Article

Development of a Fabric Phase Sorptive Extraction-High Performance Liquid Chromatography-Ultraviolet Detection Method for the Analysis of Phenyltin Compounds in Environmental Water and Canned Food Samples

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ABSTRACT: This paper reports a novel fabric phase sorptive extraction-high performance liquid chromatography-ultra violet detection (FPSE-HPLC-UV) method for the simultaneous extraction and analysis of four phenyltin derivatives that include triphenyltin hydroxide, triphenyltin acetate, triphenyltin chloride and tetraphenyltin in environmental water (agricultural waste water and municipal waste water) and canned food samples. The selected analytes were well resolved by Waters Nova pack C18 column (3.9 x 150 mm, 4 µm particle size) in isocratic elution mode within 15 minutes. The new microextraction media has been analytically evaluated using phenyltin derivatives as model compounds. The factors affecting the extraction efficiency of FPSE have been evaluated and the optimum extraction conditions were determined. Under these optimum conditions, the limits of detection (LODs) for sol-gel C18 coated fabric phase sorptive extraction (FPSE) media in combination with HPLC-UV for the analysis of the phenyltin derivatives were in the range of 10-100 ng/mL with high precision (low relative standard deviation) at 10 ng/mL concentration with good absolute recoveries. To the best of our knowledge, this is the first FPSE extraction procedure applied to environmental water and canned food samples for the simultaneous determination of phenyltin derivatives and could be readily adopted as a rapid and robust green analytical tool for routine environmental and food analytical laboratories.

Key Words: Phenyltin derivatives, Tetraphenyltin, Triphenyltin chloride, triphenyltin acetate, triphenyltin hydroxide, Fabric phase sorptive extraction (FPSE), High performance liquid chromatography (HPLC)

1. Introduction

The environment has been flooded with huge burden of toxic compounds originated from both natural and anthropogenic activities. The presence of these compounds even in minute quantities is considered critical with disastrous consequences. Organotin (OTs) compounds are the organometallic pollutants, where aryl or alkyl groups are chemically bonded to tin (Sn). The number and structure of the organic moieties bonded to Sn can significantly alter its physicochemical properties [1-2]. The industrial and agricultural utility of phenyltin compounds has led to the presence of it in food products and environment, resulting into a wide spread risk of human exposure. According to the Food and Agriculture Organization (FAO) of the United Nations and the

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World Health Organization (WHO), the maximum permissible limit for tin in canned foods is 250 mg kg-1 [3]. Phenytins can elicit a wide range of endocrine and nervous-system effects, particularly tri-substituted species [4-5]. In general, the elemental tin and its inorganic salts are considered to be harmless while the organotins like Tributyltin and Triphenyltin are very toxic and can inflict harmful effects on non-target aquatic organisms, even at a low ng/L level [6].

In this study, Tetraphenyltin [(C6H5)4Sn; TPT], Triphenyl tin acetate [(C6H5)3SnOOCH3; TPTA] Triphenyl tin hydroxide [(C6H5)3SnOH; TPTH] and Triphenyltin chloride [(C6H5)3SnCl; TPTC] were studied as target analytes because of its wide usage in insecticides, fungicides, bactericides, acaricides, preservatives, catalysts, stabilizers for PVC, antifouling paints, water repellents, fire retardants for woolen and cotton fabrics, moth repellants of textiles, disinfectors of hospitals, clothing and UV-oxidation resistance stabilizers, therefore found in numerous compartments such as water, plastic, textile and agriculture products etc. [7-13]. As a result, there is a variety of pathways for their entry into the environment and have long-term persistence in the environment. Their extreme toxicity and brutal effects on both mammals [14] and marine organism [15] including humans as well as their high bioaccumulation prospect have led to the urgency in controlling the pollution levels of phenyltin species at low concentration levels. In addition, long-term exposure to phenyltin derivatives can lead to anomalies in DNA mutation and human red blood cells [16]. Therefore, the development of rapid, precise, novel and sensitive analytical methods for the determination of these analytes is of special importance. Till now, numerous chromatographic methods including gas chromatography (GC) [17-18], capillary electrophoresis [19-20], liquid chromatography (LC) or supercritical fluid chromatography (SFC) with detection method like atomic absorption spectrometry (AAS) [21-22], atomic emission spectrometry (AES) [23], inductively coupled-plasma mass spectrometry (ICP-MS) [24-25], electron spray ionization mass spectrometry (ESI-MS) [26-27], ultraviolet (UV) [28-29] flame photometric detection (FPD) [30-31] have been successfully developed for the determination of these tin compounds. Due to their low concentration in the environment as well as the presence of numerous matrix interferents that may impact the performance of the analytical instrument, an effective sample preparation technique is inevitable prior to instrumental analysis. As such, a number of sample preparation techniques such as automated in-tube solid-phase microextraction (in-tube SPME) [26], microwave-assisted extraction [27], dispersive liquid liquid-microextraction (DLLME) [31,32] method has been successfully developed for their determination. Although these techniques are very useful but possess some serious drawbacks including laborious, time consuming, high consumption of organic solvents and sample solutions, and often lead towards significant loss of analytes and poor reproducibility (DLLME, SPE), high cost, fragility of the fiber (SPME), bubble formation results in reducing the rate of extraction. Therefore, novel, versatile and high-performance adsorbents with a simple sample preparation are still highly desirable.

Recently, Kabir et al. [33-35] has developed fabric phase sorptive extraction technique, which involves the successful integration of the advantages of rich surface chemistry of cellulose fabric and advanced material properties of sol-gel derived hybrid organic-inorganic extraction sorbents leading to the formation of a highly sensitive, solvent minimized and an efficient microextraction technique. The synergistic combination of sol-gel derived hybrid organic-inorganic sorbent and intrinsically porous network of cellulose surface as the substrate has allowed the utilization of high amount of sol-gel extraction sorbent onto the cellulose substrate matrix, which leads to a phenomenal increase in analyte retention capacity with fast extraction equilibrium. As phenyltin compounds are inherently hydrophobic compounds, the selective extraction of these analytes from aqueous sample matrix would be accelerated when a hydrophobic sorbent as the extraction phase is used. As such, sol-gel C18 coated FPSE media containing long hydrophobic C18 chains has been evaluated and successfully applied in the present study. FPSE technique using sol-gel C18 coated FPSE media is thoroughly investigated as an advanced analyte enrichment tool to develop a green sample preparation technique.

Though gas chromatography (GC) is the most common approach in phenyltin analysis, but the low volatility and pre column derivatization complicates the analysis of phenyltin compounds by GC-MS [36,37]. The derivatization process makes the sample preparation more laborious and time consuming, which increases the probability of contamination and errors, and usually accompanied by sample loss. Because of inherent simplicity, prevention of sample loss and suitability for condition of volatility, high performance liquid chromatography (HPLC) is used preferentially over gas chromatography. The concern over the toxicity of phenyltins has led to the development of precise and reliable analytical method for their determination in canned food and environmental water samples.

In this paper, we have described the synthesis, characterization of sol-gel coated FPSE media and developed the novel analytical method for the efficient extraction of trace amounts of four phenyltin species of high environmental interest from aqueous solution. The hydrophilic cellulose fabric substrate incorporated in the core of the adsorbent aids in the extraction kinetics by attracting water molecules containing phenyltin species towards its surface for a successful sorbent-analyte interaction, resulting in rapid trapping of the analytes on FPSE media. The objective of this investigation was to develop a rapid, selective and sensitive FPSE-HPLC-UV method for the determination of some important phenyltin derivatives/compounds in environmental aqueous samples and canned food.

