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Application of the experimental design of experiments (DoE) for the determination of organotin compounds in water samples using HS-SPME and GC–MS/MS

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ABSTRACT

When attempting to discover the important factors and then optimise a response by tuning these factors, experimental design (design of experiments, DoE) gives a powerful suite of statistical methodology. DoE identify significant factors and then optimise a response with respect to them in method development. In this work, a headspace-solid-phase micro-extraction (HS-SPME) combined with gas chromatography tandem mass spectrometry (GC–MS/MS) methodology for the simultaneous determination of six important organotin compounds namely monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), monophenyltin (MPhT), diphenyltin (DPhT), triphenyltin (TPhT) has been optimized using a statistical design of experiments (DOE). The analytical method is based on the ethylation with $NaBEt_4$ and simultaneous headspace-solid-phase micro-extraction of the derivative compounds followed by GC–MS/MS analysis. The main experimental parameters influencing the extraction efficiency selected for optimization were pre-incubation time, incubation temperature, agitator speed, extraction time, desorption temperature, buffer (pH, concentration and volume), headspace volume, sample salinity, preparation of standards, ultrasonic time and desorption time in the injector. The main factors (excitation voltage, excitation time, ion source temperature, isolation time and electron energy) affecting the GC-IT-MS/MS response were also optimized using the same statistical design of experiments. The proposed method presented good linearity (coefficient of determination $R^2 > 0.99$) and repeatibilty (1–25%) for all the compounds under study. The accuracy of the method measured as the average percentage recovery of the compounds in spiked surface and marine waters was higher than 70% for all compounds studied. Finally, the optimized methodology was applied to real aqueous samples enabled the simultaneous determination of all compounds under study in surface and marine water samples obtained from Valencia region (Spain).

1. Introduction

Statistical design of experiments (DOE) is superior to the traditional change-one-at-a-time approach, when different factors need to be optimized. If the factors in the design are correlated, that is if the change in response to a change in a factor level depends on the level of another factor, then it is unlikely that the optimum will be discovered and more experiments than necessary will have been done.

There are two kinds of chemical problems that need experimental design for their solution. The first is to discover which factors may significantly affect the response of an experiment, and the second to find factor values that optimise the response.

The object of the screening and robustness studies is to perform a minimum number of experiments on a maximum number of factors. These designs are done as a prelude to an optimisation, to make sure that factors being investigated do indeed significantly contribute to the response [1].

Organotin compounds are released through several routes into the environment. The major input of triorganotin compounds into aquatic systems derives from their use in anti-fouling paints. Harbor areas are specially affected by TBT contamination. In harbor sediments, flakes of anti-fouling paints from the removal of old coatings may be present and may serve as reservoirs that cause locally high concentrations of TBT. This substance is an endocrine disruptor, affecting marine organisms even at $ng\,L^{-1}$. Consequently, the determination of TBT is essential in order to assess the risks of an environmental pollution event. For other compounds such as triphenyltin (TPhT), the imput via their use as pesticides in agriculture is more important. Waters may be contaminated with organotin compounds by effluents from industrial plants. Further imputs to the environment result from the large-scale use of polyvinyl chloride (PVC), which contains mono- and diorganotin compounds as stabilizers. Leachate from landfills where organotin-containing

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wastes are dumped may contain organotin residues, as well as municipal wastewater and sewage sludge [2].

Apart from TBT, the other organotin compounds have been extensively used as active ingredients of antifouling agents commonly applied to protect boats and ships. The most toxic organotin species correspond to triorganotins, followed by di- and monoorganotin compounds [3]. Environmental studies conducted in different localities have shown that organotin compounds are present in surface water, the water column and sediment. Its distribution is influenced by factors such as the species and population density of aquatic organisms, dissolved and suspended organic material, pH, salinity, temperature and solubility in water [2].

Seasonal variations in the concentrations of organotin compounds between hot and cold seasons often occur due to the increase of anthropogenic sources during the summer (for example, tourism and use of boats increases during spring and summer) and degradation of organotin compounds. When studying surface sea waters from the Dona Paula Bay (west coast of India) collected at weekly intervals during March 2007 to April 2008, noted the occurrence of butyltin-BT compounds such as TBT (tributyltin), DBT (dibutyltin) and MBT (monobutyltin). In the study, the authors found that the concentrations of DBT and MBT were higher from October to March of 2008, while the concentratiom of TBT decreased in the same period. This difference between the concentrations of organotin compounds (BT, TBT, DBT and MBT) were attributed to recreation activities that were reduced due to expansion of the pier. This reduced the input of TBT compounds to the water, and allowed degradation of TBT to its by-products DBT and MBT [2].

Directive 2000/60/CE of the European Parliament and of the Council established a framework for the Community action in the field of water policy. This legislation included butyltins in the list of 33 priority substances [4]. Later, European Directive 2008/105/CE on environmental quality standards in the field of water policy establishes annual average and maximum allowable concentration for some priority substances and certain other pollutants [5].

