Talanta 140 (2015) 115–121

Contents lists available at ScienceDirect

Talanta

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

# Development, validation, and application of an ultra-performance liquid chromatography–sector field inductively coupled plasma mass spectrometry method for simultaneous determination of six organotin compounds in human serum

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ARTICLE INFO

*Article history:*
Received 10 February 2015
Accepted 15 March 2015
Available online 27 March 2015

*Keywords:*
Organotin compounds
Metallomics
Speciation
Ultra high pressure liquid chromatography
Inductively coupled plasma mass spectrometry
Serum
Biomonitoring

ABSTRACT

Organotin compounds (OTCs) are heavily employed by industry for a wide variety of applications, including the production of plastics and as biocides. Reports of environmental prevalence, differential toxicity between OTCs, and poorly characterized human exposure have fueled the demand for sensitive, selective speciation methods. The objective of this investigation was to develop and validate a rapid, sensitive, and selective analytical method for the simultaneous determination of a suite of organotin compounds, including butyl (mono-, di-, and tri-substituted) and phenyl (mono-, di-, and tri-substituted) species in human serum. The analytical method utilized ultra-performance liquid chromatography (UPLC) coupled with sector field inductively coupled plasma mass spectrometry (SF-ICP-MS). The small (sub-2 μm) particle size of the UPLC column stationary phase and the sensitivity of the SF-ICP-MS enabled separation and sensitive determination of the analyte suite with a runtime of approximately 3 min. Validation activities included demonstration of method linearity over the concentration range of approximately 0.250–13.661 ng $mL^{-1}$, depending on the species; intraday precision of less than 21%, interday precision of less than 18%, intraday accuracy of −5.3% to 19%, and interday accuracy of −14% to 15% for all species; specificity, and matrix impact. In addition, sensitivity, and analyte stability under different storage scenarios were evaluated. Analyte stability was found to be limited for most species in freezer, refrigerator, and freeze–thaw conditions. The validated method was then applied for the determination of the OTCs in human serum samples from women participating in the Snart-Foraeldre/MiljØ (Soon-Parents/Environment) Study. The concentration of each OTC ranged from below the experimental limit of quantitation to 10.929 ng tin (Sn) $mL^{-1}$ serum. Speciation values were confirmed by a total Sn analysis.



## 1. Introduction

Organotin compounds (OTCs) are a class of organometallic species comprised of a tin (Sn) atom bound covalently to one or more alkyl or aryl groups [1]. The number and structure of the organic substituents bound to Sn can significantly alter its physicochemical characteristics, and as a result, Sn has the greatest variety of organometallic derivatives that are currently in use by industry among any other element [2]. Since the 1940s, the plastics industry has employed mono and dialkyl OTCs as heat and light stabilizers for the production of polyvinyl chloride and other materials [3]. More recently, dialkyl OTCs have been employed in thin film, transparent conductive coatings for liquid crystal display panels [4]. Many OTCs are biocides, with maximum toxicological activity observed for trisubstituted compounds. Tributyltin (TBT) and triphenyltin (TPT) have historically been used in marine antifouling paints to minimize organism growth on ship hulls and as insecticides, miticides, or fungicides for wood preservation or agricultural crop protection [5–7].

The industrial utility of OTCs has led to significant amounts of the chemicals being found in household products and the environment, resulting in widespread potential for human exposure.

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http://dx.doi.org/10.1016/j.talanta.2015.03.022
0039-9140/

Several cases of OTC poisoning or exposure were recently reported in workers involved in leather, plastic, or other manufacturing activities [8,9], and OTCs originating from floor wax, diapers, baking paper, clothing, and other consumer products were reported in house dusts [10]. The extent of exposure in the general population is less characterized. The predominant environmental sources of TPT and TBT have been agricultural runoff and the degradation of marine antifouling paints [11,12]; both TPT and TBT are toxic and endocrine disruptors in aquatic organisms, even at sub ng $L^{-1}$ levels [13]. Numerous reports of gastropod imposex, resulting in permanent female masculinization and species decline [13–15], coupled with OTC bioaccumulation spanning several marine food chain trophic levels [5,12,16–19] have prompted regulatory agencies across the world to restrict use of tin-containing antifouling marine paints to larger ships [20,21]. Even though the use of these paints has been limited, OTCs have persisted in the environment by binding with sediments. When exposed to favorable environmental conditions, aquatic sediments can release sequestered OTCs back into the water column for biological uptake [18].