2. Materials and methods

2.1. *Apparatus*

The HPLC system consist of pump (Dionex P680, Dionex Softron GmbH, Germering, Germany) with four solvent chambers, a Waters Nova pack C18 4µm reversed phase analytical column (3.9 x 150 mm), a Dionex UVD170U detector operated at 254 nm connected to a computer loaded with Chromeleon software for data acquisition. Separations were carried out at room temperature at 20-25 °C. Sample was directly injected into a Rheodyne 20 µL loop on the valve for analysis. A digital vortex mixer (Fisher Scientific, USA) was employed for thoroughly mixing of sol solutions. An ultrasonic cleaner-2510 (Branson Inc., USA) was used to make sol solution free of trapped gas or bubbles. Centrifugation of sol solution, to obtain particle free solution, was carried out in an Eppendorf centrifuge model 5415 R. A Barnstead Nano Pure Diamond (Model D11911) deionized water system was used.

2.2. *Materials, chemicals and reagents*

Acetonitrile (HPLC grade; UV cut off 190 nm), was purchased from J.T. Baker Chemicals (USA). Triple distilled water was used as one of the elution solvent for chromatographic separation. Aqueous and non-aqueous solvents were filtered with 0.45 µm Nylon-6,6 membrane filters and 0.40 µm syringe filter (Rankem, India) in a filtration assembly (Perfit, India). Tetraphenyltin (> 97 %), triphenyl tin acetate (> 98 %), and triphenyl tin hydroxide (> 96 %) and triphenyltin chloride were purchased from Sigma aldrich (USA). Octadecyl trimethoxysilane, acetone, dichloromethane, methyltrimethoxysilane (MTMS), and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich (USA). Sodium hydroxide and hydrochloric acid were purchased from Thermo Fisher Scientific (USA). Standard stock solutions of each phenyltin compound (1000 ppm) were prepared in acetonitrile (HPLC grade). These solutions were stored at -10°C in dark bottles. Ultrasonic degassing was performed with ultrasonic bath (Sarthak Scientific Services, Panchkula, India). The required working solutions were prepared from stock solutions using acetonitrile solvent.

2.3. *Spiking procedure*

Stock solutions were prepared by dissolving each of Ph3SnCl, Ph3SnOH and Ph3SnOCOCH3 in acetonitrile (1mg/mL). Working standard solutions were prepared from stock solutions. The stock and working standard solutions were covered with aluminum foil and stored at -18°C. The spiked samples of desired concentrations of agricultural waste, municipal waste etc. were prepared from the stock solutions. The spiked samples were stirred at room temperature overnight on a magnetic stirrer (800-900 rpm/min) to obtain a homogeneous clear solution. The contents obtained were sonicated and injected into rheodyne loop for chromatographic analysis. The selected analytes were well resolved by Waters Nova Pack C18 column (3.9 x 150 mm, 4µm) in isocratic elution mode within 15 minutes. The new microextraction media has been analytically evaluated using phenyltin derivatives as model compounds.

2.4. *Sample Preparation*

2.4.1 *Preparation of agricultural waste water and municipal water samples*

Agricultural wastewater sample was collected from the agriculture field of village Sadhubela, Patiala, Punjab, India. Municipal water was collected from the area near to TN Plastic industries, Panipat, Haryana, India. Water samples were collected and transported to the lab in Pyrex borosilicate amber glass bottles. Samples were immediately stored in the freezer to avoid any microbial decomposition. Since both kind of water samples contain a lot of impurities and suspended material. Prior to analysis, a fraction of stored aqueous samples were sonicated for about 10 minutes to expel dissolved gases and then filtered using 0.22 µm nylon filters to remove the impurities. Subsequently, the samples were analyzed using the optimized FPSE-HPLC-UV procedure. Fractions of each filtered water sample were spiked with selected target analytes of phenyltin derivatives to make samples. No traces of tin were detected in the samples. The agricultural and municipal waste water were spiked with phenyltin components (100 ppb) and were analyzed with FPSE-HPLC-UV at optimized conditions.

2.4.2 *Canned food sample*

An aliquot of 0.25 g of the canned food sample was accurately weighed on a Teflon digestion vessel on a micro analytical balance and then treated with a mixture of 6 mL nitric acid and 1mL hydrogen peroxide in the microwave digestion system [38]. The extracts thus obtained were re-dissolved in 25 mL of triply distilled water, filtered using 0.22 µm nylon filters and subsequently analyzed by HPLC-UV.

2.4.3 *Urine sample*

Urine samples were acquired from a person who has consumed tin canned food. The supernatant sample collected after the process of centrifugation was stored at - 4°C in glass tube and kept in a refrigerator until the time of sample pretreatment. The urine was diluted to about ten times with triply distilled water, filtered with Nylon-6, 6 membrane filters, and was then degassed on ultrasonic bath for half an hour. Spiked Sample was prepared and pre-concentrated with FPSE media before the analysis. The FPSE eluted sample was injected into Rheodyne loop and analyzed chromatographically.

3. Results and Discussion

Malik and coworkers [39] has initiated a new era of sol-gel technology for synthesizing different variety of microextraction sorbents. Fabric phase sorptive extraction integrates the advantages of sol-gel sorbents and exploits the inherent characteristics of the fabric substrate (hydrophilic/hydrophobic). FPSE is the first extraction technique that exploits the surface property of the substrate. As such, the overall selectivity and affinity of the FPSE medium depend on (a) sol-gel precursor; (b) organic polymer; and (c) surface property of the fabric substrate. Preparation of fabric phase sorptive extraction media coated with any sol-gel sorbent involves a number of steps including (a) pretreatment of sorbent for sol-gel coating (b) preparation of sol solution (c) sol-gel coating on the target substrate.

3.1. Pretreatment of sorbent for sol-gel coating

First, the cellulose fabric was soaked in deionized water for 15 minutes under constant sonication. Then, the fabric was cleaned with ample amount of triply distilled water followed by 1M sodium hydroxide solution for 1 hour. The base treated fabric was again need to wash with profuse amount of distilled water followed by treating with 0.1 M hydrochloric acid solution for 1 hour. Finally the treated fabric was washed with deionized water and dried overnight in an inert atmosphere. The dried fabric was stored in a clean airtight glass container for the future use as a coated sorbent.

3.2. Preparation of sorbent for sol-gel coating

Taking into the consideration of nature of selected phenyltin derivatives, it is quite apparent that a nonpolar sorbent would provide the best affinity towards the target selected analytes to selectively isolate them from complex environmental water sample matrix. The substrate used in fabric phase sorptive extraction plays dynamic role in determining the overall polarity and selectivity of FPSE media. Support selected for FPSE media was 100% cellulose containing various finishing chemicals, starch softeners etc. To remove the hindrance of such chemicals, fabric requires surface treatment that would remove the chemicals and regenerate surface hydroxyl functional groups to proficiently anchor the sol-gel polymeric network. The sol solution required for creating the sol-gel coated C18 media was prepared by using the modified form of previously described formulation [35,40]. The sol solution was prepared by mixing sol-gel precursor octadecyltrimethoxysilane, sol-gel catalyst trifluoroacetic acid (TFA), dichloromethane, acetone and water. All the ingredients were mixed well in order to get homogeneous sol solution. The molar ratio between methyl trimethoxysilane and octadecyltrimethoxysilane were maintained at 1: 0.38. The mixture was then vortexed for 5 minutes and centrifuged or sonicated. The cellulose fabric substrate was then gently immersed into the homogeneous sol solution for about 2 hours.

