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Determination of methyltin compounds in urine of occupationally exposed and general population by *in situ* ethylation and headspace SPME coupled with GC-FPD

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ABSTRACT

A method for the determination of methyltin compounds in human urine samples was developed using headspace solid-phase microextration (HS-SPME) coupled with gas chromatographic separation and flame photometric detection. Three methyltin compounds, monomethyltin (MMT), dimethyltin (DMT), and trimethyltin (TMT) were *in situ* ethylated by sodium tetraethylborate ($NaBEt_4$) for SPME and GC-FPD analysis. Under the optimized condition, the detection limits of MMT, DMT, and TMT were 8.1, 2.5 and 5.6 ng Sn L^{-1}, and the relative standard deviations were 11.0%, 7.3% and 4.0%, respectively. Methyltin compounds in thirteen urine samples from occupationally exposed population and two from general population were analyzed by the proposed method. The concentrations of total methyltin in the tested urine samples of occupationally exposed population ranged from 26.0 to 7892 ng Sn L^{-1}, and the average level is higher than those of the two non-occupationally exposed individuals. The methyltins in urine were adjusted by osmolality in order to enhance the comparability of different urine samples and the feasibility of this correction method was validated.

1. Introduction

During the past decades, organotin compounds have been used worldwide in many applications, including antifouling paints, polyvinyl chloride (PVC) stabilizer, pesticides, fungicides and wood preservatives [1]. The comprehensive use of organotin compounds has caused environmental and human health concerns due to their toxicities. Previous researches have shown that trimethyltins are neurotoxic, and tri-n-butyltin compounds can cause endocrine disruption [1,2]. Furthermore, mono- and di-substituted organotin compounds, especially monomethyltin and dimethyltin, have also been found to exert neurotoxic effects on living organisms [3–5]. Methyltin compounds are more water soluble and have lower boiling points than other organotin compounds due to their smaller organic functional groups. Methyltin compounds can be eliminated from human body through emiction.

Urine is widely used for biological monitoring to assess human exposure to toxic substances, especially for those with short biologic half-lives [6]. Urine collection is considered to be noninvasive with minimum burden for the examinees [7]. However, a major disadvantage of spot urine sampling is the variability of volume and target chemical concentrations among different samples taken at different points of time. This often means that the chemical concentrations in urine need to be adjusted. Adjustment to urine creatinine concentration is commonly used although specific gravity and osmolality have also employed [6,8,9].

Detection of organotin compounds mostly combines GC with a detector such as FPD [10–12], PFPD [13,14], MS [15,16], MIP-AED [17,18] or ICP-MS [19,20]. For environmental and biological samples, derivatization and extraction procedures are often necessary before separation and detection. Several research papers have reported the development of different derivatization and extraction methods for the detection of organotin compounds in human urine samples [21–23]. Ethylation with sodium tetraethylborate is mainly chosen for its direct derivatization character in aqueous medium. Besides the typical liquid–liquid extraction, several solvent-free or low solvent consumption sample preparation methods were developed and extensively used in organotin speciation, such as solid phase extraction (SPE) [24,25], solid phase microextraction (SPME) [12,20,26] and dispersive liquid–liquid microextraction (DLLME) [27]. SPME was used by Zachariadis and Rosenberg for the detection of butyltin and phenyltin compounds in human urine [22] although methyltin compounds were not included and water balance correction was not considered in their study.

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In this paper, a fast and sensitive headspace SPME–GC-FPD method was developed for the determination of methyltin compounds in human urine samples. The concentrations of methyltin compounds in human urine were corrected by osmolality to eliminate or reduce water balance variation and enhance the comparability of different urine samples. The corrected results give us more reliable information about methyltin level in human urine. Detection results of 15 urine samples would provide some basic but valuable information about the methyltin levels in occupationally exposed and general population.

2. Experimental

2.1. Apparatus

A Shimadzu GC-2010 gas chromatograph equipped with a split/splitless injector was used for the determination of methyltin compounds in human urine samples. The separation of three target compounds was conducted on a capillary column (Rtx-5ms, 30 m × 0.25 mm × 0.25 μm) coated with 95% methyl silicone and 5% phenyl silicone. A flame photometric detector with a tin filter was used for the qualitative and quantitative analysis. High purity He (≥99.999%) was used as carrier gas. The column flow was set at 1.6 mL min^{-1}, and the purge flow at 3.0 mL min^{-1}. The injector temperature was held at 250 °C with a 1:10 split injection mode (ensure good peak shape and signal reproducibility). The oven temperature was initially held at 40 °C for 1 min, then increased at 10 °C min^{-1} to 100 °C, followed by a ramp at 30 °C min^{-1} to the final temperature 250 °C and held for 3 min. The temperature of FPD was set at 250 °C. The air and the hydrogen flows of the detector were both set at 70 mL min^{-1}. Signal collection and process were conducted by the Shimadzu GCsolution software.