A large number of extraction methods have been described for the determination of these compounds from water samples. An alternative approach to classical extraction techniques such as liquid–liquid or solid-phase extraction is the solid-phase microextraction technique (SPME). SPME is a simple, fast and solvent-free technique, which combines extraction, concentration and sample introduction into GC injector in one single device. The use of SPME is continuosly increasing in speciation analysis; which is reflected in various reviews focusing on SPME methods for the analysis of metallic and organometallic species [3]. Thus, different papers can be found reporting the SPME application to speciation of mercury [3,6,7], organotin compounds [3,7,8], organolead compounds and multielemental speciation of organometallic compounds of mercury, lead and tin [3,9,10].

Taking into account the literature, the main methods of extraction in SPME are based on the characteristics of the analyte and the matrix, and the main experimental factors that affect the efficiency of extraction are the pre-incubation time, incubation temperature, agitator speed, extraction time, desorption temperature, buffer (pH, concentration and volume), derivatization concentration, headspace volume, sample salinity, preparation of standards, ultrasonic time and desorption time in the injector.

As organometallic compounds differ significantly in volatility, stability and polarity, a suitable derivatisation technique is essential to make them amenable for GC separation. A common derivatisation method is based on in situ aqueous phase ethylation of the analytes with sodium tetraethylborate ($NaBEt_4$) prior to the extraction [3,11].

Gas chromatography is the most frequently employed technique due to its excellent separation efficiency and the availability of a number of suitable detectors. In this way, the determination of organotin compounds has been carried out using gas chromatography coupled to highly sensitive and specific detection methods, such as flame photometric detector (GC-FPD) [12], pulsed flame photometric detector (GC-PFPD) [13], inductively coupled plasma atomic emission spectrometry (GC-ICP-AES) [14], mass spectrometry [15], inductively coupled plasma mass spectrometry (GC-ICP-MS) [16], and mass spectrometry in tandem mode (GC–MS-MS) [17].

Different parameters can potentially affect the analytical response of the ITMS system. On the basis of the literature [18,19] and the experience of our laboratory five factors were selected: excitation voltage (EV), excitation time (ET), ion source temperature (IST), isolation time (IT) and electron energy (EE).

A Plackett–Burman (P–B) design was chosen as a screening method to estimate the relative influence of the selected factors that could have an influence on the analytical response [21]. The P–B design assumes that the interactions can be completely ignored and so the main effects are calculated with a reduced number of experiments (12 plus a triplicate centre point). The estimated effects of the factors and their statistical significacne at 95% confidence level ($p < 0.05$) were studied. After the selection of the factors that potentially affects to the HS-SPME and GC–MS/MS, a central composite designs (CCD) have been used and preferred to one-factor-at-a-time to optimize analytical methods [20]. The selection of the factors setting that simultaneously optimize the organotin compounds responses in the HS-SPME, was done using the "response optimiser" from response surface design in the MINITAB program.

This paper focuses on the use of the statistical design of experiments (DOE) [21–23] for the optimization of a method for the simultaneous determination of six organotin compounds in water samples, using headspace-solid phase microextraction and gas chromatography coupled to mass spectrometry in tandem (MS/MS). This detection mode, in addition to improving the selectivity of the technique with a drastic reduction of the background, has a high capability of confirmation. Thus, the advantage of headspace SPME was combined with GC–MS/MS in order to minimize matrix interferences and provide a selective method for simultaneous determination of MBT, DBT. TBT. MPhT, DPhT and TPhT in surface and marine water. Finally, this methodology has been applied to the analysis of water samples in Valencia region.

2. Experimental

2.1. Chemicals and reagents

Phenyltin trichloride (MPhT, 98%), diphenyltin dichloride (DPhT, 96%), triphenyltin chloride (TPhT, 95%), butyltin trichloride (MBT, 95%), dibutyltin dichloride (DBT, 96%), tributyltin chloride (TBT, 96%) and triphenyltin-d15 chloride (TPhTd15, 98%) were purchased from Sigma Aldrich (Steinheim, Germany). Glacial acetic acid (99.99%) and sodium acetate were obtained from Sigma-Aldrich (Bornem, Belgium) and ethanol (Suprasolv grade) from Merck (Darmstadt, Germany). Sodium tetraethylborate ($NaBEt_4$) (97%) was pursached from Sigma-Aldrich (Steinheim, Germany). Milli-Q water was obtained by purification and deionization of tap water in Milli-Q plus water system (Millipore, Bedford, MS, USA).

A 1% (m/v) solution of $NaBEt_4$ in Milli-Q water was freshly prepared daily. It was kept in desiccators into a glove bag under dry nitrogen and was always manipulated in the glove bag inside of an extraction hood. Aldrich AtmosBag two-hands, non sterile, closure type, Tape-sea was provided by Sigma-Aldrich (Steinheim, Germany). Solution was prepared every day taking certain amount

of reagent and adding the necessary volume of pure water into dark and sealed vial.

A HOAc/NaOAc buffer pH 5.3 was prepared by adding an appropriate amount of HOAc to a 0.2 M solution of NaOAc in Milli-Q water.

