Talanta 125 (2014) 196–203

Contents lists available at ScienceDirect

Talanta

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

Speciation analysis of organotin compounds in human urine by headspace solid-phase micro-extraction and gas chromatography with pulsed flame photometric detection

Aníbal Valenzuela [a,1], Gaëtane Lespes [b], Waldo Quiroz [a], Luis F. Aguilar [c], Manuel A. Bravo [a,*,1]

[a] Laboratorio de Química Analítica y Ambiental, Instituto de Química, Pontificia Universidad Católica de Valparaíso, Avenida Universidad 330, Curauma, Valparaíso, Chile
[b] Laboratoire de Chimie Analytique Bio Inorganique et Environment, IPREM, Université de Pau et des Pays de l'Adour, Avenue de l'Université, 64013 Pau, France
[c] Laboratorio de Fotofísica y espectroscopia molecular, Instituto de Química, Pontificia Universidad Católica de Valparaíso, Avenida Brasil 2950, Valparaíso, Chile

ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form
20 February 2014
Accepted 21 February 2014
Available online 28 February 2014

Keywords:
Organotin speciation
Headspace solid-phase micro-extraction (HS-SPME)
Human urine
Storage conditions
Gas chromatography
Pulsed flame photometric detection (GC-PFPD)

ABSTRACT

A new headspace solid-phase micro-extraction (HS-SPME) method followed by gas chromatography with pulsed flame photometric detection (GC-PFPD) analysis has been developed for the simultaneous determination of 11 organotin compounds, including methyl-, butyl-, phenyl- and octyltin derivates, in human urine. The methodology has been validated by the analysis of urine samples fortified with all analytes at different concentration levels, and recovery rates above 87% and relative precisions between 2% and 7% were obtained. Additionally, an experimental-design approach has been used to model the storage stability of organotin compounds in human urine, demonstrating that organotins are highly degraded in this medium, although their stability is satisfactory during the first 4 days of storage at 4 °C and pH=4. Finally, this methodology was applied to urine samples collected from harbor workers exposed to antifouling paints; methyl- and butyltins were detected, confirming human exposure in this type of work environment.

1. Introduction

The high toxicity of organotin compounds (OTCs) on aquatic organisms is widely recognized. Toxic effects have been observed at sub ng Sn L^{-1} concentrations [1,2]. Some studies have reported that OTCs such as butyl-, phenyl-, and methyltin derivatives, are potential immunotoxicants and endocrine disruptors in mammals and lead to neurological damage and anomalies in tissue and organs [3,4]. For humans, OTC can affect the central nervous system producing headache, nausea, vomiting, dizziness, and sometimes convulsions and loss of consciousness [5,6]. Moreover, OTC can irritate the eyes, respiratory tract and skin, and some organotins can cause cerebral edema and cardiovascular effects [2]. Recent studies have implicated OTC in human obesity due to their actions on the endocrine system [7,8].

Organotin compounds have been used principally as active components in anti-fouling paints, wood preservation materials and PVC stabilizers, allowing OTC to enter aquatic and terrestrial environments [6]. As a result, OTC are ubiquitous in the environment and are commonly present in water [9], sediments [10,11], seafood [12], textiles [13], plastic [14] and house dust [15]. According to the Agency for Toxic Substances and Disease, humans can be exposed to OTC by eating seafood from coastal waters or from contact with household products, such as polyurethane, plastic polymers and silicon-coated baking parchment, that contain organotins [16]. The occurrence of organotins in human blood [17], urine [18], liver and human breast milk [19] provides direct evidence of human exposure.

To assess the impact of organotin compounds on humans, reliable and selective analytical methodologies are necessary to quantify these compounds in human samples such as urine and blood. Several analytical methodologies have been proposed for OTC determination in environmental samples, such as sediments, natural waters and biological samples [20–22]. However, the

* Corresponding author. Tel.: +56 32 2274916; fax: +56 32 2274939.
E-mail address: manuel.bravo@ucv.cl (M.A. Bravo).
[1] These authors contributed equally to the manuscript.

http://dx.doi.org/10.1016/j.talanta.2014.02.054
0039-9140

number of methodologies developed to assess OTC levels in human samples, such as urine, is scarce, and no systematic evaluation of organotin stability during storage has been conducted in these types of samples.

In general, tin speciation analysis is performed using chromatographic separation techniques, principally gas chromatography, coupled to sensitive and selective detection methods. The most frequently used detectors for OTC determination are mass spectrometers (MS) [23,24], inductively coupled plasma-mass spectrometers (ICP-MS) [25], flame photometric detectors (FPD) [26] and pulsed flame photometric detectors (PFPD) [27,28]. According to the literature, ICP-MS is the most selective and sensitive detector, followed by PFPD and MIP-AED. Furthermore, the use of preconcentration techniques, such as Liquid–Liquid Extraction (LLE) [29] and Solid-Phase Microextraction (SPME) [30,31], reduce detection limits and enable common concentration levels in environmental samples to be evaluated.

The aim of the present work is to develop an analytical methodology for organotin determination, including methyl-, butyl-, phenyl-, and octyltins, in human urine using HS-SPME followed by GC-PFPD to systematically investigate the optimization of the storage conditions for organotin determination in human urine samples using an experimental design methodology. To the best of our knowledge, no similar studies have been carried out for tin speciation analysis in this sample type.

2. Experimental

2.1. Instrumentation and materials

A Varian 3800 Gas Chromatograph (Walnut Creek, USA) equipped with a PFPD detector system and a Varian 1079 split/splitless with a temperature-programmable injector was used. The conditions for chromatographic separation have been reported previously [32]. Briefly, the separation was performed on a DB-1 fused-silica capillary column (30 m × 0.32 mm i.d.) coated with 100% polidimethylsiloxane (0.25 μm film thickness), using nitrogen as the carrier gas at a flow rate of 2.0 mL min^{-1}. The temperature program applied for OTC separation was: the oven temperature was initially held at 50 °C for 0.5 min; it was then programmed to increase at 10 °C/min to 200 °C and at 30 °C/min to the final temperature of 290 °C, at which it was held for 4 min. The splitless injector port was kept at 307 °C, and the temperature of the PFPD detector was 350 °C. A high-transmission band filter (λ: 320–540 nm; BG 12, Schott, France) was selected to measure the signal corresponding to Sn–C emission, and a gate delay of 4.0 ms and a gate width of 2.0 ms was employed.