The SPME procedure was conducted by manual SPME device with a fused silica fiber coated with 75 μm polydimethylsiloxane/carboxen (PDMS/CAR) (Supelco, Bellefonte, PA, USA), which was reported to have very high sensitivity for the analysis of methyltin compounds [26].

The osmolality of urine samples were directly determined by a Vapro 5520 vapor pressure osmometer (Wscor Inc., USA). Only about 80 s was needed for one sample to measure the osmotic pressure.

2.2. Reagents and materials

Monomethyltin trichloride (MMT, 98%), dimethyltin dichloride (DMT, 98%), and trimethyltin chloride (TMT, 98.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The stock standard solutions were prepared as 1 g L^{-1} (as Sn) in methanol and stored at −20 °C in dark. Fresh working solutions of 1 mg L^{-1} (as Sn) were prepared daily by a proper dilution of the stock solution with ultra pure water.

Methanol (HPLC grade) used for preparation of stock solutions was purchased from J.T. Baker Chemicals Co., USA. Tetrahydrofuran anhydrous (THF, 99.9%, free of inhibitor) was obtained from Sigma–Aldrich. Sodium acetate (NaAc, 99%, Sigma–Aldrich) and acetic acid (HAc, 99.8%, CNW Technology GmbH, Germany) were used for buffer preparation (0.2 M, pH = 5.0). The derivatization reagent, sodium tetraethylborate ($NaBEt_4$, 98%, Strem Chemicals, USA), was prepared as 20% (m/v) stock solution in THF and stored in refrigerator. Fresh working solutions of 2% (m/v) was prepared daily with ultra pure water. Both the stock and working solutions were stored in dark brown glass vials with PTFE septum caps, which allowed for an easy transfer via a syringe without handling under inert gas.

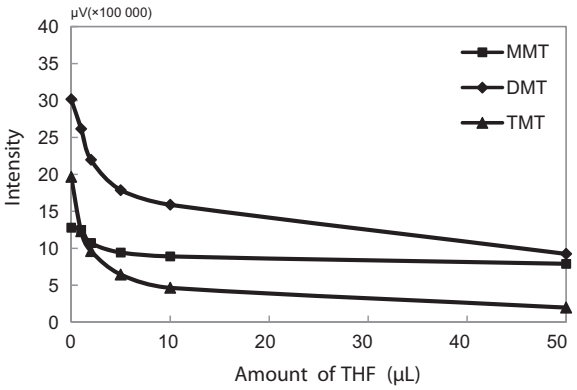

Fig. 1. Effects of amount of tetrahydrofuran on signal intensity (peak height) of MMT, DMT and TMT. The urine matrix used was spiked with methyltin species (400 ng Sn L^{-1} each).

All other reagents and solvents used in this study were of analytical grade or higher. The glassware was cleaned with deionized water, soak overnight in 50% (v/v) nitric acid solution and rinsed with ultra pure water.

2.3. SPME procedures

Urine sample (20 mL) and HAc–NaAc buffer (5 mL, 0.2 M, pH = 5.0) were transferred into a 50-mL glass vial in which a glass stir bar was added. After addition of 20 μL $NaBEt_4$ solutions (2%, m/v) into the vial, the SPME fiber was exposed to the headspace of the solution and the mixture was magnetically stirred at 900 rpm under room temperature for 15 min. The SPME fiber was immediately injected into the GC inlet for analysis after the extraction procedure.

For quantitative determination, an external standard calibration method was utilized. Pooled analyte-free urine samples (prevalidated by the proposed method.) were used as matrix. After spiking with suitable amounts of methyltin standards, the derivatization and HS-SPME procedure were performed as described above.

3. Results and discussion

3.1. Optimization of HS-SPME parameters

The optimum condition for the ethylation and headspace solid-phase microextraction mainly depends on pH of the solution, temperature, amount of derivatizing agent ($NaBEt_4$), extraction time and salt concentration. Therefore, these parameters were optimized in this study.