3.3. Characterization of sol-gel C18 coated FPSE media

Mechanism of extraction of an analyte by fabric phase sorptive extraction media largely depends on the porous nature of the sol gel sorbent. As can be seen from the SEM images, each cellulose fabric is constituted of various macro pores, which make an easy access of sol solution into the cellulose fabric matrix formed uniformly on the surface of microfibrils of cellulose. Thus, it accomplishes the faster extraction equilibrium in a very short period. Fig. 1 represents the scanning electron micrographs (SEM) of (a) uncoated cellulose substrate; (b) sol-gel C18 coated fabric phase sorptive extraction media.

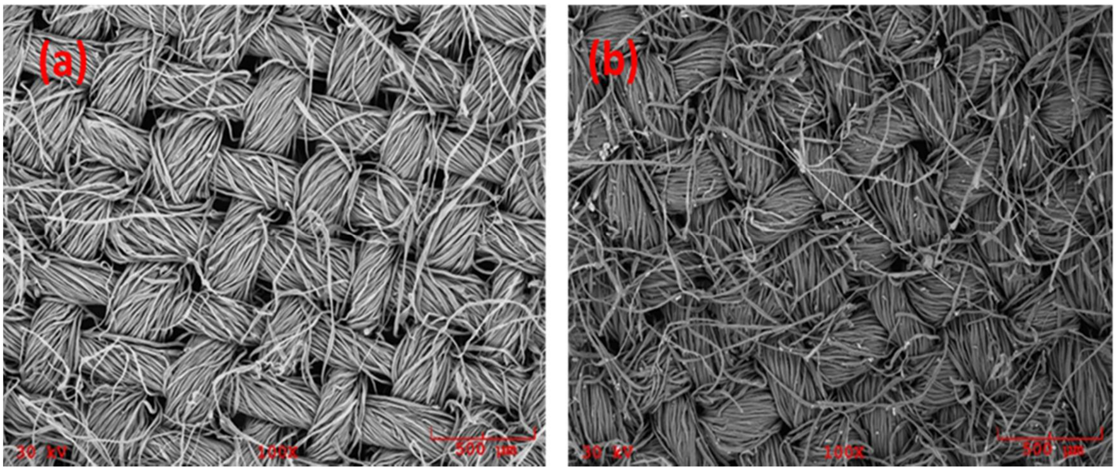
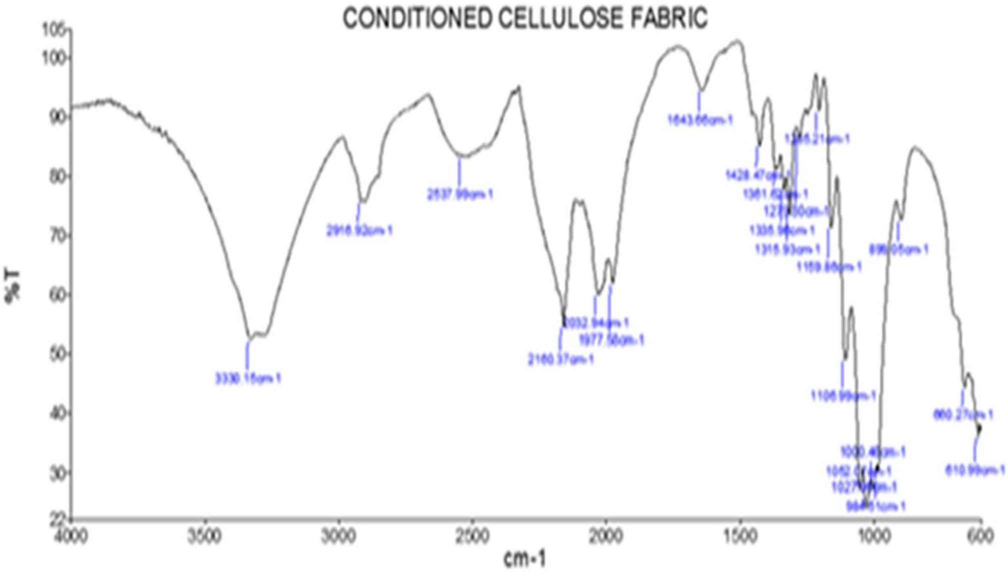


Fig. 1: **S**canning electron micrographs (SEM) of (a) uncoated cellulose substrate; (b) sol-gel C18 coated fabric phase sorptive extraction media

Fig.2 represents two FT-IR spectra of uncoated cellulose substrate (top) and sol-gel C18 coated FPSE media (bottom).



C-18 MTMS

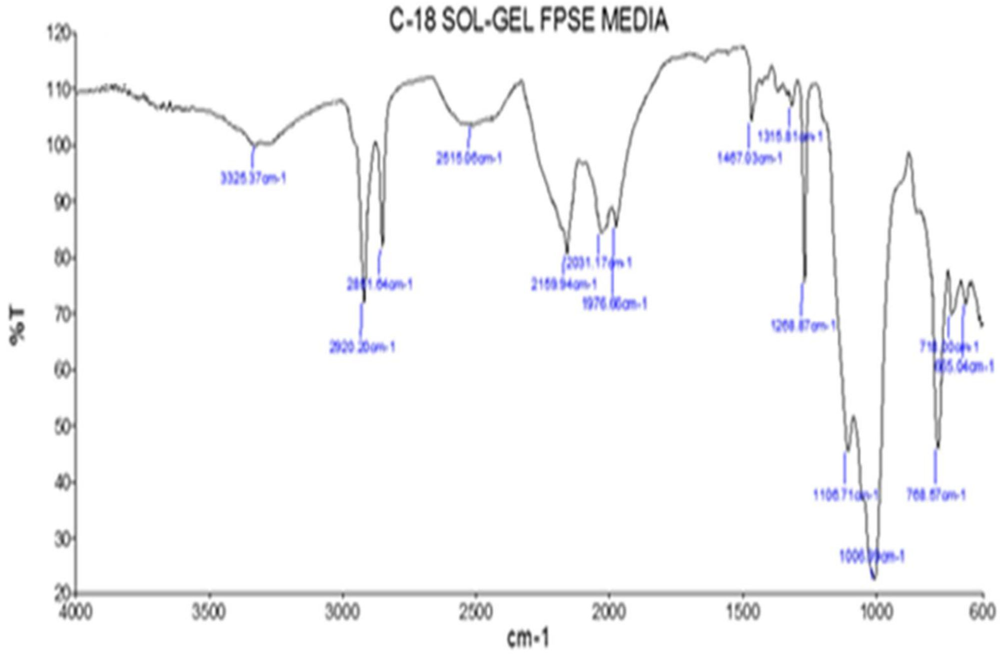


Fig. 2: FT-IR spectra of (top) uncoated cellulose substrate; (bottom) sol-gel C18 coated FPSE medium.