Deuterated organotin standard (TPhT d15) was used as internal standard for all compounds. For internal standardization, a stock solution of 10 μg mL^{-1} in ethanol was used to spike water samples.

Glassware was rinsed with Milli-Q water, decontaminated overnight in 10% (v/v) nitric acid solution and rinsed again. This procedure for cleaning is important in order to avoid possible contamination [13,31].

Four stock solutions of 10,000, 100, 0.1 and 0.01 μg mL^{-1} from native organotin compounds were prepared in ethanol. Ethanol was selected as a solvent in the standard preparation because it is more environmentally friendly than methanol [24,28]. All stocks and working solutions were stored in the dark at 4 °C in a refrigerator. Standards (1, 2, 3, 4, 5 and 6 μg L^{-1}) were obtained by a set of weekly and daily dilutions in ethanol.

2.2. Sample preparation

Aqueous test samples were prepared by adding an appropriate amount of organotin working solutions for preparing the standards and fortified samples or 5 mL for preparing the unknown samples to a mixture of 5 mL Milli-Q water and 5 mL buffer solution (pH=5.3) in a closed-cap headspace vial of 20 mL. Later, 66 μL of the internal standard was added, resulting in a concentration of approximately 3.5 μg mL^{-1}.

Derivatization is performed by adding 300 μL of a 1% $NaBEt_4$ solution. The derivatisation step involves the ethylation of organotin in order to obtain thermally stable tetrasubsituted species sufficiently volatile for GC separation. This step has been previously optimized and validated [13]. The vial volume is completed with a total volume of 11 mL using Milli-Q water.

Sample vials are vigorously shaken and they are introduced in an ultrasonic bath during 10 min. Finally, they are placed in the MPS-2 autosampler for headspace-SPME extraction.

2.3. Optimization of headspace-solid phase microextraction conditions

The fiber was introduced in the headspace of a glass vial containing the standard samples of organotins. Afterwards, the SPME device was placed into the GC interface and the organotins were desorbed from the fiber under static mode during 1 min.

A SPME holder with replaceable extraction fibers was used for extraction of organotin compounds from water samples. The fiber used in the study was coated with 100 μm thickness polydimethylsiloxane (PDMS), needle size 23 Ga, fused silica, red hub plain. The SPME holder and the fibers were obtained from Supelco (Bellefonte, PA, USA). After each sample exposition, the fiber is cleaned during 10 min at 250 °C.

Taking into account the literature, the main factors affecting the HS-SPME are the following: pre-incubation time, incubation temperature [11,13,24–28], incubation, pre-incubation agitator speed [13,24–26,29], extraction time [24], desorption temperature [11,13,24,25,27–29], buffer pH [11,13,24,25,28,30,31], buffer concentration [25], buffer volume, derivatization concentration [25,31], headspace volume [11,24,28,29], sample salinity [25], preparation of standards, ultrasonic time [24], and desorption time in the injector [11,13,24,25,27–29].

The relative influence of these factors on the analytical response (arbitrary units of peak area of the ions with majority relative abundance) was studied with a Plackett–Burman (P–B) design [21] (Table SD-1). This screening design allows us to find the parameters that have the largest influence with a reduced number of experiments.

The minimum and maximum values used in the P–B are: pre-incubation time (0.10–5 min), incubation temperature (70–90 °C), pre-incubation agitator speed (250–750 rpm), extraction time (15–45 min), desorption time (30–90 s), desorption temperature in the injector (225–300 °C), buffer pH (4.5–6), buffer concentration (0.1–0.3 M), buffer volume (4–6 mL), derivatization concentration (0.5–1.5%), headspace volume (9–11 mL), sample salinity (0–3%), preparation of standards and ultrasonic time (5–15 min).

The in situ ethylation with $NaBEt_4$ was also used in other works [32], to overcome the difficulties associated with extracting ionic organotin analytes from an aqueous matrix. Ikonomou et al. [32] shows the loss incurred when performing a separate liquid–liquid extraction with hexane and then derivatization, as opposed to the in situ derivatization and simultaneous extraction. Monobutyltin and monophenyltin compounds suffer the largest recovery loss when separate extraction and derivatization procedures are employed, especially when the less polar extraction solvent was used. However, in situ extraction/derivatization with $NaBEt_4$ provided the most quantitative extraction and derivatization of organotins.