The manual SPME holder and fibers were obtained from Supelco (Bellefonte, USA). The SPME fiber coating used in this study was carboxen-polydimetilsiloxane (CAR/PDMS, 75 μm thickness). The fiber was conditioned prior to use by heating the injector system of the chromatographic system according to the conditions recommended by the manufacturer.

For the derivatization/extraction procedure, 70-mL glass reaction vials closed with polytetrafluoroethylene (PTFE)-coated silicone rubber septa (Supelco, Bellefonte, PA, USA) were used. Mechanical shaking during this procedure was performed using a mechanical elliptical table (Prolabo, KS 2502 basic, France).

Filtration of urine samples was performed using a membrane of cellulose ester (0.20 μm and 0.45 μm, Millipore).

2.2. Reagents and standards

Monomethyltin trichloride (MMT, 97%), dimethyltin dichloride (DMT, 97%) and trimethyltin chloride (TMT, 97%), were obtained from Sigma-Aldrich (Germany). Monobutyltin trichloride (MBT, 95%), dibutyltin dichloride (DBT, 96%), tributyltin chloride (TBT, 96%), monophenyltin trichloride (MPhT, 98%), diphenyltin dichloride (DPhT, 96%), triphenyltin chloride (TPhT, 95%), tripropyltin chloride (TPrT, 98%) and diheptyltin dichloride (DHepT, 98%) were obtained from Strem Chemicals (France). Monooctyltin trichloride (MOcT, 97%) and dioctyltin dichloride (DOcT, 97%) were obtained from Lancaster (France). Stock solutions of these reagents (1000 mg L^{-1} of Sn) were prepared in methanol and stored at −20 °C in the dark. Working standards were prepared by dilution with deionized water weekly for solutions of 1–10 mg Sn L^{-1} and daily for 1–100 μg Sn L^{-1}. These working standards were stored at 4 °C in the dark.

High-quality water (18 MΩ cm) obtained from a Millipore system (Millipore, Bedford, MA, USA) was used to prepare solutions throughout the study. Sodium tetraethylborate ($NaBEt_4$) was obtained from Galab (Germany), and an aqueous solution of this reagent (1%, w/v) was prepared immediately prior to analysis and stored at 4 °C in the dark. Acetic acid, sodium hydroxide, methanol, nitric acid and hydrochloric acid were obtained from Merck (Darmstadt, Germany).

Creatinine in urine samples was determined using a spectrophotometric system, with picrate and buffer, using a commercial kit (Kinetic Creatinine Kit, Valtek diagnostics, Chile).

All glassware and plastic materials used in this study were rinsed with deionized water, decontaminated overnight in 20% (v/v) nitric acid solution and then rinsed again with deionized water.

2.3. Derivatization and headspace solid-phase micro-extraction procedure

For the analysis of human urine, an aliquot of 5–20 mL of urine sample (unfiltered or filtered) was directly introduced into an 80-mL derivatization reactor containing 10 mL of acetic/acetate buffer (0.5 mol L^{-1}; pH 4.8). Subsequently, 50 μL of 1% m/v $NaBEt_4$ solution was added to the reactor vial, and the mixture was stirred for 10 min at 420 rpm to reach equilibrium. Next, the SPME fiber was exposed to the headspace volume, and the mixture was stirred again for 40 min at the same agitation rate, according to the optimized conditions previously reported by our research group [31]. The SPME fiber was then directly introduced into the GC-PFPD injector port, where the analytes were thermally desorbed for 5 min.

2.4. Analytical performance evaluation

Tripropyltin (TPrT) and diheptyltin (DHepT) were used as internal standards (I.S) for the quantification of OTC, as previously reported [33]. For figures of merit evaluation, a pooled urine sample was prepared with samples collected from some volunteers and the absence of organotins was verified before analysis. Analytical performance parameters, such as the limit of detection (LOD), limit of quantification (LOQ) and precision (relative standard deviation, % RSD), were calculated according to IUPAC recommendations [27,28] using standard addition from 0.05 ng to 100 ng (Sn) depending of each OTC.

Recovery assays were carried out by spiking the urine samples with all organotins. For this purpose, Milli-Q water, unfiltered and filtered urine sample (0.45 μm) were fortified with OTC concentrations ranging from 7.5 to 250 ng Sn L^{-1}, depending on the analyte sensitivity.

2.5. Stability study of OTC in human urine

To study the stability of OTC in urine samples and to evaluate the possible degradation of these compounds the chemometric

approach of experimental design was used to optimize the storage conditions for organotin speciation in this type of sample. A Doehlert design was selected, and the time of storage (X1: days) and pH (X2) were the two variables/factors studied. Optimization was carried out by evaluating the response surface. All experiments were performed in duplicate to ensure the reliability of the results.

Urine samples were collected from different volunteers and mixed to obtain a pooled analyte-free sample. One portion of the sample was fortified with mono-substituted OTC (portion 1), another portion was fortified with di-substituted OTC (portion 2), and the last portion was fortified with tri-substituted OTC (portion 3) in concentrations ranging from 7.5 to 250 ng Sn L^{-1}, depending on each OTC. In addition, each spiked portion of urine sample was sub-divided into three portions with different pHs, which were adjusted with diluted HCl, according to the experimental design (pH=1.5; pH=3.5; pH=5.5). Samples with pH=5.5 were adjusted with acetic acid/acetate buffer (0.5 mol L^{-1}). All portions of urine samples were kept at +4 °C until analysis.

Validation of the modeling and optimization of storage conditions were evaluated by analyzing two spiked urine samples fortified with tri-substituted OTC at two different concentration levels (low level: 5–7.5 ng Sn L^{-1}; high level: 20–30 ng Sn L^{-1}). Sample analyses were performed throughout the 36 days of study.

The Statgraphics Centurion XV software package was used for statistical and mathematical calculations, providing a flexible step-by-step approach.