It has been found that sodium tetraethylborate ($NaBEt_4$) is unstable in aqueous solution, while in THF, it can be stable for more than one month [28]. The stock solution of $NaBEt_4$ has therefore been prepared in THF, and as a consequence the daily prepared aqueous working solutions also contained some amounts of THF. The volatile THF might compete with the analytes for adsorption sites of certain fiber coatings and could thus affect the extraction performance. Therefore, the effect of THF on the extraction of methyltin compounds by 75 μm PDMS/CAR fiber was studied. A solution of 2% (m/v) $NaBEt_4$ was prepared directly in ultra pure water without THF. This $NaBEt_4$ solution was used for the HS-SPME procedure, in which different volumes of THF were added. Fig. 1 shows the negative effect of THF on signal intensities of the three methyltin compounds. THF and its alkylborate derivative complexes have seemingly evaporated into the headspace and adsorbed onto the SPME fiber, which decreased the extraction efficiency of the derivatizated methyltin compounds. Less amount of

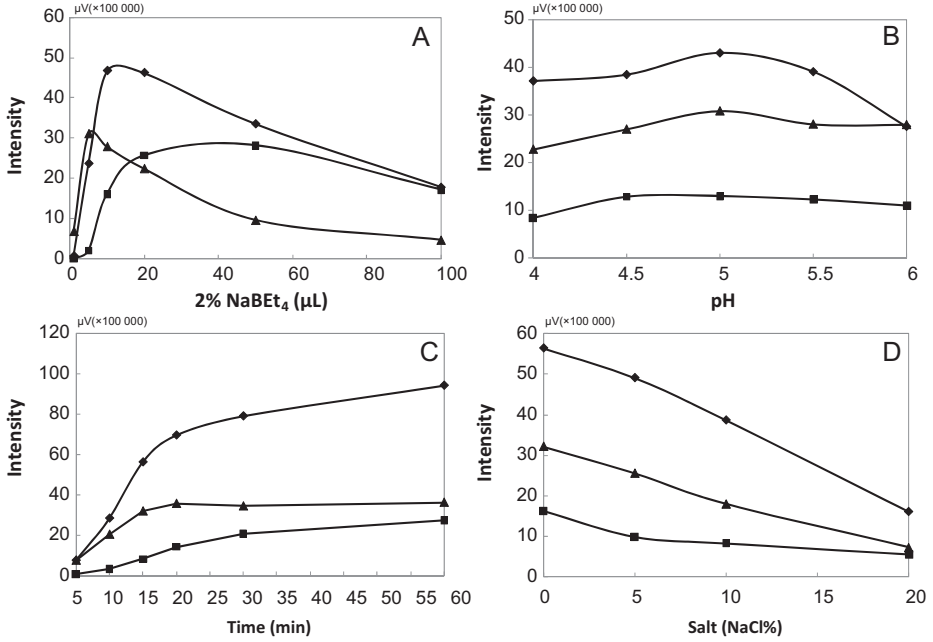

Fig. 2. Effects of amount of derivatizing reagent, pH, extraction time and salt concentration on signal intensity (peak height) of MMT (■), DMT (♦) and TMT (▲). Sample pH was adjusted by HAc–NaAc buffer in a pooled urine matrix spiked with methyltin species (500 ng Sn L^{-1} each).

THF in the reaction system would therefore lead to better results. To reduce the unavoidable negative effect, a high concentration of $NaBEt_4$ in the stock solution is necessary. Therefore, a concentration of 20% (m/v) was chosen since this approximates the solubility of $NaBEt_4$ in THF at room temperature.

The amount of the 2% $NaBEt_4$ working solution used for the derivatization reaction was optimized. According to Fig. 2A, 20 μL of $NaBEt_4$ (2%, m/v) solution was chosen so as to provide satisfactory sensitivity for all three methyltin compounds.

According to various reports, ethylation of organotin compounds by $NaBEt_4$ is favorable at a pH range from 4 to 5.3 depending on the selected buffer systems and the target organotin species [28–30]. For human urine analysis, adjustment of pH was not necessary [22] because the pH of the detected urine samples (pH = 5.2–5.6) was suitable for derivatization. However, in our investigation, the pH values of urine samples were at a relatively wider range (5.2–6.0). In order to improve the sensitivity and reproducibility, an adjustment of pH was conducted. The pH value of the urine samples was adjusted by HAc–NaAc buffer (0.2 M). As shown in Fig. 2B, the optimum pH is 5.0.