2.4. Optimization of gas chromatography-ion trap mass spectrometer

Analyses were performed on a Finnigan ion trap mass spectrometer Polaris Q (Austin, TX, USA). The mass spectrometer was connected by a heated transfer line to a Thermoquest Trace GC 2000 (Waltham, MA, USA) gas chromatograph equipped with a Combi Pal Autosampler from CTC Analytics AG (Zwingen, Zwitzerland). Xcalibur 1.2 was used for data acquisition. The analysis were carried out with a 30 m × 0.25 mm i.d., 0.25 μm film thickness SGE-BPX5 capillary column (Austin). The carrier gas was helium (constant pressure 70 hPa, 41 cm s^{-1} at 50 °C). A liner silcosteel PTV 1 × 2.75 × 120 was installed in the split/splitless injector and the temperature was set at 250 °C. High Pressure Microseal Septum was pursached from Supelco (Bellefonte, PA, USA). The oven was programmed from 50 °C (1 min) at 10 °C min^{-1} to 300 °C (4 min). After each sample exposition column is heated at 310 °C during 6 min for removing possible contaminated interferences. Transfer line was set at 300 °C. The electron impact (EI) ionization mode was selected working with an electron energy of 70 eV. The ionization source temperature was set at 230 °C.

Taking into account the literature [23], the main factors affecting the GC-IT-MS/MS are the following: excitation voltage (EV), excitation time (ET), ion source temperature (IST) [11,24,29], isolation time (IT), electron energy (EE) [11,29].

The relative influence of these factors on the analytical response (arbitrary units of peak area of the ions with majority relative abundance) was studied with a Plackett–Burman (P–B) design [21] (Table SD-2). This screening design allows us to find the parameters that have the largest influence with a reduced number of experiments. In order to have generous degrees of freedom for testing the statistical significance of the estimated effects, 12 runs plus a triplicate centre point were used.

The minimum and maximum values used in the P–B are: EV (0.1–1.05–2 V), ET (5–27.5–50 ms), IST (150–200–250 °C), IT (4–19–34 ms) and EE (35–55–75 eV).

Numerical analysis of data resulting from the experimental design was carried out by means of the statistical package MINITAB for Windows, Release 14, Minitab Inc., USA.

3. Results and discussion

3.1. Optimization of MS–MS parameters

Tandem mass spectrometry (MS–MS) combined with ion trap instruments has been scarcely used for the determination of organotin compounds in water [3,33]. Many developed methods for organotin compounds in water consist on a HS-SPME combined with gas chromatography–mass spectrometry (GC–MS) working in SIM mode [11,24,25]. In this work, we report a method for the determination of six organotin compounds including three butyls and three phenyltins using GC–MS/MS.

Precise optimization of MS–MS parameters is needed in order to maximize the signal for each organotin. The first step of the MS–MS optimization was the selection of the most selective and abundant ion (as the precursor ion) from each compound performing a full scan spectra.

Table 1 shows the precursor ion selected for each organotin compound. Precursor ions were isolated in the ion trap and fragmented by collision-induced dissociation (CID) [18] and the two most abundant product ions for each compound were selected. Selected quantification ions were chosen for each organotin and other characteristic ions were also selected in order to identify each compound. This MS–MS experiment was carried out with the default operating parameters provided by ITMS system. Table 1 shows quantification and identification ions used for the studied organotins.

Different parameters can potentially affect the analytical response of the ITMS system. On the basis of the literature [18,19] and the experience of our laboratory five factors were selected: resonance excitation voltage (EV), isolation time (IT), excitation time (ET), ion source temperature (IST) and electron energy (EE).

A Plackett–Burman (P–B) [21] design was chosen as a screening method to estimate the relative influence of the five factors indicated before on the analytical response, taken as arbitrary units of peak area of the product ions for each compound. The P–B design assumes that the interactions can be completely ignored and so the main effects are calculated with a reduced number of experiments (12 plus a triplicate center point). The estimated effects of the five factors and their statistical significance at 95% confidence level ($p < 0.05$) are shown in Table 2.

As can be seen in Table 2, Excitation Voltage (EV), Electron Energy (EE) and Excitation Time (ET) had a significant effect on the analytical response for most of compounds studied. To optimize these parameters the variation of the response (peak area) at different values of EV, EE and ET was studied using a Central Composite Design (CCD) [21]. This design allows to obtain a more accurate optimization of the three significant parameters (Excitation Voltage, Electron Energy and Excitation Time). This design consists of a full factorial design (8 hypercube points, 2 axial points and 10 central points). The 20 runs were randomized to provide protection against the effect of hidden variables. The values corresponding to every factor in each experiment and the responses for each compound are shown in Table SD-3. This type of experimental design permits the response surface to be built and the factor settings or operating conditions that maximize organotin response to be found. The factor settings that individually maximize the responses for each of the six compounds for excitation voltage and excitation time were selected using the response optimizer in the Minitab program. The response optimizer parameters were as follows, “goal”: maximize; “low”: the minimum response for each compound obtained in the Central Composite Design experiments and “target”: the maximum response for each compound obtained in the Central Composite Design experiments. In the case of electron energy, the factor settings that simultaneously maximize the responses of the six compounds were selected using the same response optimizer parameters. It is important to mention that only one electron energy could be selected in each injection.