2.6. Kinetic degradation study

A pooled OTC-free urine sample, as detailed above, was divided into three portions that were spiked with tri-, di-, and mono-organotins, respectively, and stored at 4 °C until analysis. They were further sub-divided into three portions at the same pH levels mentioned above. Monitoring of each OTC concentration was performed throughout the 36 days of the study using standard addition from 0.05 ng to 100 ng (Sn), depending on each OTC.

2.7. Analysis of organotin speciation in human urine samples

To validate the analytical methodology, spiked urine samples were analyzed. For this experiment, three different samples (HU1, HU2 and HU3) were spiked with all OTC at two concentrations levels (8–100 ng Sn L^{-1} and 15–200 ng Sn L^{-1}) and stored at 4 °C in the dark for 4 days before analysis. These samples were previously analyzed to demonstrate that they were OTC free.

In addition, six urine samples were collected from occupationally exposed volunteers who worked in a harbor in the Valparaiso region of Chile, where ship repairing and painting activities are commonly carried out. The samples were collected in clean polyethylene flasks, which are commonly used in clinical analysis, and immediately transported at 4 °C to our laboratory, where they underwent pH adjustment and were stored at 4 °C until analysis (for 4 days).

For each urine sample, the derivatization and HS-SPME procedure described above was carried out. The relative chromatographic areas (analyte/internal standard area-ratio) were evaluated, and the organotin concentrations were determined using the method of standard addition. All analyses were run in triplicate.

The concentration of organotins in human urine was also corrected for the creatinine content in the samples, as this is a recommended correction parameter for urine dilution [34]. For this purpose, urine-creatinine levels (expressed as g creatinine L^{-1}) were determined using a commercial kit (Valtek diagnostics, Chile) with spectrophotometric detection at 510 nm. To adjust the organotin concentrations for creatinine, the corrected concentrations have been expressed as ng Sn/g creatinine in each urine sample.

3. Results and discussion

3.1. Evaluation of figures of merit for OTC speciation in human urine

To evaluate matrix effects with HS-SPME-GC-PFPD for OTC speciation in human urine, calibration curves in Milli-Q water and unfiltered and filtered pooled urine (0.45 μm) were obtained. In the case of urine matrix (filtered and unfiltered), the slopes of the calibration curves were statistically lower than those in Milli-Q water (Student's t-test; $\alpha=0.05$). The SPME extraction is sensitive to matrix variations and several factors such as pH, salt concentrations, compounds in excess or organic solvents may affect analyte recovery and reproductibility [35]. Besides, urine is a highly variable matrix and liquid intake could cause variations in urine ionic strength and pH. However, as previous results suggest, the mixing of this matrix with a buffer and an internal standard prior to SPME procedure allows to overcome these difficulties [31,33].

For solid SPME-coatings as Carboxen-PDMS a competitive adsorption process occurs and the matrix composition affects the amount of analyte extracted by the fiber [36]. Then, the extraction of volatile compounds commonly present in human urine such alcohols, drugs, ketones, amines or sulfur compounds could affect the OTC-mass extracted by the SPME fiber. However, even if a significant matrix effect is detected, the chromatographic separation is not affected. Thus, a typical chromatogram is obtained without interferences and without overlapping signals, a result that is likely due to the adequate selectivity of the PFPD detector. Finally, OTC quantification in this type of sample has been performed using the standard addition method to ensure reliable results.

The figures of merit, limits of detection, limits of quantification and precision values (expressed as % RSD) were evaluated

Table 1
Analytical performance of the HS-SPME-GC-PFPD methodology for organotin speciation in human urine.

Compound	Detection limit (ng Sn L^{-1})	Quantification limit (ng Sn L^{-1})	Linear range(ng Sn L^{-1})	Determination coefficient (R^2)	Repeatability (RSD, %)
MMT	0.5	1.7	L.O.Q–180	0.9990	2
DMT	0.8	2.5	L.O.Q–180	0.9988	7
TMT	0.8	2.7	L.O.Q–170	0.9991	4
MBT	0.9	2.8	L.O.Q–40	0.9999	3
DBT	1.0	3.3	L.O.Q–40	0.9981	6
TBT	0.8	2.8	L.O.Q–50	0.9997	3
MPhT	1.9	6.3	L.O.Q–50	0.9999	6
DPhT	2.7	9.0	L.O.Q–600	0.9991	2
TPhT	4.9	16	L.O.Q–600	0.9988	6
MOcT	2.5	8.3	L.O.Q–600	0.9997	5
DOcT	4.5	15	L.O.Q–150	0.9980	2

Table 2
Recovery assays for analysis of filtered urine, unfiltered urine and Milli-Q water spiked with all organotin compounds.

Organotin	Spiked value (ng Sn L^{-1})	Filtered urine (0.45 μm)		Unfiltered urine		Mili-Q water	
		Value found (ng Sn L^{-1})	Recovery (%)	Value found (ng Sn L^{-1})	Recovery (%)	Value found (ng Sn L^{-1})	Recovery (%)
MMT	25	22.3 ± 0.9	89 ± 4	22.1 ± 0.7	87 ± 2	22.2 ± 0.8	89 ± 3
DMT	25	22 ± 1	87 ± 3	23.0 ± 0.8	91 ± 3	22.8 ± 0.7	91 ± 3
TMT	25	22 ± 2	87 ± 3	22 ± 1	89 ± 4	22 ± 0.5	89 ± 2
MBT	7.5	7.2 ± 0.2	96 ± 3	7.3 ± 0.5	97 ± 6	7.4 ± 0.3	98 ± 4
DBT	7.5	7.1 ± 0.3	94 ± 4	7.1 ± 0.5	94 ± 1	7.4 ± 0.2	99 ± 3
TBT	7.5	7.0 ± 0.2	93 ± 2	7.0 ± 0.2	93 ± 2	7.2 ± 0.3	97 ± 4
MPhT	15	14.7 ± 0.7	97 ± 4	14.3 ± 0.5	95 ± 3	13.9 ± 0.5	93 ± 3
DPhT	200	186 ± 9	93 ± 4	186 ± 4	93 ± 2	194 ± 5	97 ± 3
TPhT	250	235 ± 12	94 ± 5	235 ± 8	94 ± 3	240 ± 8	96 ± 3
MOcT	10	8.9 ± 0.5	89 ± 5	8.9 ± 0.1	89 ± 1	10.4 ± 0.7	104 ± 7
DOcT	200	174 ± 7	87 ± 3	176 ± 6	88 ± 3	184 ± 8	92 ± 4

according IUPAC criteria, and the results are presented in Table 1. Good linearity was obtained for all organotins, with determination coefficients (R^2) ranging from 0.9980 to 0.9999. Similarly, the precision values reached from 2% to 7%, satisfactory for the ultra-trace determination of OTC in this complex matrix. Clearly, as reported in previous work [27], the use of two internal standards (I.S.), such as tripropyltin (TPrT) and diheptyltin (DHepT), is necessary for the simultaneous determination of volatile and semi-volatile OTCs. Additionally, DHepT improved the repeatability for octyltins (which were not evaluated previously), showing that the use of this I.S. improves precision for less volatile organotins.

