH, the spectrophotometric method using Fujiwara reaction[11] is usually adapted because of its simplicity of operation. Head space method is adapted for more accurate determination of VHH. However, this method needs incubation time and accurate pre-treatment for gas-liquid equilibrium and even large volume of sample (20-30 m*l*) for an analysis. So, few information for analysis of urine containing trace level of VHH is available due to above problems.

To get over these problems, purge trap/on-column cryofocusing method[12] which has been developed for the trace analysis of VHH in aqueous sample such as rain water were modified and applied to the risk assessment of human exposure to VHH[13]. This method doesn't need precise treatment, incubation time or equilibrium temperature those are essential for head space method. Free form of 1,1,2-trichloroethylene (TRI), 1,1,2,2-tetrachloroethylene (PER) in human urine[14] from airborne enviroment were analyzed with capillary gas chromatograph-^{63}Ni electron capture detector (ECD). The final metabolites of VHH are well known as trichloroethanol (TCE) and trichloroacetic acid (TCA)[15]. These compounds were also analyzed because they could be the indices of biological monitoring[16]. The urine samples were diluted with distilled water, sealed in vials. TRI, PER and TCE were purged out

by inert gas and trapped in Tenax^R GC tube through the clean up tube and analyzed. Being undetectable to GC directly, TCA was methyl esterificated (TCA-M) according to Breimer's method[17] and analyzed identically. The optimal incubation time was investigated. The concentrations of TRI, PER, TCE and TCA obtained from 5 healthy volunteers are also described. The obtained results indicate that this analytical method could be successfully applied to monitor the VHH and their metabolites as the indices of human exposure to VHH.

EXPERIMENTAL METHODS

Materials & sample collection

Analytical grade reagents (sulfuric acid, dimethyl sulfuric acid, sodium carbonate and methanol) were purchased from the Wako Pure Chem. (Tokyo, Japan). The standards (1,1,2-trichloroethylene, 1,1,2,2-tetrachloroethylene, 2,2,2-trichloroethanol and trichloroacetic acid methyl ester) were purchased from the Tokyo Kasei (Tokyo, Japan). Distilled water was heated and purged out with nitrogen gas (purity: 99.9998%) to eliminate any possibility of VHH contamination. Possible contamination of the equipments used (Tenax^R GC, trap tube, test tube, vial, septa) was eliminated by washing with distilled water, heating and drying in oven.

Five healthy male volunteers, aged 22-56 years, working for Japanese National Institute of Public Health were selected for urine specimen collection. They had no experience to be exposed to VHH at least for 6 months. The volume of urine excretion for 24 hours was not considered because this method was examined for possibility of analysis. Five or 10 m*l* of human urine was taken in test tube and no sooner air tightly capped. These test tubes were kept below 4℃ prior to pre-treatment.

Pre-treatment of sample

Urine specimen, 1 m*l* was taken into a vial and diluted with 1 m*l* of distilled water. Then, 1 m*l* of H_2SO_4 was added to hydrolyze the conjugated compounds and sealed air tightly with Teflon^R faced silicone septa. The sample vial was purged out and trapped to gas trap device for TRI, PER and TCE analysis. For TCA analysis, diluted urine sample with 1 m*l* of H_2SO_4 was sealed and incubated at 60℃ for 3 hours. And 0.1 m*l* of $(CH_3O)_2SO_2$ was spiked and incubated again at 60℃ for 4 hours. Then the TCA-M was purged out and trapped to gas trap device, as shown in Fig. 1.

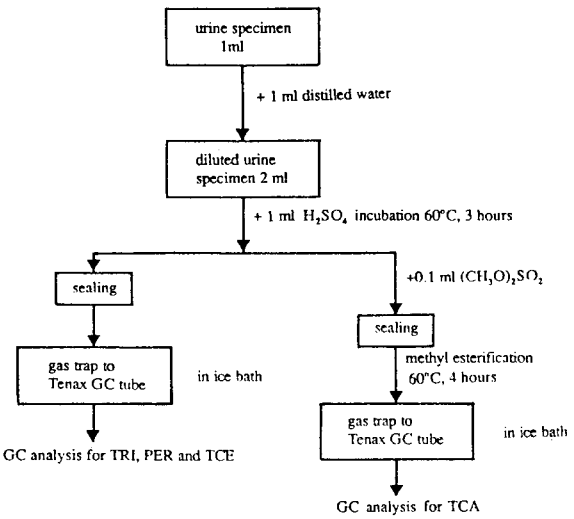

Fig. 1. Pre-treatment of urine specimen prior to analysis.

Gas trap device

Fig. 2 shows the diagram of gas trap device. The clean up tube containing sodium carbonate was used to eliminate the mist of sulfuric acid and dimethyl sulfate. Being kept at 0℃, the vial was purged by nitrogen gas. The purging flow rate was 20 m*l*/min for 4 min as our previous report[12].