Considering the lower boiling points of the ethylated methyltin compounds (MMT, 106 °C, DMT, 93 °C, and TMT, 84 °C at 760 mmHg pressure) [26], the derivatization and extraction temperature cannot be too high. It was observed that all three analytes had satisfactory sensitivity and reproducibility at room temperature (23 ± 2 °C) which was chosen in this work.

Equilibrium extraction times for three methyltin compounds were 20 min or more. However, a significant fraction of ethylated methyltin compounds was extracted after 15 min and the reproducibility was also acceptable (Fig. 2C and Table 1). Consequently an extraction time of 15 min was selected.

Salt concentration is an important factor that affects the analytical performance. The effect of salinity was checked by adding different amounts of NaCl into the urine samples which were spiked with the same amount of standards. The result in Fig. 2D shows that the detector signals of the analytes decreased with the increase of salt concentrations. It was therefore considered that addition of salt was not necessary in the reaction system.

3.2. The performance of methyltin speciation by HS-SPME and GC-FPD

The calibration results of methyltin compounds obtained under the optimum conditions are presented in Table 1; representative chromatograms of the standards and a urine sample are shown in Fig. 3. According to the results listed in Table 1, the proposed method is very sensitive with detection limits for the three methyltin compounds at the ng Sn L^{-1} level. The detection limits of three methyltin compounds are comparable or much lower than the former reports (Table 2) [31–35] although a split (1:10) mode was used in this work which sacrificed parts of the sensitivity. As shown in Fig. 3, the separation of three methyltin compounds is complete within 7 min. The total analytical time for one sample is less than 30 min.

As a short summary, the HS-SPME coupled with GC-FPD provides a rapid, simple and sensitive method for the analysis of methyltin compounds in human urine. This method has the poten-

Table 1
Analytical performance for the determination of methyltin compounds in human urine samples by HS-SPME–GC-FPD.

Species	Concentration range (ng Sn L^{-1})	Correlation coefficient, r	LOD[a] (ng Sn L^{-1})	RSD[b] (%)	Recovery[c] (%)
MMT	50–10,000	0.998	8.1	11.0	116
DMT	10–10,000	0.997	2.5	7.3	108
TMT	20–10,000	0.995	5.6	4.0	97

[a] Limit of detection, the concentration of three times S/N.
[b] Relative standard deviation, $n = 5$.
[c] Mean value, $n = 5$.

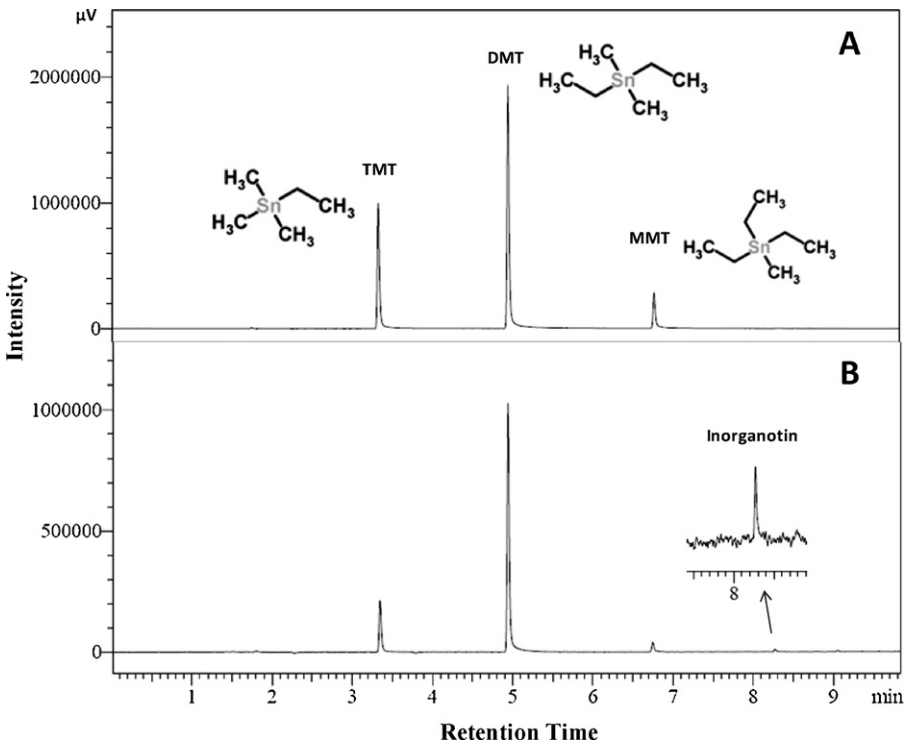

Fig. 3. Typical chromatograms of the ethylated methyltin compounds (chemical structures were also shown.) obtained by *in situ* ethylation and HS-SPME–GC-FPD. (A) Blank urine matrix spiked with methyltin standards in amounts of 200 ng Sn L^{-1} for each species. (B) Human urine sample No. 11.