The detection limits obtained in urine samples in this study are similar to those reported in previous work using different environmental matrices [31]. Compared to other studies [5–7] in which similar detector systems and SPME procedures are used, the values obtained are considerably lower and allow adequate monitoring of these compounds in this human fluid at ultra-trace levels.

Due to the lack of urine samples with certified concentrations of organotins, recovery assays have been carried out to evaluate the accuracy of the developed methodology. Table 2 shows the recovery rate for each OTC in unfiltered urine, filtered urine (<0.45 μm) and Milli-Q water spiked with all of the organotins. The concentrations levels indicated by these results are statistically similar to the spiked concentrations (p value <0.05), reaching recovery rates that range from 87% to 104%. However, even in an aqueous synthetic medium (Milli-Q water), methyltins display lower recovery rates compared to other OTC. Given the lowest boiling points of methyltins, the low recovery rates may be attributed to their loss during the HS-SPME procedure. More volatile internal standards, such as monoheptyltin and tetrapropyltin, could be evaluated to correct this inconvenience. However, our previous work has shown that no improvements are obtained when different internal standards are used [31]. Finally, the recovery rates are statistically similar for filtered and unfiltered urine samples, such that subsequent measurements and determinations were made in unfiltered urine (to minimize the number of steps in the analysis).

3.2. Stability of OTC in human urine

Sample preservation and integrity is one of the main considerations in chemical speciation analysis [37,38]. However, there have been no comprehensive studies pertaining to the stability of OTC in fluids or biological matrices such as human urine. According to the literature, many parameters can affect or alter the initial sample composition and allow the degradation of different organotins in biological or environmental matrices.

3.2.1. Factors affecting the stability of OTC in human urine samples

Preliminarily, the effect of temperature on OTC stability was evaluated. First, the spiked urine sample was frozen at −20 °C, after which it was thawed at room-temperature for several hours. After several hours, an insoluble amber-yellow material, persistent even after sample sonication, was observed. When the effect of frozen-thawed urine on the stability of organotin was evaluated, only 60% of the spiked amount was recovered. A possible explanation for this result is that precipitation and/or adsorption occurred during the frozen storage of the urine. As a result, the temperature selected for the stability study of organotin compounds was 4 °C.

The other variables evaluated in OTC stability studies (at 4 °C) were pH and storage time. This preliminary evaluation showed significant degradation of OTC, especially in the case of tri-substituted compounds, when the sample was stored in acidic media (pH < 3.5). Previous studies indicated that the use of mineral acids may reduce degradation by controlling microbiological activity in urine samples [39]. However, in our case, HCl is not suitable for this purpose because acidification at pH = 1.5 promotes rapid degradation of OTC. According to the literature, this effect can be attributed to the presence of mineral acid(s), such as HCl or HNO_3, that produce chemical cleavage of the Sn–C bond [6]. The storage time also significantly affects the stability of organotin compounds. The degradation ratios of OTC ranged from 18% to 54%, depending of the compound, and tri-substituted compounds showed the highest degradation rates. For example, TMT and TPhT showed 54% and 51% degradation, respectively, in acidified urine during the 36 days of storage. The action of a biotic process (i.e., enzymatic reactions) could explain OTC degradation during storage as observed in several environmental samples [40].

3.2.2. Modeling OTC stability in human urine samples

The stability of organotin compounds has been studied by modeling concentration changes in human urine over time. Empirical models based on quadratic polynomials, obtained using a Doehlert experimental design, have been proposed. The response selected for this study corresponds to the relative concentration of each OTC, and it is expressed as follows:

$$[\mathrm{OTC}]_{t,\%} = \frac{[\mathrm{OTC}]_t}{[\mathrm{OTC}]_{t=0}} 100 \quad (1)$$

where $[\mathrm{OTC}]_{t,\%}$ is the relative OTC concentration at time t; $[\mathrm{OTC}]_t$ is the OTC concentration at time t; and $[\mathrm{OTC}]_{t=0}$ is the initial OTC concentration at $t=0$ days.

The results obtained using this mathematical model are presented in Table 3, which shows the effect of each factor and the statistical parameters for model validation. As shown by these results, the mathematical model presents an adequate fit with experimental data ($R^2 > 0.97$). In addition, the residue analysis

Table 3
Estimated effects of storage conditions on OTC stability and optimal storage conditions.

OTC	Estimated effects							Optimal conditions	
	Time (d)	pH	Time × Time	pH × pH	Time × pH	Average	R^2 modeling	Time (d)	pH
TMT	−15.60	7.13	4.23[a]	−0.89[a]	4.53[a]	80.39	0.9997	<4.0	4.10
TBT	−15.77	6.74	0.96[a]	−7.92	3.06	83.46	0.9968	<4.3	3.92
TPhT	−17.17	3.21	−1.38[a]	−9.58	4.99[a]	86.41	0.9972	<4.2	3.55
Deseability								<4.4	4.02
DMT	−15.48	1.79	−0.67[a]	−5.27	3.83	86.82	0.9978	<3.9	3.85
DBT	−14.48	9.51	−4.93	−3.49[a]	3.89	88.45	0.9969	<4.0	3.56
DPhT	−16.59	3.42[a]	6.98	9.68	0.41[a]	74.30	0.9729	<4.0	4.30
DOcT	−9.10	5.97	0.73	−12.98	3.11	90.27	0.9998	<4.5	3.81
Deseability								<4.0	4.08
MMT	−15.37	8.36	4.18[a]	3.77[a]	0.55[a]	79.68	0.9952	<5.1	4.60
MBT	−13.67	8.14	3.825[a]	−7.54	3.56[a]	82.56	0.9998	<5.1	3.78
MPhT	−12.675	2.04[a]	1.16[a]	−7.26[a]	0.79[a]	87.67	0.9876	<5.3	3.86
MOcT	−11.43	−1.49[a]	4.52[a]	0.56[a]	−6.99	84.735	0.9936	<4.9	4.60
Deseability								<5.1	3.83