The FT-IR spectra of uncoated cellulose fabric have characteristic absorption between 2894 cm^{-1} and 1308 cm^{-1}; 3000 cm^{-1} and 3300 cm^{-1} ; and at 1024 cm^{-1} correspond to C–H, O–H and C–O vibration or C–H bending vibration, respectively. The distinctive peaks of sol-gel C18 coated FPSE media (bottom) appeared at 2890 cm^{-1} and 2851 cm^{-1} which represent symmetric vibration of $–CH_2$ and asymmetric vibration of $–CH_3$, respectively. These peaks are also seen in uncoated cellulose fabric media. In addition, the presence of peaks approximately 1467 cm^{-1}, 1268 cm^{-1} and 1977 cm^{-1} in the sol-gel C18 FPSE media strongly suggests the successful integration of octadecyl moieties into the sol-gel network. The considerable reduction of the O-H stretching vibrations (at 3325 cm^{-1}) in sol-gel C18 coated FPSE media compared to uncoated cellulose indicates the chemical assimilation of the sol-gel C18 network to the cellulose structure via the process of condensation. FPSE offers remarkably superior thermal and stable due to the chemical integration of sol-gel sorbent to the substrate surface. This technique has innovatively incorporated both the solid phase microextraction (SPME) and solid phase extraction (SPE) into a single technology podium.

3.4. FPSE Method

Optimization of FPSE conditions

The method using fabric phase sorption extraction was used with sol-gel silica C_{18} as a sorbent material. To optimize FPSE procedure, factors affecting the recovery such as the extraction time, back extraction time, effect of ionic strength, composition and volume of the elution solution and carryover effects were studied.

3.4.1. Optimization of eluting solvent

Effect of different eluting solvents on elution recoveries of phenyltin derivatives was studied. Once the target analytes were extracted onto the FPSE media, a quantitative desorption into a suitable organic solvent/solvent-system was required. Methanol, ethanol, acetonitrile, toluene and hexane (ranging from high polarity to low polarity) were tried for quantitative desorption of target analytes from sol-gel coated FPSE media (Fig. 3). Ethanol and methanol (100%) were not found to be suitable for the extraction. Toluene has a definitive advantage for the extraction of phenyltin compounds with well resolved and fine peaks. Therefore, toluene was selected as the back extracting solvent for further experimentation. Recoveries with toluene were further increased when the aqueous phase was treated with salts like sodium chloride.

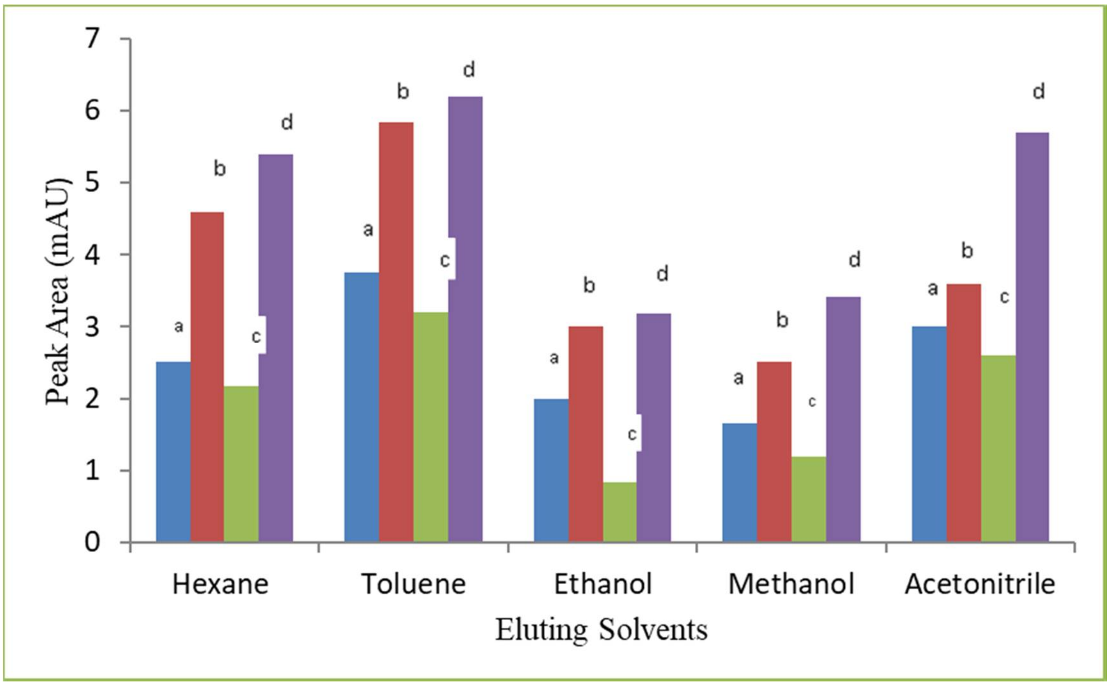


Fig. 3: Effect of eluting solvent on extraction recoveries of phenyltins

3.4.2. Optimization of extraction time

Extraction time is duration required to reach the state of extraction equilibrium between the extraction media and the matrix. The extraction time was studied by exposing the samples to PSE media from time 5 to 30 min. Recovery of analytes increases with increase in extraction time up to 20 min and it becomes constant later on (Fig. 4). So the extraction time of 20 min was selected for the subsequent analysis.

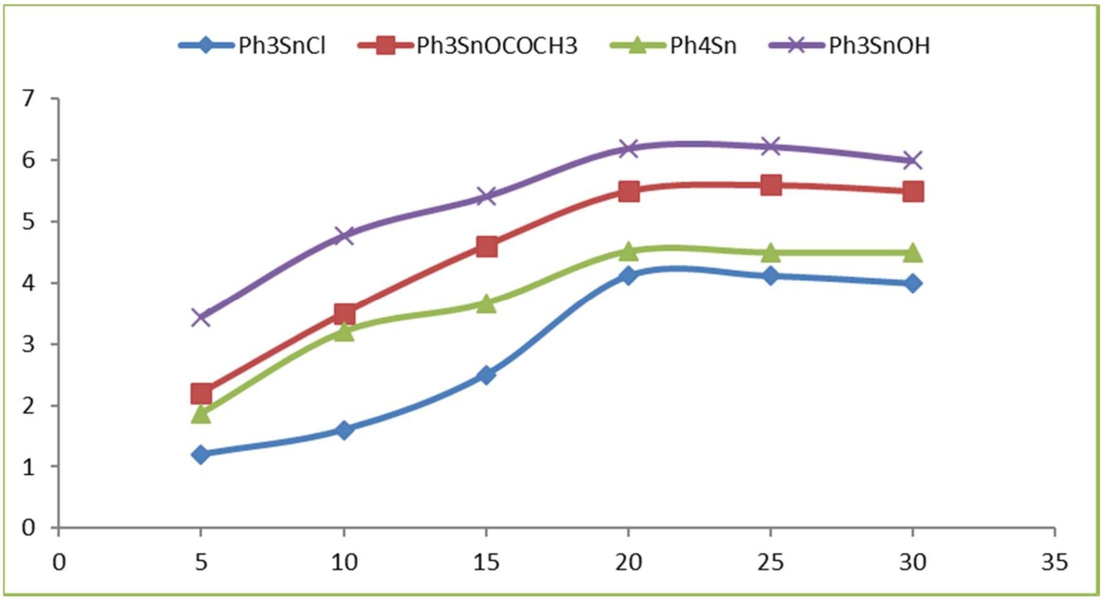


Fig. 4: Effect of extraction time on recoveries of phenyltins

3.4.3. Optimization of back extraction time

The influence of back extraction time on analytes recovery was tested by varying time from 5 to 25 min. Maximum recovery was obtained at 15 min, while slightly reduced recoveries were obtained at 20 and 25 min, which probably may be due to re-adsorption process on fabric (Fig. 5). So, back extraction time of 15 min was optimized for further studies.