Analytical method

The apparatus used were a Shimadzu GC-15A gas chromatograph with ^{63}Ni-ECD and Shimadzu C-R4A integrator. DKK GAS-20 with DKK-OMRON thermal desorption unit was used as purge trap/on-column cryofocusing system for urinalysis as shown in Fig. 3. Purge gas was inserted to gas trap tube heating at 200℃ for thermal desorption. Flow rate was 20 m*l*/min for 4 min. Following analytical procedures were identical with those of our previous report[18].

Tenax^R GC used was beads of polymeric 2,6-diphenyl-*p*-phenylene oxide (35/60 mesh). The capillary column used was Shimadzu CBP-1 (midbore: 0.25 mm i.d.×25 m *L*.) fused silica column chemically bonded with 0.25 μm layer of methyl silicone. The same one with 60 cm length was used as cryo-

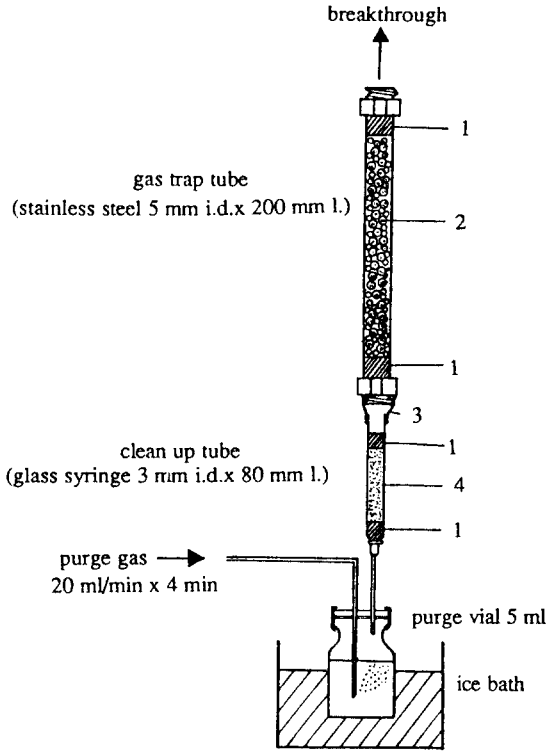

Fig. 2. Diagram of gas trap device.
1. glass wool; 2. Tenax[R] GC (35/60 mesh beads of polymeric 2,6-diphenyl-*p*-phenylene oxide); 3. polyethylene tube; 4. sodium carbonate (Na_2CO_3).

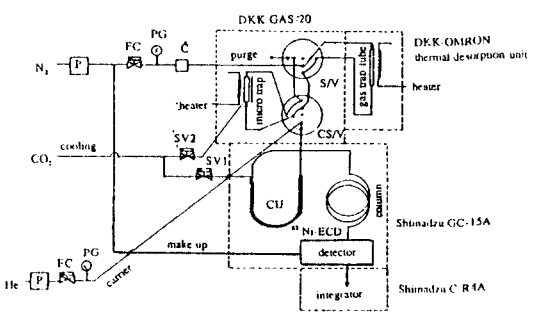

Fig. 3. Schematic diagram of analytical apparatus for urinalysis.
p: purifier; FC: flow controller; PG: pressure guage; SV: solenoid vale; C: charcoal filter S/V, CS/V: 6 port chromatographic valves; CU: on-column cryofocusing unit.

focusing column. The initial column oven temperature was 45℃ and raised to 145℃ at the end of

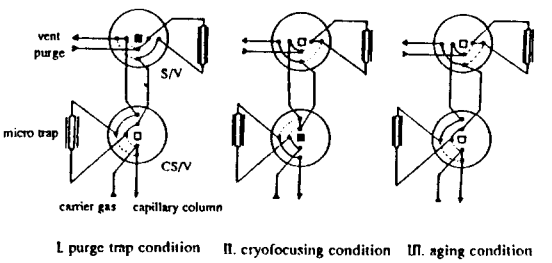

Fig. 4. Operation of 6 port chromatographic valves to switch the pathway for gas trapping.
purge gas: N_2 (99.9998%); carrier gas: He (99.9999%); ■ valve on; □ valve off

Table I. Detection limits of volatile halogenated hydrocarbons in aqueous sample by purge trap/on-column cryofocusing method

Compound	D. able	D. limit	
		ng/*l* level	ppb level
1,1,2-trichloroethylene	0.445	1.336	0.230
1,1,2,2-tetrachloroethylene	0.018	0.055	0.004

D. able; detection ability calculated as $D_1+D_2+D_3+\cdots D_n/n$
D. limit; detection limit calculated as D. able ×3

50 min (2℃/min). The operation of 6 chromatographic valves is shown in Fig. 4.

Calibration curves were constructed by spiking 5 μ*l* of standard solution (Each m*l* contains; ClCH-CCl_2 1 μg, CCl_2: CCl_2 1 μg, CCl_3CH_2OH 1 μg and CCl_3COOCH_3 1 μg). The procedures for pre-treatment and analysis were similar to those of sample preparation. The detection limits and reproducibilities of VHH by this method were also verified.