[a] Non-significative.

indicates no autocorrelation or random distribution (Durbin–Watson test: $p<0.05$), validating the assumptions of the least-squares method. Storage time is shown to have a significant negative effect on the preservation of all organotins, while pH presents a less significant effect (positive effect). Moreover, a quadratic relationship between the response and the pH can be observed, suggesting a decrease in organotin stability, as indicated in the previous section. According to the models, the optimal response (maximum response for all OTC: 98.8–101.8%) is obtained during the first 4 days, when the sample is stored at pH of 3.6–4.6. Using the desirability function for all organotins, the maximal response (D: 0.98–1.0%) is obtained during the first 4 days at pH 4.0.

To validate the stability model for OTC in human urine, the quality of prediction was evaluated by analyzing an independent set of samples. For this purpose, two independent urine samples were spiked with organotins (urine sample A: low level; and urine sample B: low and high levels) and analyzed at different storage times, and the results were compared with values predicted by a previously validated model. Because tri-substituted organotins were the fastest degradable species, only TMT, TBT and TPhT were included in this validation step. Fig. 1 shows the typical variations in tri-organotin concentrations (expressed as recovered concentration with respect to initial spiked concentration) at different storage times, as calculated using the model and obtained via experimental analysis of spiked urine samples. The model satisfactorily predicts the concentrations of selected organotins under normal storage conditions. Furthermore, with regard to urine sample B, the results show that for every tri-substituted organotin, the duration of degradation does not depend of the concentration level in the studied range.

Finally, the results show that modeling satisfactorily evaluates and predicts the degradation rates that can occur in human urine samples during 36 days of storage. Based on this model, OTC speciation analysis in human urine can be performed during the first 4 days (stored at 4 °C and pH=4) with a 5% degradation rate, which is considered satisfactory.

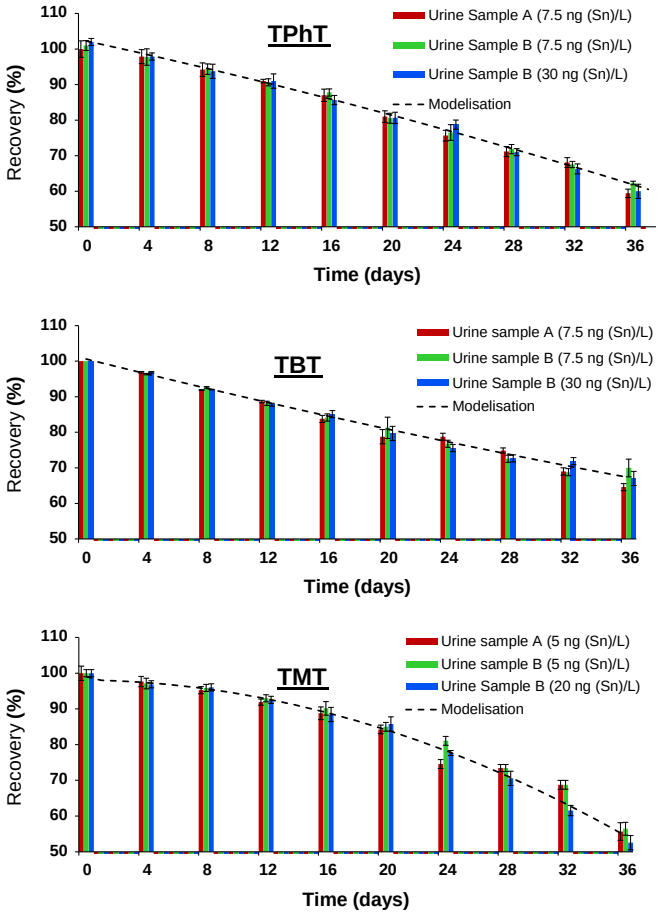

Fig. 1. Recoveries of tri-organotin compounds in urine samples for different sample storage conditions calculated using the experimental design model (dashed line) and independent spiked urine samples (bars). TBT: tributyltin; TPhT: triphenyltin; and TMT: trimethyltin.

3.2.3. *Kinetic degradation of OTC in human urine samples*

To understand the degradation processes of organotin compounds in urine, their transformations during storage at different pHs were kinetically modeled. Although different equation rates were considered, the best fit with experimental data was obtained with a first-order reaction ($R^2>0.98$, see Table 3). These results agree with previous degradation studies involving other environmental matrices [6]. The final model considered is described by the equation:

$$[\mathrm{OTC}]_t = [\mathrm{OTC}]_0 e^{-kt} \quad (2)$$

where $[\mathrm{OTC}]_0$ and $[\mathrm{OTC}]_t$ correspond to the initial organotin concentration ($t=0$) and to time "t", respectively, and k corresponds to the apparent kinetic constant. Using this model, the half-life time ($t_{1/2}$) has been calculated for each organotin, and the

Table 4
Kinetic parameters for OTC degradation in human urine.