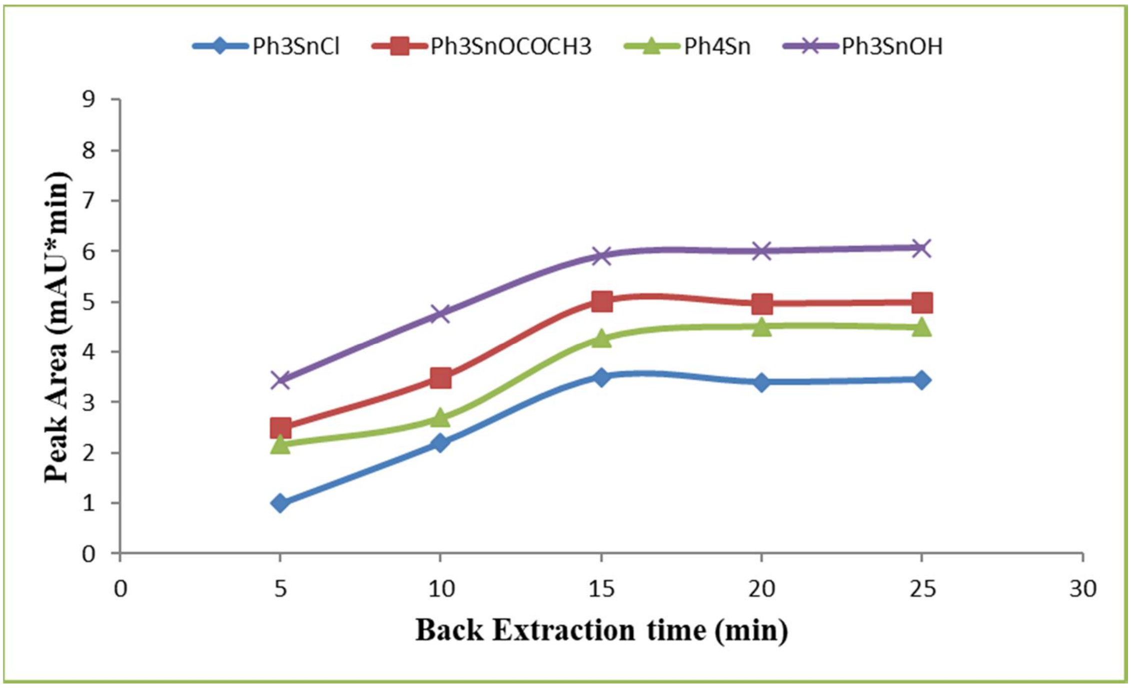


Fig. 5: Effect of back extraction time on recoveries of phenyltins

3.4.4. Effect of pH

The effect of pH of the sample matrix was examined on the extraction of phenyltin derivatives for a pH range 3 to 9. It was observed that the maximum extraction sensitivity was at pH 7.0.

The pH of 7.0 was observed for the aqueous solution after the addition of analytes to the water sample without adding any buffer solution. The extraction was decreased both in acidic and alkaline condition shown in (Fig.6).

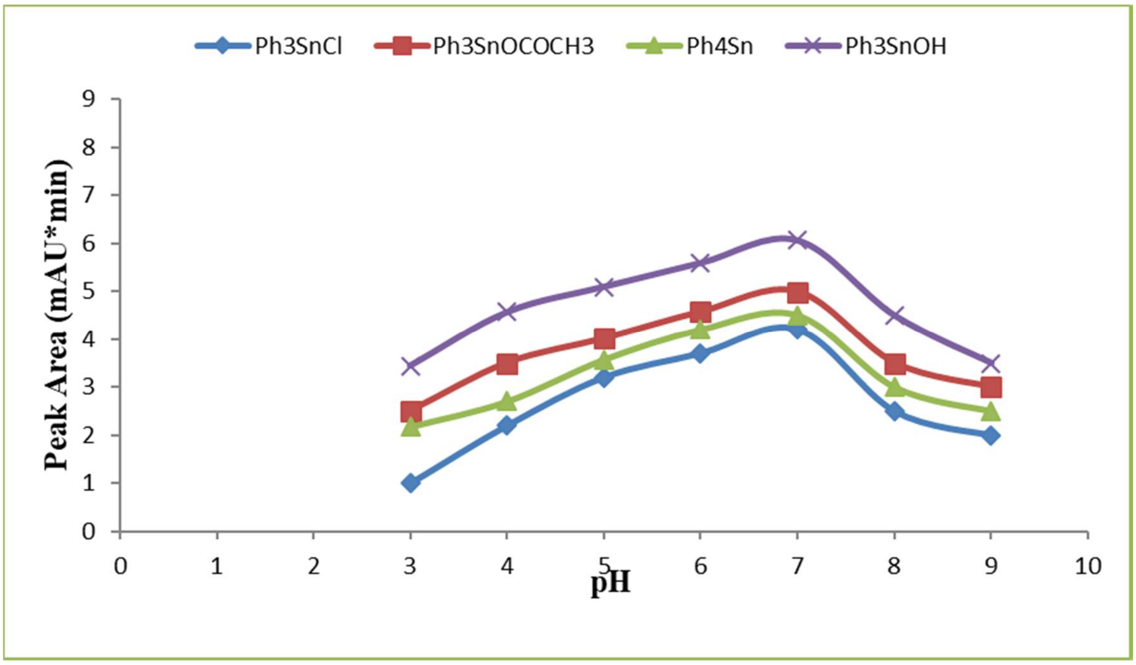


Fig. 6: Effect of pH on extraction efficiency

3.4.5. Carryover effect

To avoid the carryover effect, the fabric was washed with eluting solvent 2-3 times and then with water before subsequent experiment. The carryover was checked by injecting elution solvent to the chromatographic instrument. To eliminate the memory effect, 2 mL x 3 of toluene was incorporated for washing followed by 2 mL acetonitrile to clean the fabric, before analyzing the next sample. However, due to their easy availability and economic nature, FPSE media can be used as a single use microextraction device to lessen the solvent consumption in washing.

3.4.6. Effect of salt addition

Addition of salt is a common practice to decrease the solubility of target analytes into the sample matrix so that they are induced to interact with the sorption media, resulting in adsorption. The effect of addition of sodium chloride on adsorption characteristics of phenyltin compounds was studied. Salt addition might effects in two different but opposite ways. Addition of salt decreases the solubility of the analytes and compels the analytes to interact with the extraction medium. On the other hand, the salt increases the viscosity of the sample matrix and consequently, retards the mass transfer rate of the analyte from the bulk solution to the sorption medium, resulting in longer extraction equilibrium time. Different concentrations of sodium chloride (1-10%) were added to check the response. Probably due to nonpolar nature of target analytes, there was no appreciable change in the adsorption characteristics and extraction efficiency. However, this observation indicated that the sol gel C18 fabric phase sorptive extraction media is stable in various conditions and extraction efficiency for phenyltin compounds is independent of salinity.

