Talanta 85 (2011) 1028–1033

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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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ARTICLE INFO

Article history:
Received 4 April 2011
Accepted 6 May 2011
Available online 13 May 2011

Keywords:
Methyltin
Urine
Headspace solid-phase microextraction
Gas chromatography-flame photometric detection
Occupationally exposed population

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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doi:10.1016/j.talanta.2011.05.018

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.

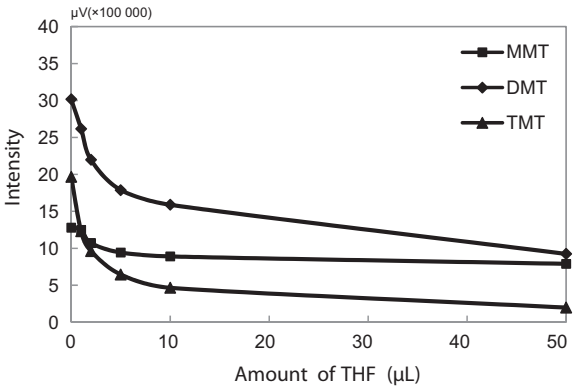

Fig. 1. Effects of amount of tetrahydrofuran on signal intensity (peak height) of MMT, DMT and TMT. The urine matrix used was spiked with methyltin species (400 ng Sn L^{-1} each).

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