	MMT	DMT	TMT	MBT	DBT	TBT	MPhT	DPhT	TPhT	MOcT	DOcT
Regression coefficient R^2											
pH=1.5	0.9896	0.9944	0.9914	0.9919	0.9976	0.9927	0.9879	0.9900	0.9917	0.9843	0.9798
pH=3.5	0.9976	0.9896	0.9934	0.9872	0.9897	0.9900	0.9951	0.9882	0.9983	0.9792	0.9913
pH=5.5	0.9879	0.9941	0.9891	0.9801	0.9899	0.9881	0.9812	0.9912	0.9915	0.9920	0.9832
Kinetic constant, k (d^{-1}) $\times 10^{-4}$											
pH=1.5	120 ± 3	150 ± 4	191 ± 2	129 ± 3	120 ± 3	190 ± 4	133 ± 2	110 ± 2	200 ± 5	71 ± 2	121 ± 6
pH=3.5	113 ± 2	98 ± 3	140 ± 3	91 ± 3	122 ± 2	120 ± 1	81 ± 1	140 ± 2	111 ± 2	70 ± 4	64 ± 3
pH=5.5	81 ± 4	102 ± 4	112 ± 2	108 ± 1	101 ± 4	130 ± 4	90 ± 2	102 ± 1	130 ± 3	99 ± 4	59 ± 3
Half-life time $t_{1/2}$ (d)											
pH=1.5	58 ± 1	46 ± 1	36.3 ± 0.4	54 ± 1	58 ± 2	36.5 ± 0.8	52.1 ± 0.8	63 ± 1	34.6 ± 0.8	98 ± 3	57 ± 3
pH=3.5	61 ± 1	71 ± 2	49.2 ± 0.8	76 ± 3	56.8 ± 0.9	57.7 ± 0.4	85 ± 1	49.5 ± 0.8	62 ± 1	98 ± 4	108 ± 5
pH=+5.5	86 ± 4	68 ± 3	61 ± 1	64.2 ± 0.6	69 ± 3	53 ± 1	77 ± 2	68 ± 1	53.3 ± 0.9	70 ± 3	117 ± 6

resulting values are displayed in Table 4. Based on these results, the stabilities of organotin compounds vary according to their degree of substitution, as has been reported for other environmental samples [40,41]. In our case, the tri-organotins presented the lowest $t_{1/2}$ values, while the mono-organotins appear slightly more stable than the di-organotins. Furthermore, the acidic medium produces a slight decrease in the half-life times obtain for the majority of OTC. The same behavior has been observed in aqueous samples such as natural freshwaters [37], and, as discussed previously, it can be attributed to the hydrochloric acid that is added to acidify the urine samples.

To understand the mechanism of the organotin degradation process in urine samples, the appearance of less-substituted species was monitored during the degradation study of the tri-organotins TBT, TPhT and TMT. Generally, the postulated mechanism of organotin degradation in the environment considers a progressive loss of organic groups bonded to the Sn cation [6]. However, in other environmental media, the direct or successive dealkyl or dearylation process can be simultaneously observed in the formation of di- and monosubstituted compounds [42]. In this study, the first mechanism was observed only for TBT, while for the other compounds the simultaneous presence of di- and mono-organotins is observed from 8th day of storage, suggesting the second alternative. Unfortunately, the elucidation of individual mechanisms was not possible due to the limited data available. A more systematic study should be undertaken to clarify the degradation mechanisms for organotin compounds in human fluids.

3.3. Application to organotin speciation in human urine samples

The developed methodology has been applied to three different human urine samples (HU1, HU2 and HU3) spiked with all OTC at three concentration levels. The spiked samples were stored at 4 °C and maintained at pH=4 during 4 days, according to the optimized conditions. The results obtained (recovery rates) after analysis of the urine samples are summarized in Fig. 2. As observed, the values determined at the three concentration levels are statistically similar ($p < 0.05$) to spiked levels for all samples. The recoveries for all samples ranged between 89% and 104%, while the repeatability (% RSD) did not exceed 10%, which are satisfactory results for these spiked levels in urine samples.

Finally, to demonstrate the applicability of this method to human exposure studies, six real urine samples were analyzed. These samples were collected from people working in dry-dock facilities in the Valparaiso region and were stored at 4 °C and pH 4.0 until analysis (first 4 days). The analytical results obtained are presented in Table 5, where the presence of methyl-, butyl- and phenyl-derivatives is observed. For butyltins, the reported values are higher than those obtained in previous studies [43], suggesting

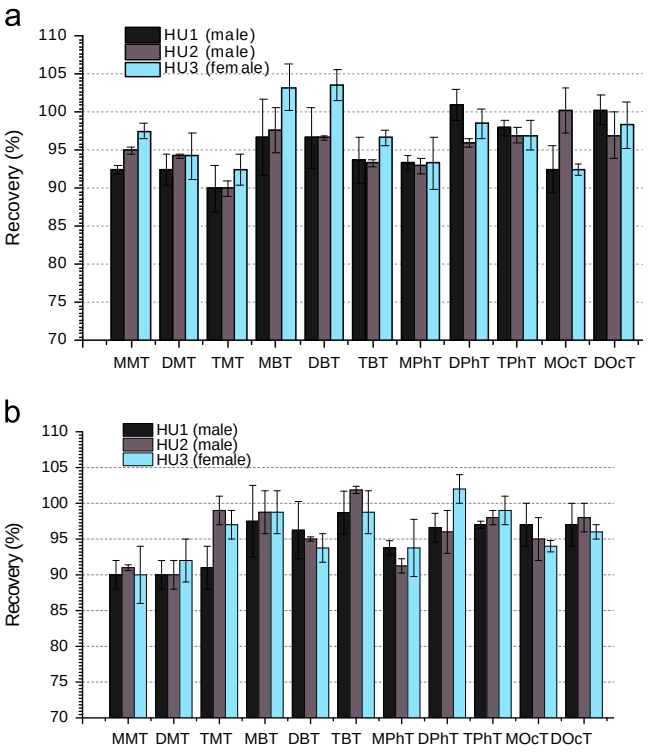

Fig. 2. Recovery study for organotin compounds in urine samples spiked to (a) low concentration (8–100 ng Sn L^{-1}) and (b) medium concentration levels (15–200 ng Sn L^{-1}).

that port workers, who perform repairing and painting activities on boats or ships, could be exposed to OTC from antifouling paints. Furthermore, the MBT levels, ranging from 55 to 108 Sn ng L^{-1}, were higher than DBT and TBT concentrations in the samples, suggesting the degradation of tri- and/or dibutyltin to lower-substituted compounds. Obviously, this degradation could take place before the intake of organotins or as a result of their metabolization. The (bio)transformation of organotin compounds in humans has been scarcely studied. However, some studies in mammals indicate that gastric digestion produces negligible degradation of TBT, while the liver and kidney can degrade tributyltin to produce DBT and MBT, which are commonly excreted in urine [41]. A similar mechanism may occur in human beings. However, these results are insufficient and a systematic study of degradation/metabolization of organotins in human samples is necessary to confirm this hypothesis.