3.5. Method Performance

3.5.1. Preparation of Calibration graph

The optimized FPSE-HPLC-UV conditions (Table 1) were used to prepare calibration curves for the spiked samples (10-100 ng/mL). The calibration curves of phenyltin compounds were obtained by performing linear regression analysis on spiked agricultural, municipal and canned food samples ranging from 10-100 ng/mL and were found linear over this range. The calibration curves were described by the equation; $y = mx + c$, where y is peak area ratio, x is the concentration, m is the slope, and c is the intercept. The developed method was validated to deliver optimal analytical performance for the overall target analyte suite. Reproducibility was studied by evaluating the relative standard deviations for three replicate injections for each concentration of target analytes. The repeatability of the method was evaluated intra- and inter-day using a triplicate analysis of each sample. Detection limits were calculated from a signal that was three times the noise. The LOD was defined as the lowest concentration that gave a signal to noise ratio that was equal to 3. The LOQ was defined as the lowest concentration that gave a signal to noise ratio that was equal to 10.

Table 1: Optimized FPSE-HPLC-UV conditions for phenyltin derivatives

	Fabric sol-gel coating	**C18 fiber**
FPSE	Eluting solvent	Toluene
	Extraction time	20 min
	Back extraction time	15 min
	pH	7.0
	Salting effect	Nil
HPLC	Pump	Dionex P680
	Column	Waters spherisorb C18
	Mobile Phase	90:10 (acetonitrile: water)
	Injection volume	20μL
	Flow Rate	1.00 mL/min

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3.5.2. Linearity, Accuracy and Precision

The method was validated in order to deliver the optimal analytical performance for the target analytes. The linearity of the method for the phenyltin derivatives was determined using the standard calibration curve and other analytical parameters (Table 2). The accuracy and precision of FPSE-HPLC-UV method were evaluated for each analyte by analyzing a standard of known concentration and quantifying it using the calibration curve. The precision expressed as relative standard deviation, RSD was evaluated under intraday and interday conditions at 10 ng/mL concentration. As can be seen in Table 2, the repeatability (n = 3) ranged from 2% to 3%. The reproducibility ranged from 2.7 to 4.2%. The accuracy of phenyltin compounds attributed to the degradation during the analysis of standard prepared compounds after 3-4 days. So regarding the stability of compounds, it is recommended to carry out sample preparation and instrumental analysis on the same day. The developed method is considered selective, since no interference of foreign substances in the concerned region was observed. Once optimization was done, the proposed analytical method was applied for the determination of the target phenyltin derivatives in environmental water samples. A recovery study was performed in order to assess the applicability of the proposed method to determine phenyltin derivatives in agricultural and municipal waste water. Since the target analytes were not detected, the samples were spiked with the target analytes at a concentration of 100 ng/mL.

Table 2: Analytical characteristics of developed FPSE-HPC-UV method for determination of Phenyltin compounds (10-100 nm)

Parameter	**$Ph_3SnOCOCH_3$**	**Ph_3SnOH**	**Ph_4Sn**	**Ph_3SnCl**
Regression Equation	$0.126x + 0.398$	$0.153x + 0.369$	$0.088x + 0.92$	$0.11x + 0.335$
Coefficient of determination	0.987	0.991	0.989	0.987
Retention time (min)	3.5	4.1	5.1	5.9
Intra-day RSD (%)	2.5-3.0	2.0-2.5	2.2-2.6	2.0-2.4
Inter-day RSD (%)	3.6-4.2	2.7-3.2	3.0-3.5	3.3-3.9
Limit of detection (LOD) (ng/mL)	0.29	0.26	0.34	0.30
Limit of Quantification (LOQ) (ng/mL)	0.96	0.86	0.11	0.10
Retention factor	0.66	0.95	1.42	1.80
Selectivity	1.42	1.5	1.26	2.71

3.6. Applications

Research and development over the years has provided reliable and selective analytical techniques that can be used for water analysis research, monitoring and health risks assessment including sampling, testing and validation, although some challenges still remain unsolved in regard to the accessibility of efficient and economic sampling techniques. To assess the wider applicability of developed FPSE based method, it was applied to agricultural waste and municipal waste water. The performances of the new analytical method were tested in real urine samples collected from healthy volunteers after single oral administration of canned food. Samples were extracted by FPSE and then analyzed using HPLC-UV according to the method herein reported. The sample of this target canned food was protein powder and amount of tin content found was 0.152 mg/kg. However, this content is very low but may also present in higher amount in some kind of acidic canned foods. Although the maximum permissible limit of tin 200 ppm was not exceeded, but when these cans remained open for certain hours then the dissolution of the tin from the can surface occurred and tin concentration more than 200 ppm were observed. Therefore, it is recommended that after opening the can the contents should immediately be transferred to some other glass vessels.

The recoveries from real samples were determined by comparing the recovered amounts of phenyltin with original spiked concentrations. The relative recoveries obtained from the spiked waste water sample (Fig. 7) were around 90 to 102%. The recoveries range more than 90% obtained were quite satisfactory for a broad concentration range (Table 3).

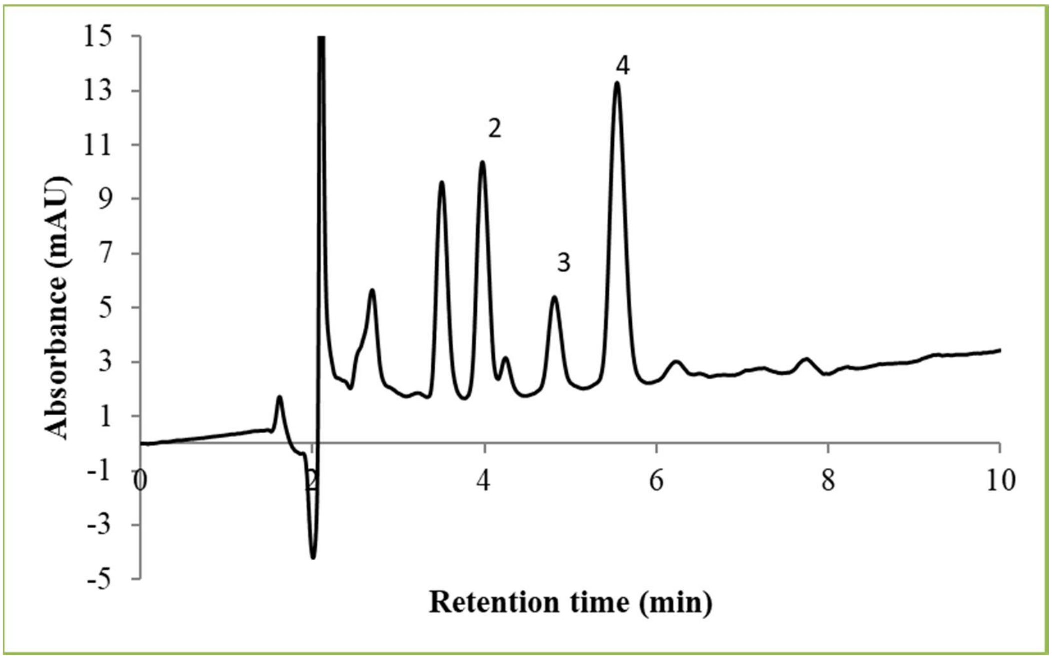

Fig. 7: FPSE-HPLC-UV Chromatogram in agricultural waste water sample of spiked 1-triphenyltin acetate; 2- triphenyltin hydroxide; 3-tetraphenyltin; 4- triphenyltin chloride.