The optimized excitation voltage was as follows for each compound: butyltin trichloride, 0.23 V; dibutyltin dichloride, 1.03 V; tributyltin chloride, 1.35 V; phenyltin trichloride, 1.45 V; diphenyltin dichloride, 1.14 V; triphenyltin chloride, 0.78 V and triphenyltin-d15 chloride 0.95 V. The optimized excitation time was 25 ms for butyltin trichloride; dibutyltin dichloride, 17 ms; tributyltin chloride, 17 ms; phenyltin trichloride, 17 ms; diphenyltin dichloride, 14 ms; triphenyltin chloride, 34 ms and triphenyltin-d15 chloride 36 ms. The other factors were fixed for all compounds as follows: electron energy, 70 eV; ion source temperature, 200 °C and isolation time, 20 s.

Fig. 1.1, 1.2 and 1.3 shows, as an example, some response surfaces obtained by using the three dimensional response surfaces for MBT, MPhT and TPhT. Three dimensional response surfaces show the effect of two independent variables (excitation voltage and excitation time) on a given response, at a constant value of the other independent variable (electron energy).

Table 1
Selected GC–MS/MS experimental parameters for organotin compounds studied.

Compound	Time (min)	Precursor ion (m/z)	Product ions (m/z)
Butyltin trichloride (MBT)	9.57	235	**179**-151
Dibutyltin dichloride (DBT)	11.77	263	**207**-151
Tributyltin chloride (TBT)	13.66	291	**179**-235
Phenyltin trichloride (MPhT)	13.18	255	**227**-199
Diphenyltin dichloride (DPhT)	18.21	303	**275**-197
Triphenyltin chloride (TPhT)	22.35	351	**197**-120
Triphenyltin chloride d15 (TPhT d15)	22.29	366	**202**-120

Other conditions (default values): isolation with, 1.0; isolation time, 12 ms; excitation time, 15 ms; ion source temperature, 250 °C; electron energy, 75 eV; maximum ion time, 25 ms; microescans, 3. Bold indicates quantification ions.

Table 2
Estimated effects and p-values ($\alpha = 0.05$) of the five main factors obtained from Plackett–Burman design used in the optimization of GC-IT–MS/MS.

Compounds	Factors									
	EV effect	EV p-value	ET effect	ET p-value	IST effect	IST p-value	IT effect	IT p-value	EE effect	EE p-value
MBT	54,666	0.002	12,180	0.343	−513	0.967	2,728	0.827	−22,519	0.099
DBT	12,298	0.013	−480	0.904	361	0.928	−7,162	0.102	−9,264	0.044
TBT	9,767	0.026	2,693	0.474	−2,930	0.437	−8,221	0.051	−8,765	0.040
MPhT	70,270	0	444	0.965	−7,320	0.478	3,695	0.717	−32,058	0.012
DPhT	335,695	0	−73,648	0.042	35,883	0.361	−26,674	0.492	−130,555	0.008
TPhT	62,247	0	−15,459	0.181	23,105	0.060	−20,124	0.093	−18,909	0.111

EV (Excitation voltage), ET (Excitation time), IST (Ion source temperature), IT (Isolation time) and EE (Electron energy).

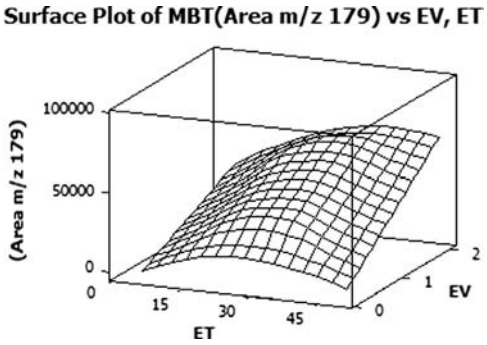

Fig. 1.1. Response surface for MBT. Fixed conditions: electron energy, 70 eV.

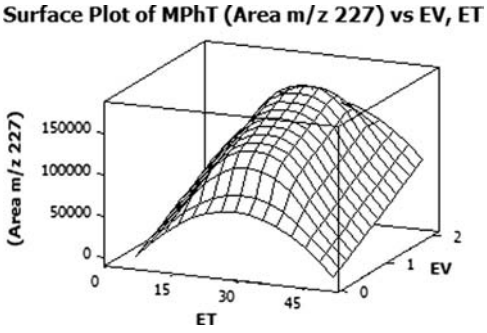

Fig. 1.2. Response surface for MPhT. Fixed conditions: electron energy, 70 eV.

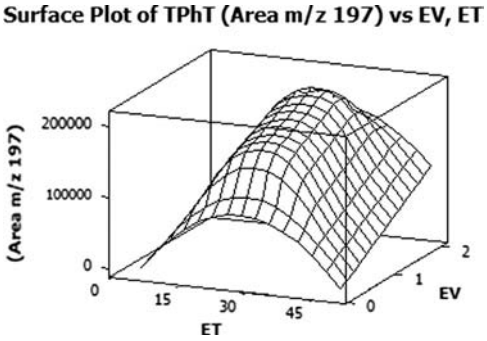

Fig. 1.3. Response surface for TPhT. Fixed conditions: electron energy, 70 eV.