With respect to methyltins, DMT is the most abundant in the urine samples analyzed (with a concentration ranging from 21 to 312 ng Sn L^{-1}), while TMT, which is the most toxic, was detected

Table 5
OTC concentrations in human urine samples collected from occupationally exposed individuals.

OTC	Urine samples (ng Sn L^{-1})						Urine samples (ng Sn g^{-1} creatinine)[a]					
	A	B	C	D	E	F	A	B	C	D	E	F
MMT	n.d	98 ± 2	21 ± 2	n.d[b]	167 ± 4	96 ± 4	n.d	113 ± 2	11 ± 1	n.d	291 ± 7	103 ± 4
DMT	179 ± 8	246 ± 11	67 ± 5	312 ± 23	206 ± 8	n.d	187 ± 8	285 ± 13	34 ± 2	438 ± 32	359 ± 14	n.d
TMT	n.d	35 ± 1	n.d	198 ± 9	n.d	n.d	n.d	41 ± 1	n.d	278 ± 13	n.d	n.d
MBT	101 ± 7	76 ± 2	55 ± 2	n.d	67 ± 4	108 ± 8	105 ± 7	88 ± 2	28 ± 1	n.d	117 ± 7	116 ± 8
DBT	124 ± 8	n.d	98 ± 3	n.d	n.d	n.d	129 ± 8	n.d	49 ± 1	n.d	n.d	n.d
TBT	n.d	n.d	48 ± 3	n.d	90 ± 7	87 ± 2	n.d	n.d	24 ± 2	n.d	156 ± 12	104 ± 2
MPhT	145 ± 4	79 ± 5	n.d	179 ± 5 ± 8	n.d	118 ± 10	151 ± 4	92 ± 6	n.d	251 ± 7	n.d	127 ± 11
DPhT	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
TPhT	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
MOcT	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
DOcT	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

[a] OTC concentrations in human urine corrected by creatinine concentration.
[b] Non-detectable.

in only two samples. Such a predominance of DMT has also been reported for urine samples collected from volunteers working in an organotin production plant, where this compound is the main product [18]. Furthermore, the detection of TMT has been attributed to methylation of DMT, as observed in mammals such as rats and mice [44]. In the present case, the presence of methyltins is unexpected, considering that the selected volunteers are principally exposed to butyltin from antifouling paints. Methylation in the body is a possible source of these compounds in urine. The detection of methylated compounds of arsenic or antimony in human urine after exposition to inorganic forms of these species has been widely reported [45,46]. However, the methylation of organotin compounds has not been elucidated in humans and further investigations are necessary to confirm this process.

Finally, the concentrations of OTC in human urine were determined and then corrected for creatinine concentration. Not performing this correction has several disadvantages, such as variability in total urinary output, the water balance, dilution and/or dehydration. It is fortunate that we can compensate for this variability by adjusting for the urine creatinine level [34]. Furthermore, this correction has been used for pesticides and metals in other studies with satisfactory results [47,48]. The measured and corrected OTC concentrations in the urine samples (as ng Sn g^{-1} creatinine) are presented in Table 5. To ensure adequate correction, the American Conference of Government Industrial Hygienists (ACGIH) has recommended urine creatinine levels ranging from 0.5 to 3.0 g L^{-1}. In our case, the creatinine level ranged between 0.71 and 1.98 g L^{-1} in all of the urine samples, validating our use of this approach. To the best of our knowledge, this is the first study that has applied this adjustment strategy. Only one similar approach has been reported in the literature, where methyltin concentrations in urine were corrected using an osmolality approach [18]. Obviously, the corrected concentrations reported in this similar study have neither the same magnitude nor units as our results, demonstrating the urgent need to standardize the adjustment strategy for pollutant analyses in urine samples.

4. Conclusions

The methodology developed has been validated and applied to the analysis of human urine samples, demonstrating that this SMPE technique is appropriate for the simultaneous determination of 11 different organotins in human urine. This methodology allows the determination of OTC at ultra-trace levels (ng L^{-1}) and with adequate precision (% RSD less than 10%) for monitoring these compounds in this biological matrix.

The stability study of organotins has demonstrated that the behavior of OTC in human urine differs with the degree of substitution. Tri-substituted species exhibited the highest degradation rates for the time period studied, while mono-substituted organotins were stable for a longer time in this matrix. These results are consistent with those reported in the literature.

Finally, the validated methodology was applied to the quantitation of organotins in human urine samples, demonstrating its analytical potential for human biomonitoring studies. Moreover, the corrected OTC concentrations detected in exposed volunteers evidenced the lack of data regarding degradation/metabolization of organotins in the human body. Further investigations are clearly necessary to establish a comparative approach for the adjustment of OTC concentrations in urine.

Acknowledgments

The authors gratefully acknowledge FONDECYT (Project 1120541). A. Valenzuela also thanks to CONICYT and Dirección de Investigación (PUCV, Chile) for doctoral and research-travel fellowships.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.02.054.

References

[1] K. Fent, Crit. Rev. Toxicol. 26 (1996) 3–117.
[2] B. Antizar-Ladislao, Environ. Int. 34 (2008) 292–308.
[3] M.M. Whalen, B.G. Loganathan, K. Kannan, Environ. Res. 81 (1999) 108–116.
[4] K. Grote, B. Stahlschmidt, C.E. Talsness, C. Gericke, K.E. Appel, I. Chahoud, Toxicology 202 (2004) 145–158.
[5] M. Nath, Appl. Organomet. Chem. 22 (2008) 598–612.
[6] M. Hoch, Appl. Geochem. 16 (2001) 719–743.
[7] F. Grun, B. Blumberg, Endocrinology 147 (2006) 50–55.
[8] F. Grun, Obesogens, Curr. Opin. Endocrinol. 17 (2010) 453–459.
[9] G.B. Jiang, Q.F. Zhou, J.Y. Liu, D.J. Wu, Environ. Pollut. 115 (2001) 81–87.
[10] M. Bravo, A. Valenzuela, W. Quiroz, M. Pinto, M. Flores, H. Pinochet, Talanta 81 (2010) 1034–1039.
[11] C.R. de Oliveira, D. dos Santos, L.A.D. Madureira, M.R.R. de Marchi, J. Hazard. Mater. 181 (2010) 851–856.
[12] A.C. Belfroid, M. Purperhart, F. Ariese, Mar. Pollut. Bull. 40 (2000) 226–232.
[13] X. Wang, H.Y. Jin, L. Ding, H.R. Zhang, H.Q. Zhang, C.L. Qu, A.M. Yu, Talanta 75 (2008) 556–563.
[14] H. Ohno, M. Suzuki, S. Nakashima, T. Aoyama, K. Mitani, J. Food Hyg. Soc. Jpn. 43 (2002) 208–214.