Table 2
Detection limits of the proposed method compared to several former reports.

Ref.	Method	LOD (ng Sn L^{-1})		
		MMT	DMT	TMT
[31]	SPME–GC-FPD[a]	12	10	11
[32,33]	PTI–GC-FPD	18	12	3
[34]	HPLC–ICP/MS	266	95	39
[35]	HPLC-FPD	1.69×10^3	0.51×10^3	0.36×10^3
This work	SPME–GC-FPD	8.1	2.5	5.6

[a] Use PDMS (100 μm) fiber.

tial to be an effective method to evaluate environmental and occupational exposure to methyltin compounds.

3.3. Correction of urine methyltin concentration by osmolality

The results of urinalysis may be of great variation due to differences in water balance, dilution or dehydration [36]. As discussed above, creatinine concentration, specific gravity and osmolality were commonly used to adjust the fluid balance. Osmolality indicates the apparent molality of the solution. It is considered an index of osmotic pressure and can be easily and rapidly determined by an osmometer with minimal labor. This method was therefore chosen to adjust the concentrations of methyltin compounds in human urine samples. Although it has already successfully been used in previous studies for the correction of lead, mercury and other metal compounds [36,37], to our knowledge, this study is the first to use osmolality to correct urine methyltin level.

To demonstrate the feasibility of osmolality as a correction parameter, three urine samples were collected during a period of 12 h from one male volunteer at different physiological status. Sample A with dark yellow color was the first-morning void collected just after the volunteer woke up. Sample B was acquired in a normal physiological state in the morning, and the color was yellow. Sample C was obtained about half an hour after the volunteer purposely

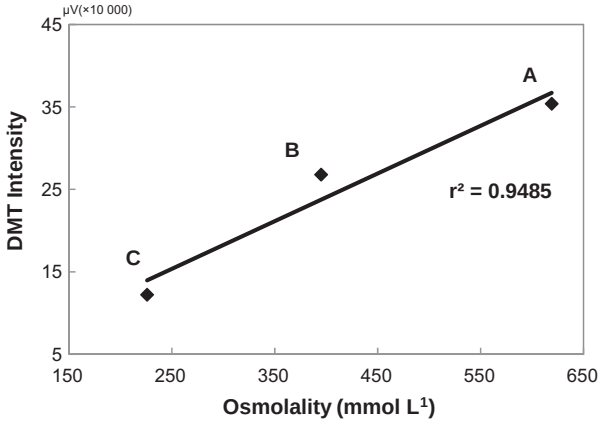

Fig. 4. The linear correlation between intensity of DMT and osmolality. Urine samples (A–C) were collected from a volunteer during 12 h.

drank a large volume of water (about 1.2 L) in the afternoon and the sample color was light yellow. The methyltin concentrations and the osmolalities of these samples were determined immediately after collection. Only DMT were detected in all the three samples. Fig. 4 shows a good linearity between DMT intensity and osmolality, indicating that the osmolality is a satisfactory correction parameter. The osmolality adjustment might further be extended to the determination of other organometal compounds in human urine samples.

3.4. Methyltin compounds in human urine

The proposed method was successfully used in the determination of methyltin compounds in human urine. Fifteen urine samples were collected and immediately analyzed within 24 h. The urine samples must be analyzed as soon as possible after collection because it was found in our preliminary study that the results might

Table 3
Concentrations of methyltin compounds in human urine samples. Sample Nos. 1–13 were collected from occupationally exposed individuals; sample Nos. 14 and 15 were collected from general population.