RESULTS AND DISCUSSION

Detection limits and reproducibilities of VHH

The response to amount sampled from a standard solution was observed to be linear in the range required. To verify the detection limits of VHH by this method, the responses to amount sampled from aqueous solution were calculated in according to following equation referred to JEMCA method[19].

$$\bar{D}=t(n-1,\ 0.005)\times\delta R/n\times dC/dR$$

where $\bar{D}$=detection ability

Table II. Reproducibilities of volatile halogenated hydrocarbons in aqueous sample by purge trap/on-column cryofocusing method

Compound	Amount sampled(ng/m*l*)	Response (Peak area, mV)					Coefficient of variance
		1	2	3	4	5	
1,1,2-trichloroethylene	0.23	692,017	766,825	656,023	764,290	601,886	9.1
1,1,2,2-tetrachloroethylene	0.11	1,697,605	1,784,913	1,818,917	1,525,977	1,871,707	4.8
						mean	6.9%

Table III. Concentrations of volatile halogenated hydrocarbons and their metabolites in urine specimen (unit : μg/*l*)

Volunteers	Sex	Age	Body weight(kg)	n[a]	Free form		Metabolites	
					TRI	PER	TCE	TCA[b]
FM	Male	56	60	3	1.24± 0.08	0.82± 0.02	0.74± 0.08	0.69± 0.09
HK	Male	28	71	4	3.26± 0.12	2.90± 0.10	1.08± 0.07	0.78± 0.08
TF	Male	22	74	3	1.03± 0.07	0.94± 0.06	0.92± 0.03	0.95± 0.04
WT	Male	29	88	3	1.80± 0.07	0.95± 0.04	0.99± 0.01	0.63± 0.05
NH	Male	38	65	3	2.64± 0.30	1.33± 0.05	1.42± 0.11	1.78± 0.06
			Mean		1.99	1.22	1.03	0.96

[a]Number of analysis, (The data are given as mean± SD)
[b]TCA-M was expressed in term of TCA.

t(n−1, 0.005)
="t" value with 95% confidence
δR=standard deviation
n=number of analysis
dC=amount sampled
dR=average response

As shown in Table I, detection limits by this method were much lower than 1-10 μg/*l* for TRI, 0.04-4 μg/*l* for PER provided for head space method.[20] Many reports about the determination of VHH using head space method have been reported,[14-17,21] but sensitivity with approximate 1-5 μg/*l* of detection limit is not enough to determinate using small volume of aqueous sample, because this method uses only a part of target compound which are in gaseous phase equilibrated from aqueous phase. Reproducibilities of VHH by this method were also inspected (Table II) by repeated analysis with spiking the standard solution. This method showed average 6.9% coefficient of variance which is more reproducible than 10-20% provided for head space method. In a matter of fact, VHH determination using head space method has been reported to have approximate 10.7% coefficient of variance[22].

The optimal time for hydrolysis and methyl esterification

Vials each of which containing 1 m*l* of distilled water and 1 m*l* of urine divided from the same specimen were prepared and added H_2SO_4 1 m*l*. Then each 3 of the vials were incubated at 60℃ for 1, 2, 3, 4, 5 and 6 hours, respectively. The best recoveries of TRI, PER and TCE were obtained by 3 hours' incubation. After 3 hours' hydrolysis, each 3 of them were methyl esterificated by adding 0.1 m*l* of $(CH_3O)_2SO_2$ and incubating at 60℃ for 1, 2, 3, 4, 5 and 6 hours with mechanical shaking. In methyl esterification, the best recoveries were obtained at 4 hours' incubation at 60℃ as shown in Fig. 5. Therefore, this results were applied for the analysis of all specimen.

VHH concentrations in human urine

Table III shows the concentrations of free VHH and their metabolites in human urine. The concentration of TCA-M was expressed in term of TCA. The minimum and maximum ranges were 0.63 μg/*l*-3.26 μg/*l*, respectively. The average urine concentration from 5 healthy volunteers were 1.99 μg/*l* for

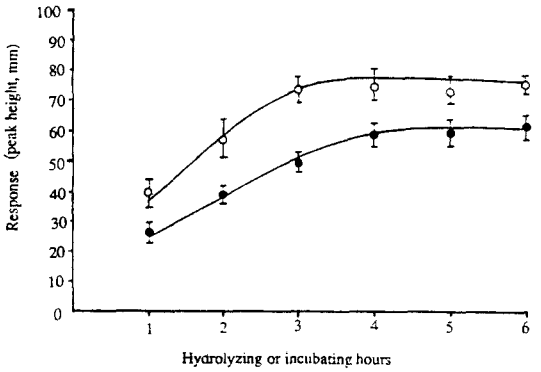

Fig. 5. The optimal conditions for hydrolysis and methyl esterification.
○ trichloroethanol (TCE); ● trichloroacetic acid methyl ester (TCA-M); The data are given as mean± SD by 3 determinations.

TRI, 1.22 μg/*l* for PER, 1.03 μg/*l* for TCE and 0.98 μg/*l* for TCA. The concentrations were relatively lower than those reported by Hajimiragha[23]. As a conclusion, our biological monitoring method using dynamic purge trap system could be applied to determine the trace level of VHH in the urine from the human who exposed at low level in domestic place. This method is expected to be used risk assessment of VHH at very trace levels.

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