[15] K. Kannan, S. Takahashi, N. Fujiwara, H. Mizukawa, S. Tanabe, Arch. Environ. Cont. Tox. 58 (2010) 901–907.
[16] ATSDR, in, Toxicological profile for tin and tin compounds, Agency for Toxic Substances and Disease Registry 2005, ⟨http://www.atsdr.cdc.gov/toxprofiles/tp55.pdf⟩.
[17] K. Kannan, K. Senthilkumar, J.P. Giesy, Environ. Sci. Technol. 33 (1999) 1776–1779.
[18] Z.Y. Cui, K.G. Zhang, Q.F. Zhou, J.Y. Liu, G.B. Jiang, Talanta 85 (2011) 1028–1033.
[19] Y. Mino, F. Amano, T. Yoshioka, Y. Konishi, J. Health Sci. 54 (2008) 224–228.
[20] J.L. Gomez-Ariza, E. Morales, I. Giraldez, D. Sanchez-Rodas, A. Velasco, J. Chromatogr. A 938 (2001) 211–224.
[21] C. Dietz, J. Sanz, E. Sanz, R. Munoz-Olivas, C. Camara, J. Chromatogr. A 1153 (2007) 114–129.
[22] M. Abalos, J.M. Bayona, R. Compano, M. Granados, C. Leal, M.D. Prat, J. Chromatogr. A 788 (1997) 1–49.
[23] N. Cardellicchio, S. Giandomenico, A. Decataldo, A. Di Leo, Fresenius J. Anal. Chem 369 (2001) 510–515.
[24] L. Dunemann, H. Hajimiragha, J. Begerow, Fresenius J. Anal. Chem. 363 (1999) 466–468.
[25] F. Bianchi, M. Careri, M. Maffini, A. Mangia, C. Mucchino, J. Anal. Atom. Spectrom. 21 (2006) 970–973.
[26] Q.R. Ou, C.W. Whang, Anal. Bioanal. Chem. 386 (2006) 376–381.
[27] M. Bravo, G. Lespes, I. De Gregori, H. Pinochet, M.P. Gautier, Anal. Bioanal. Chem. 383 (2005) 1082–1089.
[28] M. El Ati-Hellal, G. Lespes, M. Dachraoui, Int. J. Environ. Anal. Chem. 86 (2006) 733–742.
[29] C. Bancon-Montigny, G. Lespes, M. Potin-Gautier, J. Chromatogr. A 896 (2000) 149–158.
[30] S. Aguerre, C. Bancon-Montigny, G. Lespes, M. Potin-Gautier, Analyst 125 (2000) 263–268.
[31] M. Bravo, A. Valenzuela, E. Fuentes, W. Quiroz, J. Chromatogr. A 1223 (2012) 9–14.
[32] M. Bravo, L.F. Aguilar, W. Quiroz, A.C. Olivieri, G.M. Escandar, Microchem. J. 106 (2013) 95–101.
[33] M. Bravo, G. Lespes, W. Quiroz, I. De Gregori, H. Pinochet, M. Potin-Gautier, J. Chil. Chem. Soc. 54 (2009) 171–174.
[34] T. Arndt, Forensic Sci. Int. 186 (2009) 48–51.
[35] C. Grote, K. Levsen, The applications of SPME in water analysis, in: J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, The Royal Society of Chemistry, Cambridge, 1999, pp. 169–187.
[36] T. Górecki, Solid versus liquid coatings, in: J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, The Royal Society of Chemistry, Cambridge, 1999, pp. 92–110.
[37] C. Bancon-Montigny, G. Lespes, M. Potin-Gautier, Water. Res. 35 (2001) 224–232.
[38] T. Lindemann, A. Prange, W. Dannecker, B. Neidhart, Fresenius J. Anal. Chem. 368 (2000) 214–220.
[39] J. Feldmann, V.W.M. Lai, W.R. Cullen, M.S. Ma, X.F. Lu, X.C. Le, Clin. Chem. 45 (1999) 1988–1997.
[40] E. Tessier, D. Amouroux, A. Morin, L. Christian, E. Thybaud, E. Vindimian, O. F. Donard, Sci. Total Environ. 388 (2007) 214–233.
[41] P. Rodriguez-Gonzalez, J.R. Encinar, J.I.G. Alonso, A. Sanz-Medel, Anal. Bioanal. Chem. 381 (2005) 380–387.
[42] S. Dubascoux, G. Lespes, L. Denaix, M.P. Gautier, Appl. Organomet. Chem. 22 (2008) 481–487.
[43] G.A. Zachariadis, E. Rosenberg, Talanta 78 (2009) 570–576.
[44] K. Furuhashi, M. Ogawa, Y. Suzuki, Y. Endo, Y.H. Kim, G. Ichihara, Chem. Res. Toxicol. 21 (2008) 467–471.
[45] F. Cubadda, F. Aureli, M. D'Amato, A. Raggi, A.C. Turco, A. Mantovani, Pure Appl. Chem. 84 (2012) 203–214.
[46] W. Quiroz, H. Arias, M. Bravo, M. Pinto, M.G. Lobos, M. Cortes, Microchem. J. 97 (2011) 78–84.
[47] H.J. Mason, I.M. Calder, Occup. Environ. Med. 51 (1994) 287.
[48] D.T. Mage, R.H. Allen, A. Kodali, J. Expo. Sci. Environ. Epidemiol. 18 (2008) 360–368.