3.2. Optimization of HS-SPME

Standard solutions (3000 ng L^{-1} of each compound) containing a mixture of the organotins evaluated were prepared in a 22 mL vial as follows: 5 mL of Milli-Q water, 5 mL of buffer solution (pH=5.3), 300 μL of a 1% $NaBEt_4$ solution and 66 μL of the internal standard, for performing 20 experiments in the screening design (Plackett–Burman design). The vial volume was completed with a total volume of 11 mL using Milli-Q water.

Table 3 shows the estimated effects of pre-incubation time, incubation temperature, pre-incubation agitator speed, extraction time, desorption time, desorption temperature in the injector, buffer pH, buffer concentration, buffer volume, derivatization concentration, headspace volume, sample salinity, preparation of standards and ultrasonic time, on the optimization of HS-SPME, as well as their statistical significance at 95% confidence level (α=0.05). The parameters that had a significant effect ($p<0.05$) on the response were incubation temperature and buffer pH. Derivatization concentration obtained a p-value very close to stadistically significance for TBT (p=0.054) and MPhT (p=0.053). So, finally we decided to select these three factors for further optimization. The non-significant factor was fixed at the minimum or maximum value checked (5 a.u.) depends on its negative or positive effect on all compounds.

To obtain a more accurate optimization of the three significant parameters (incubation temperature, buffer pH and derivatization concentration), a central composite design (CCD) [21] was carried out. This design consists of a full factorial design (8 hypercube points, 2 axial points and 10 central points). The 20 runs were randomized to provide protection against the effect of hidden variables. The values corresponding to every factor in each experiment and the responses for each compound are shown in Table 4. This type of experimental design permits the response surface to be built and the factor settings or operating conditions that maximize organotin response to be found.

The following step was to select the factor settings (values for incubation temperature, buffer pH and derivatization concentration) that maximize organotin response. The factor settings that simultaneously maximize the responses of the six compounds were selected using the "response optimizer" in the MINITAB program. The response optimizer parameters were as follows, "goal": maximize; "low": the minimum response for each compound obtained in the Central Composite Design experiments and "target": the maximum response for each compound obtained in the Central Composite Design experiments.

As we have multiple responses (one for each organotin), and as the response surfaces are different for each compound, it is necessary to find a factor setting that simultaneously maximizes the desirability for each response. The desirability is 0.0 for the lowest values obtained in the CCD. This number increases as response values increase, with 1.0 being the highest response value obtained in the experiments. For this reason, a composite desirability was maximized to combine the individual desirability of all the response variables into a single measure, taking into account that all the response variables have the same importance. The optimized conditions (optimized factor settings) were as follows: incubation temperature, 80 °C; buffer pH, 5.3; derivatization concentration, 1% (m/V).

Devos et al. [24] also developed a methodology for the analysis of the same six organotin compounds (MBT, DBT, TBT, MPhT, DPht and TPhT) in water and sediment samples. They performed analyses at different temperatures and the optimum was also obtained at 80 °C. Extraction at lower temperatures resulted in much lower extraction recoveries especially for TPhT. However, Bianchi et al. [34] optimized the extraction temperature at 30 °C because they found that temperature values higher than 70 °C could lead to analyte desorption from the fibre. In the same way, Centineo et al. [11] studied the temperature effect in the HS-SPME. This process involves two equilibrium steps: the first step is the partitioning of the analyte between the fiber coating and the headspace gas phase, with a partitioning coefficient K_1; the second step involves analytes partitioning between the gas phase and the liquid sample phase, with a partitioning coefficient K_2. To some extent, heating is a convenient method to improve extraction efficiency since heating the sample helps to release analytes from matrix to headspace. Of course, lower temperature facilitates the physical adsorption process on the fibers coating. If temperature increases, the ability of fibers to adsorb analytes will decrease. Therefore, the total extraction efficiency depends on both, the fiber (its affinity character) and the compound (volatility). An increase in temperature will mainly affect their first equilibrium step (K_1), but hardly the second one (K_2) for the most volatile compounds. However, the amount of MBT, DBT and TBT extracted increased with the temperature due to their higher boiling points, i.e. the increase of K_2 values with increasing temperatures is much higher than the decrease of K_1 values. Centineo et al. [11] studied the temperature effect between 20 and 80 °C. Because extraction at

Table 3
Estimated effects and *p*-values (α=0.05) of the main factors obtained from Plackett-Burman design used in the optimization of the HS-SPME conditions.