Table 3: Recoveries of phenyltins in environmental water, canned food and urine samples

Analyte	**Added**	**Agricultural waste Water**		**Municipal waste Water**		**Canned food**		**Urine sample**	
		Found (RSD)	Recovery (%)	Found (RSD)	Recovery (%)	Found (RSD)	Recovery (%)	Found (RSD)	Recovery (%)
TPTA	0.1	0.092 (3.5)	92.00	0.094 (3.7)	94.00	0.102 (3.6)	102.00	0.092 (3.9)	92.00
	0.5	0.467 (3.2)	93.40	0.488 (3.5)	97.60	0.501 (3.4)	100.20	0.489 (3.7)	97.80
	10	9.274 (3.0)	92.74	9.755 (3.4)	97.59	9.967 (3.2)	99.67	9.897 (3.6)	98.97
TPTH	0.1	0.088 (2.9)	88.00	0.097 (2.8)	97.00	0.099 (3.2)	99.00	0.097 (3.0)	97.00
	0.5	0.475 (2.7)	95.00	0.482 (2.6)	96.40	0.499 (3.0)	99.80	0.477 (2.8)	95.40
	10	9.336 (2.5)	93.36	9.864 (2.4)	98.60	9.987 (2.9)	99.87	0.980 (2.7)	98.00
TPT	0.1	0.092 (3.6)	92.00	0.095 (3.7)	95.00	0.099 (3.5)	99.00	0.097 (3.9)	97.00
	0.5	0.452 (3.4)	90.40	0.491 (3.5)	98.20	0.496 (3.3)	99.20	0.495 (3.7)	99.00
	10	9.696 (3.2)	96.69	0.978 (3.3)	97.8	9.899 (3.1)	98.99	0.987 (3.5)	98.70
TPTC	0.1	0.091 (3.4)	91.00	0.095 (3.1)	95.00	0.103 (3.6)	103.00	0.096 (3.2)	96.00
	0.5	0.483 (3.2)	96.60	0.491 (3.1)	98.20	0.503 (3.2)	100.60	0.493 (3.1)	98.60
	10	9.675	96.75	9.765	97.65	10.12	101.20	9.784	97.84

	(3.1)	(2.8)	(3.0)	(2.9)

4. Conclusion

Fabric phase sorptive extraction is a promising solvent-minimized sample preparation technique for the high-efficiency extraction of different analytes from various matrices. By choosing the suitable FPSE sorbent chemistry and optimizing sorption and elution conditions, FPSE can be used in solving many difficult and unsolved analytical problems. In the current study, all the parameters related to FPSE, such as extraction and desorption times, eluting solvent, effect of salt addition, pH of the sample have been optimized in order to get better recoveries for all compounds. The inherent porosity of sol-gel sorbent and characteristic permeability of flexible cellulose fabric substrate results in rapid extraction of phenyltin derivatives and accomplishes extraction equilibrium in a short period of time. Since it is a rapid, solvent-minimized, user-friendly and direct method that simplifies the overall sample preparation workflow, it can be used in routine analysis with high sample throughput at low cost. The flourished FPSE-HPLC-UV method shows a good sensitivity and selectivity and it offers low detection limits that ranged from 2.4-3.6 ng/mL and lower relative standard deviation which are appropriate in the analysis of phenyltin derivatives.

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5. References

1. J.B. Graceli, G.C. Senaa, P.F.I. Lopesa, G.C. Zamprognob, M.B. da Costab, A.F.L. Godoic, D.M. dos Santosd, M.R. Rodrigues de Marchie and M.A. dos Santos Fernandezfet, Organotins: A review of their reproductive toxicity, biochemistry, and environmental fate, Reproduc. Toxicol 36 (2013) 40-52, https://doi.org/10.1016/j.reprotox.2012.11.008.
2. A.O. Sunday, B.A. Alafara and O.G. Oladele, Toxicity and speciation analysis of organotin compounds, Chem. Spec. Bioavailab 24 (2012) 216-226, http://dx.doi.org/10.3184/095422912X13491962881734.
3. J. Fao, Joint FAO/WHO food standards programme codex committee on contaminants in foods fifth session, The Hague, Netherlands, (2011).
4. S. Dobson, P. Howe and P. Floyd, WHO, Mono and disubstituted methyltin, butyltin, and octyltin compounds, Concise International Chemical Assessment Document No. Geneva, 73 (2006).
5. G.A. Zachariadis and E. Rosenberg, Speciation of organotin compounds in urine by GC-MIP-AED and GC-MS after ethylation and liquid-liquid extraction, J. Chromatogr. B 877 (2009) 1140-1144, https://doi.org/10.1016/j.jchromb.2009.02.065.
6. K. Fent, Ecotoxicology of organotin compounds, Crit. Rev.Toxicol 26 (1996) 3-117, http://dx.doi.org/10.3109/10408449609089891.
7. F. Cima, P.J. Craig, C. Harrington, in: P.J. Craig, Organometallic Compounds in the Environment, Wiley & Sons, Chichester, 101 (2003)

8. J. Kuballa, R.D. Wilken, E. Jantzen, K.K.K. Wan and Y.K. Chau, Speciation and genotoxicity of butyltin compounds, Analyst 120 (1995) 667-673, http://dx.doi.org/10.1039/AN9952000667.
9. Y. Suzuki, Y. Endo, M. Ogawa, Y. Kim, N. Onda and K. Yamanaka, Development of an analytical method to confirm toxic trimethylated tin in human urine, J. Chromatogr. B 868 (2008) 116-119, https://doi.org/10.1016/j.jchromb.2008.04.032.
10. M. Furdek, M. Vohcic, J. Scancar, R. Milacic, G. Kniewald, N. Mikac, Organotin compounds in seawater and Mytilus galloprovincialis mussels along the Croatian Adriatic Coast, Marine Poll. Bullet 64 (2012) 189-199, https://doi.org/10.1016/j.marpolbul.2011.12.009.
11. Y. Liu, Y. Wan and L. Guo, Determination of Organotin Compounds in Wine by Microwave-Assisted Extraction and High Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry, Anal. Lett 47 (2014) 343-355, http://dx.doi.org/10.1080/00032719.2013.834445.
12. M. Hoch, Organotin compounds in the environment- an overview, Appl. Geochem 16 (2001) 719-743, https://doi.org/10.1016/S0883-2927(00)00067-6.
13. K.E. Levine, D.J. Young, S.E. Afton, J.M. Harrington, A.S. Essader , F.X. Weber, R.A. Fernando, K. Thayer, E.E. Hatch, V.G. Robinson and S. Waidyanatha, 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, Talanta, 140 (2015) 115-121, https://doi.org/10.1016/j.talanta.2015.03.022.
14. Y. Suzuki, Y. Endo, M. Ogawa, Y. Kim, N. Onda and K. Yamanaka, Development of an analytical method to confirm toxic trimethylated tin in human urine, J. Chromatogr. B 868 (2008) 116-119, https://doi.org/10.1016/j.jchromb.2008.04.032.
15. M. Furdek, M. Vohcic, J. Scancar, R. Milacic, G. Kniewald, N. Mikac, Organotin compounds in seawater and Mytilus galloprovincialis mussels along the Croatian Adriatic Coast, Marine Poll. Bull 64 (2012) 189-199, https://doi.org/10.1016/j.marpolbul.2011.12.009.
16. J. Du, S. Chadalavada, Z. Chen and R. Naidu, Environmental remediation techniques of tributyltin contamination in soil and water: A review, Chem. Eng. J 235 (2014) 141-150, https://doi.org/10.1016/j.cej.2013.09.044.
17. P. Rodriguez-Gonzalez, J.I.G. Alonso and A. Sanz-Medel, Single and multiple spike procedures for the determination of butyltin compounds in sediments using isotope dilution GC-ICP-MS, J. Anal. Atomic Spectrom 20 (2005) 1076-1084, https://doi.org/10.1039/B505653E.
18. M. Leermakers, J. Nuyttens and W. Baeyens, Organotin analysis by gas chromatography–pulsed flame-photometric detection (GC–PFPD), Anal. Bioanal. Chem 381 (2005) 1272-1280, https://DOI 10.1007/s00216-004-3050-y.
19. L. Guo, F.M. Matysik and P. Glaser, Speciation of organotin compounds by capillary electrophoresis: comparison of aqueous and mixed organic-aqueous systems, Anal. Bioanal. Chem 380 (2004) 669-676, https://DOI 10.1007/s00216-004-2800-1.
20. A.M. Leach, M. Heisterkamp and F.C. Adams, Gas chromatography-inductively coupled plasma time-of-flight mass spectrometry for the speciation analysis of organometallic compounds, J. Anal. Atomic Spectrom 15 (2000) 151-155, https://10.1039/A905825G.
21. P. Rychlovsky, P. Cernoch and M. Sklenickova, Application of a heated electrospray interface for on-line connection of the AAS detector with HPLC for detection of organotin and