Sample	Concentration[a] (ng Sn L^{-1})				Osmolality[b] (mmol L^{-1})	Calibrated total MT (ng Sn $mmol^{-1}$)	Inorganotin
	TMT	DMT	MMT	Total MT			
1	137.8	998.5	D.[c]	1136	992	1.15	+[e]
2	416.4	920.0	D.	1336	588	2.27	+
3	30.0	297.4	N.D.[d]	327.4	262	1.25	+
4	1277	6353	261.9	7892	1041	7.58	+
5	178.6	415.4	68.9	662.9	462	1.43	+
6	128.4	977.5	78.0	1184	274	4.32	+
7	900.1	3133	134.9	4033	1065	3.79	+
8	460.4	3187	103.4	3647	943	3.87	+
9	22.1	106.4	N.D.	128.6	346	0.37	+
10	D.	101.1	N.D.	101.1	748	0.14	+
11	44.5	113.8	D.	158.3	188	0.84	+
12	46.1	183.8	N.D.	229.9	387	0.59	+
13	N.D.	26.0	N.D.	26.0	433	0.06	–
14	N.D.	N.D.	N.D.	N.D.	619	N.D.	–
15	102.2	154.7	N.D.	256.9	536	0.48	+

[a] Mean value, $n = 2–4$ depending on the urine volume.
[b] Mean value, $n = 5$.
[c] Detected but lower than the limit of quantification (the concentration of ten times S/N).
[d] Non-detectable.
[e] +/–: species detected but not quantified/not detected.

vary after 48 h even as the samples were stored in refrigerator at 4 °C. Among the fifteen samples, thirteen were from occupationally exposed subjects who worked in the same organotin production plant, and the other two are from randomly selected individuals of the general population.

The analytical results are shown in Table 3. DMT was detected in all the urine samples of the occupationally exposed volunteers and the concentrations ranged from 26.0 to 6353 ng Sn L^{-1}. This result is corresponding to the fact that the main products of the plant are DMT compounds. The high toxic TMT was also detected in almost all the samples except for No. 13, and the concentrations ranged from lower than the limit of quantification to 1277 ng Sn L^{-1}. This should be seriously taken into consideration for the health risk assessment of the occupational workers because the reported acute toxicity of TMT is much higher than DMT (The LC_{50} values for TMTC and DMTC on *Artemia franciscana* were 0.22 and 80.7 mg Sn L^{-1}, respectively.) [38]. Considering DMT is the main substance the workers exposed to, the detection of high level TMT in the urine samples indicate that the methylation of DMT might possibly occurred in human bodies which is in accord with the findings in mice and rats [39]. While due to lacking of data, further investigations are needed to confirm the illation. MMT was also detected in several samples with the highest concentration at 261.9 ng Sn L^{-1}. The source of MMT might possibly be the byproducts or the degradation products of DMT. As to the two volunteers of general population, different results were observed. None of the methyltin species was detected in sample No. 14, while DMT and TMT were detected in sample No. 15.

The total methyltin compounds in tested urine samples were calculated, and then corrected by osmolality. Effects from this correction were observed, e.g. the uncorrected total methyltin of sample No. 3 was lower than that of sample No. 1 while an opposite trend was found after correction. The corrected results might be much more reliable for each individual and also much more comparable among different individuals than the uncorrected results. Seen from the results of the corrected total methyltin, it is obvious that the MT level of occupationally exposed population is markedly higher than that of general population. The results of occupationally exposed population are also work-related: the sample Nos. 1–8 were collected from workers who worked on the process lines and thus exposed to the MT compounds extensively, while the sample Nos. 9–13 were collected from individuals who worked as technicians, supervisors or maintenance people. The MT levels detected in the former group are obviously higher than those in the latter.

Because of the non-selectivity for tin compounds, other tin species could also be detected by the proposed method. Besides the three methyltin compounds, a peak corresponding to inorganic tin also appeared in the chromatograms of several urine samples (see Fig. 3). Inorganic tin was detected (but not quantified) in all urine samples except for sample Nos. 13 and 14.

4. Conclusions

Urine is an excellent bio-indicator for monitoring occupational and environmental exposure to organic and inorganic pollutants. Up to date, only a few papers have reported the detection of organotin compounds in human urine samples. The proposed method, using headspace solid-phase microextraction coupled with gas chromatography and flame photometric detection, was found to be of merit for the detection of methyltin compounds in urine samples. Under the optimum condition, the detection limits of MMT, DMT, and TMT were 8.1, 2.5 and 5.6 ng Sn L^{-1}, and the relative standard deviations were 11.0%, 7.3% and 4.0%, respectively. Correction of methyltin levels in human urine by osmolality was conducted and the feasibility was validated by the good correlation between dimethyltin level and osmolality in urine samples from a volunteer. The corrected total methyltin concentrations of the thirteen urine samples from occupationally exposed subjects ranged from 0.06 to 7.58 ng Sn $mmol^{-1}$, and the average level is higher than that of the non-occupationally exposed individuals. The potential health risks of methyltin compounds to occupationally exposed and general population should be concerned.

Acknowledgements

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