Factors	Compounds					
	MBT (*m/z* 179)	DBT (*m/z* 207)	TBT (*m/z* 179)	MPhT (*m/z* 227)	DPhT (*m/z* 275)	TPhT (*m/z* 197)
Pre-incub. time (min)	3,193	1,192	872	15,001	18,123	−58,754
	(0.507)	(0.521)	(0.791)	(0.241)	(0.607)	(0.404)
Incub. temp. (°C)	−27,824	−12,044	−17,943	−47,299	−64,768	73,129
	(0.003)	(0.002)	(0.004)	(0.012)	(0.117)	(0.311)
Pre-inc. agitator speed (rpm)	7,062	4,208	7,118	3,728	18,606	18,911
	(0.182)	(0.068)	(0.082)	(0.750)	(0.597)	(0.779)
Extraction time (min)	2,214	3,803	6,686	9,945	8,086	56,710
	(0.640)	(0.088)	(0.096)	(0.413)	(0.816)	(0.419)
Desorption temp. (°C)	431	1,605	2,887	7,940	14,669	92,102
	(0.926)	(0.397)	(0.402)	(0.507)	(0.675)	(0.218)
Buffer: pH	3,560	1,697	2,135	44,456	64,134	67,678
	(0.462)	(0.373)	(0.527)	(0.015)	(0.120)	(0.344)
Buffer: conc. (M)	1,094	889	512	−16,898	−42,826	895
	(0.815)	(0.627)	(0.876)	(0.196)	(0.258)	(0.989)
Buffer: vol. (mL)	−5,779	−1,705	−6,078	−16,150	−28,135	−59,266
	(0.258)	(0.371)	(0.120)	(0.213)	(0.435)	(0.401)
Derivat. conc. (%)	2,290	3,311	8,346	29,663	28,499	−10,767
	(0.629)	(0.122)	(0.054)	(0.053)	(0.430)	(0.873)
HS volume (mL)	−2,175	418	2,876	9,875	−28,859	−47,478
	(0.646)	(0.817)	(0.404)	(0.416)	(0.424)	(0.494)
Sample salinity (%)	6,479	2,364	5,160	23,322	56,781	85,540
	(0.213)	(0.235)	(0.170)	(0.099)	(0.155)	(0.247)
Stand. Preparation	−1,267	1,571	−1,295	−5,444	−13,109	−50,091
	(0.787)	(0.406)	(0.696)	(0.644)	(0.707)	(0.472)
Ultrasonic time (min)	−2,571	−1,359	−1,878	−6,741	6,291	21,347
	(0.589)	(0.468)	(0.576)	(0.570)	(0.856)	(0.752)
Desorption time (s)	−2,078	−1,196	−3,984	−2,461	837	2,884
	(0.660)	(0.519)	(0.266)	(0.832)	(0.981)	(0.966)

Table 4
Experimental conditions and response (peak area) of the Central Composite Design (CCD) used for the HS-SPME optimization.

RunOrder	Incub. Temp. (°C)	Buffer pH	Derivat. Conc. (%)	MBT (*m/z* 179)	DBT(*m/z* 207)	TBT(m/z 179)	MPhT(*m/z* 227)	DPhT(*m/z* 275)	TPhT(*m/z* 197)
1	86	5.7	1.3	31,827	9,461	23,085	71,149	196,760	332,920
2	80	5.3	1.0	30,302	12,433	23,130	57,337	234,401	278,113
3	80	5.3	1.0	29,979	10,942	21,890	60,647	226,501	264,112
4	80	5.3	1.0	30,092	11,690	22,147	56,264	234,264	272,388
5	74	4.8	0.7	38,189	12,631	19,508	43,131	209,219	215,151
6	86	5.7	0.7	29,197	9,915	16,785	40,015	214,856	408,986
7	80	5.3	0.5	31,679	13,531	27,298	27,645	244,295	381,318
8	80	5.3	1.0	31,941	11,926	22,345	52,825	241,901	311,429
9	80	5.3	1.5	30,294	10,457	19,761	57,475	231,091	279,085
10	86	4.8	1.3	26,907	8,806	17,135	34,632	195,379	307,878
11	74	5.7	1.3	38,767	13,272	19,148	82,903	212,729	151,543
12	90	5.3	1.0	23,438	8,393	17,196	34,984	204,184	427,233
13	70	5.3	1.0	43,875	16,132	21,212	66,118	221,774	158,838
14	80	4.5	1.0	31,143	11,191	17,615	13,049	128,667	232,624
15	86	4.8	0.7	27,009	10,544	19,002	16,689	173,460	392,050
16	74	4.8	1.3	40,837	14,685	19,312	46,228	191,760	148,739
17	74	5.7	0.7	43,551	15,365	21,724	58,834	220,907	217,329
18	80	6.0	1.0	38,063	14,339	19,158	96,029	263,963	321,926
19	80	5.3	1.0	34,245	12,890	20,000	45,441	223,938	311,601
20	80	5.3	1.0	33,879	12,886	20,646	43,364	217,845	326,788

higher temperatures is more tedious (and time consuming), extraction were accomplished at 20 °C.