The differential toxicity, industrial utility, environmental prevalence, and potential for human exposure have fueled the demand for analytical methods capable of determining the concentrations of OTCs in a variety of matrices. These methods must be capable of differentiating target organotin species at very low concentrations in an environmental or biological matrix of interest. Fluorescence spectrometry and bioluminescent assays have recently been reported for detection of organotin compounds [22,23], but the most common analytical approach remains coupling of chromatography with a specific detector. Gas chromatography (GC) coupled to mass spectrometry (MS) [12], tandem mass spectrometry (MS/MS) [13], high resolution MS [24], atomic absorption spectrometry (AAS) [3], flame photometric detection (FPD) [25], pulsed flame photometric detection (PFPD) [20], atomic emission detection (AED) [26], and inductively coupled plasma mass spectrometry (ICP-MS) detection [5] have all been used to quantify organotin species in a variety of matrices. Regardless of the instrumental technique used for detection, OTCs must be extracted and converted to fully alkylated, volatile species to allow analysis by GC. Extraction of ionic OTC species into non-polar solvents has been achieved with complexing agents like tropolone or dithiocarbamate, followed by sample cleanup and concentration. A second analytical approach involves the use of ethylating agents including sodium tetraethylhydroborate and Grignard reagents to convert OTCs to volatile forms that are amenable to analysis by gas chromatography (GC) [20,27]. More recently, headspace solid-phase microextraction (HS-SPME) with in situ derivitization has been employed to simultaneously volatilize, extract, and concentrate OTCs prior to GC separation [28–31].

Although GC has been successfully employed for OTC determinations, challenges associated with the method have prompted a search for alternative methods. Extraction, clean-up, and concentration procedures for OTC measurements by GC can be tedious and time-consuming [32,33]. In addition, GC methods require derivatization of extracted OTCs to more volatile forms, and the extent of derivatization can be dependent on the sample matrix and Sn species present [34,35]. Consolidated extraction and derivatization HS-SPME techniques are promising, but are prone to inconsistent matrix effects for OTC measurements [26]. In contrast, liquid chromatography (LC) does not require derivatization prior to analysis and could be capable of separating common OTC environmental contaminants when used with complexing agents. Further, coupling of LC with ICP-MS could provide sensitive detection of OTCs and relatively low limits of detection compared with other detection methods. The objective of this investigation was to develop and validate a rapid, sensitive, and selective ultra-performance liquid chromatography (UPLC) method coupled with sector field inductively coupled plasma mass spectrometry (SF-ICP-MS) detection for the determination of a suite of OTCs, including monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), monophenyltin (MPT), diphenyltin (DPT), and triphenyltin (TPT) (structures presented in Supplementary information, Table S1) in human serum collected from women participating in the Snart-Foraeldre/MiljØ (Soon-Parents/Environment) Study, a cohort of Danish women who have recently discontinued birth control in order to become pregnant.

## 2. Experimental

### 2.1. Reagents

The human serum matrix used throughout this investigation to prepare matrix standards and quality control (QC) samples was pooled from six adult female donors and was received and stored frozen (nominal −20 °C) from BioChemed Services (Winchester, VA, USA). Several ampules of a custom organotin standard containing nominal 2000 μg $mL^{-1}$ concentrations of MBT, DBT, TBT, MPT, DPT, and TPT chlorides in methylene chloride were obtained from Restek (Bellefonte, PA, USA). Semiconductor grade methanol, Ultrex grade acetic acid, and high-purity deionized water (~18 MΩ, DI $H_2O$) for the mobile phase were obtained from Sigma-Aldrich (St. Louis, MO, USA), J.T. Baker (Center Valley, PA, USA), and Pure Water Solutions (Hillsborough, NC, USA), respectively. High-purity (≥99%) tropolone and triethylamine were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Acros Organics (Geel, Belgium), respectively. Ultrex grade hydrochloric and nitric acids were obtained from J.T. Baker, and a National Institute of Standards and Technology (NIST)-traceable, 10 μg $mL^{-1}$ Sn standard was purchased from High Purity Standards (Charleston, SC, USA).

### 2.2. Contamination control

With recent advances in instrument technology, the sample preparation method has become the most important source of error in analytical measurements [36]. In order to successfully determine the concentrations of organotin analytes at biologically relevant, sub-ng $mL^{-1}$ levels, control of the laboratory environment to minimize the potential for contamination and analyte degradation is an absolute necessity. All sample handling activities occurred in a high-efficiency particulate air (HEPA)-filtered environment, and work was conducted under UV-free lighting conditions to minimize the potential for photodegradation [37]. In order to minimize Sn background from labware [24], glass UPLC vials (Waters Corporation, Milford, MA, USA) were filled with concentrated hydrochloric acid for at least two hours, rinsed multiple times with high-purity DI water, and were dried and stored under HEPA-filtered air until use. All other labware was soaked in 20% (v/v) hydrochloric acid for a minimum of two hours, rinsed with high-purity DI water, and dried and stored under HEPA-filtered air until use. Multiple lots of each reagent were obtained from several vendors and were screened for total Sn content by SF-ICP-MS. Briefly, reagents were digested in a mixture of nitric and hydrochloric acids prior to analysis against external calibration standards. A total Sn screening procedure was also conducted for blood collection tubes. Tubes were extracted with water and acid for comparison of water-leachable Sn and acid-leachable Sn. Tubes were rinsed three times with high-purity DI water and each rinse was analyzed for total extracted Sn. Tubes were then rinsed with a 2.5% hydrochloric acid/2.5% nitric acid solution and the extract was analyzed for total extracted Sn. The average total water-leachable Sn for six replicates of two lots of commercially-obtained collection tubes was 0.115 and 0.207 ng $tube^{-1}$. The average sum of water-leachable