organolead compounds, Anal. Bioanal. Chem 374 (2002) 955-962, https://DOI 10.1007/s00216-002-1558-6.

22. D.N. Van, R. Lindberg and W. Frech, Redistribution reactions of butyl-and phenyltin species during storage in methanol, J. Anal. Atomic Spectrom 20 (2005) 266-272, https://DOI 10.1039/B416570E.
23. S. Aguerre, C. Pecheyran and G. Lespes, Validation, using a chemometric approach, of gas chromatography–inductively coupled plasma–atomic emission spectrometry (GC-ICP-AES) for organotin determination, Anal. Bioanal. Chem 376 (2003) 226-235, https://DOI 10.1007/s00216-003-1898-x.
24. *S.J. Kumar, S. Tesfalidet and J.P. Snell,* A simple method for synthesis of organotin species to investigate extraction procedures in sediments by isotope dilution-gas chromatography-inductively coupled plasma mass spectrometry Part 2. Phenyltin species, J. Anal. Atomic Spectrom 19 (2004) 368-372, https://DOI//10.1039/B313787B.
25. P. Rodriguez-Gonzalez, J.R. Encinar and J.I.G. Alonso, Monitoring the degradation and solubilisation of butyltin compounds during in vitro gastrointestinal digestion using “triple spike” isotope dilution GC-ICP-MS, Anal. Bioanal. Chem 381 (2005) 380-387, https://DOI 10.1007/s00216-004-2833-5.
26. J. Wu, Z. Mester and J. Pawliszyn, Determination of tributyltin by automated in-tube solid-phase microextraction coupled with HPLC-ES-MS, J. Anal. Atomic Spectrom 16 (2001) 159-165 https://DOI 10.1039/B007321K.
27. E. Gonzalez-Toledo, R. Compano, M.D. Prat and M. Granados, Determination of triorganotin species in water samples by liquid chromatography–electrospray-mass spectrometry, J. Chromatogr. A 946 (2002) 1-8, https://doi.org/10.1016/S0021-9673(01)01519-9
28. S.K. Lal, G.H. Tan, N.H. Tioh and V.G. Kumar Das, Speciation of phenyltin (IV) compounds using high-performance liquid chromatography: Part 1. The direct analysis of mixed standard solutions of tetraphenyltin, triphenyltin chloride, triphenyltin hydroxide and tripheyltin acetate, Appl. Organomet. chem 16 (2002) 250-257, https://DOI: 10.1002/aoc.292.
29. X. Wang, L. Ding, H. Zhang, J. Cheng and Y. Li, Development of an analytical method for organotin compounds in fortified flour samples using microwave-assisted extraction and normal-phase HPLC with UV detection, J. Chromatogr. B 843 (2006) 268-274, https://doi.org/10.1016/j.jchromb.2006.06.013.
30. E Gonzalez-Toledo, R Compano, M Granados and M.D Prat, Determination of butyltin and phenyltin species by reversed-phase liquid chromatography and fluorimetric detection, J. Chromatogr. A, 878 (2000) 69-76, https://doi.org/10.1016/S0021-9673(00)00278-8.
31. A.P. Birjandi, A. Bidari, F. Rezaei, M.R.M. Hosseini and Y. Assadi, Speciation of butyl and phenyltin compounds using dispersive liquid–liquid microextraction and gas chromatography-flame photometric detection, J. Chromatogr. A 1193 (2008) 19-25, https://doi.org/10.1016/j.chroma.2008.04.003.
32. M.A. Bravo, S. Parra, C. Vargas and W. Quiroz, Determination of organotin compounds in sediment samples by dispersive liquid-liquid microextraction followed by gas chromatography-pulsed flame photometric detection (DLLME-GC-PFPD), Microchem. J 134 (2017) 49-53, https://doi.org/10.1016/j.microc.2017.05.009.
33. *A. Kabir and K.G. Furton*, Fabric phase sorptive extractors (FPSE). US Patent: 9,557,252 (2017)

34. A. Kabir, K.G. Furton and F.A. Malik, Innovations in sol-gel microextraction phases for solvent-free sample preparation in analytical chemistry, Trends. Anal. Chem 45 (2013) 197-218, https://doi.org/10.1016/j.trac.2012.11.014.
35. R. Kumar, Gaurav, Heena, A.K. Malik, A. Kabir and K.G.Furton, Efficient analysis of selected estrogens using fabric phase sorptive extraction and high performance liquid chromatography-fluorescence detection, J. Chromatogr. A 1359 (2014) 16-25, https://doi.org/10.1016/j.chroma.2014.07.013.
36. G.A. Zachariadis and E. Rosenberg, Speciation of organotin compounds in urine by GC-MIP-AED and GC-MS after ethylation and liquid–liquid extraction, J. Chromatogr. B 877 (2009) 1140-1144, https://doi.org/10.1016/j.jchromb.2009.02.065.
37. C. Coscolla, S. Navarro-Olivares, P. Marti and V. Yusa, Application of the experimental design of experiments (DoE) for the determination of organotin compounds in water samples using HS-SPME and GC-MS/MS, Talanta, 119 (2014) 544-552, https://doi.org/10.1016/j.talanta.2013.11.052.
38. E. de Oliveira, Sample Preparation for Atomic Spectroscopy: Evolution and Future, J. Braz. Chem. Soc 14 (2003) 174-182, http://dx.doi.org/10.1590/S0103-50532003000200004.
39. A. Kabir, K.G. Furton, A. Malik, Innovations in sol-gel microextraction phases for solvent-free sample preparation in analytical chemistry, Trends Anal. Chem 45 (2013) 197-218, https://doi.org/10.1016/j.trac.2012.11.014.
40. A. Kabir, C. Hamlet and A. Malik, Parts per quadrillion level ultra-trace determination of polar and nonpolar compounds via solvent-free capillary microextraction on surface-bonded sol-gel polytetrahydrofuran coating and gas chromatography-flame ionization detection, J. Chromatogr. A 1047 (2004) 1-13, https://doi.org/10.1016/j.chroma.2004.06.078.