Devos et al. [24] obtained optimum derivatization yields for the butyl- and phenyltins at a pH of 5.3 and 8, respectively. A multi-residue method, however, requires selection of one pH only. A pH of 5.3 was chosen because this was the best compromise for both the butyl- and phenyltins. Following this, highest derivatization yields for simultaneously determination of various organometallic compounds of mercury, lead and tin in natural water samples by Centineo et al. [11] were obtained at pH 5.3, which is in agreement with our results. Ikonomou et al. [32] studied the effect of buffer pH on derivatization efficiency of organotins with $NaBEt_4$. It has been empirically determined that a pH of 4.5 be used for the derivatizations works well. Segovia-Martínez et al. [25] prepared sodium chloride solution and sodium acetate buffer solution in order to adjust the salinity and the pH in the derivatization process. The buffer solution was prepared by dissolving the necessary amount of sodium acetate in pure water to get 0.1 M concentration and then adding acetic acid to adjust the pH to 5. Bianchi et al. [34] optimized the pH=4. It is known that the derivatization reaction, being a nucleophilic substitution, is better performed under moderately acidic conditions. Lower pH values could produce the formation of organotin hybrids.

Segovia-Martínez et al. [25] prepared a 2% (w/v) aqueous solution of $NaBEt_4$ for derivatization. $NaBEt_4$ was kept in desiccators into a glove bag under dry nitrogen. However, Shioji et al. [35]

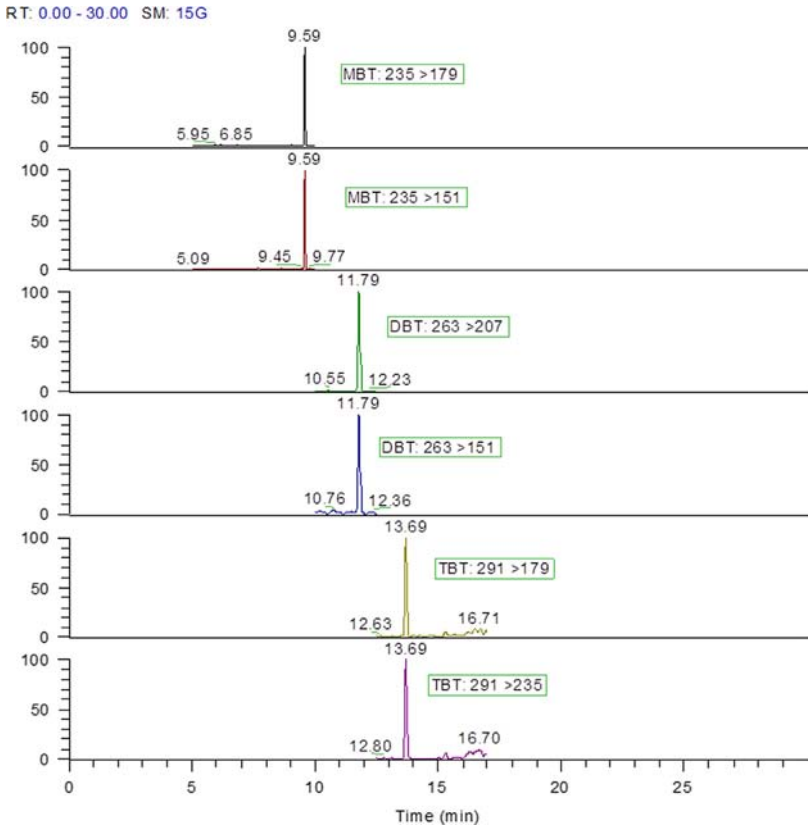

Fig. 2.1. Chromatogram of spiked surface water at 4000 ng L^{-1} for butyltin compounds (MBT, DBT and TBT) under optimum conditions.

also investigated the amount of sodium tetraethylborate adding 50 μL of an aqueous solution containing varying amounts of sodium tetraethylborate. The peak area ratios of ethyl TBT and TPhT decreased by adding an excess amount of the tetraethylborate (over 0.05%). For this reason, 0.05% was chosen for the concentration of sodium tetraethylborate.

3.3. Performance characteristics of the method

Analytical figures of merit for the quantitative determination of organotin compounds by in situ ethylation and simultaneous headspace solid-phase micro-extraction followed by GC–MS/MS were derived from matrix-matched calibration, using spiked solutions of an analyte-free pooled water sample matrix. Chromatograms of a typical standard addition procedure are presented in Fig. 2.1 and 2.2.

The linearity of the HS-SPME and GC-IT-MS–MS response was investigated injecting four series of a five-points calibration solution containing concentrations from 1000 to 6000 ng L^{-1}. Good linearity was achieved with coefficients of determination (R^2) higher than 0.99 for all compounds.

The precision and accuracy of the method was tested by analyzing a series of water sub-samples spiked with the same amount of multi-compound (levels 1000, 2000, 3000, 4000, 5000 and 6000 ng L^{-1}) and was found satisfactory for all species as can be seen in Table 5, which shows the recoveries and the relative standard deviation for the different compounds. The relative standard deviation varied in the range 1–25% and recoveries from 70 to 130% for the individual species.

The quantification limit (LoQ) was determined as the lowest concentration giving good recoveries and precision for each compound. LoQ of the developed method was 1000 ng L^{-1} for TBT, MPhT and TPhT, and 2000 ng L^{-1} for MBT, DBT and DPhT.

3.4. Matrix effect study

Signal suppression or enhancement as a result of matrix effect (ME) can severely compromise quantitative analysis of organotins at trace levels. Matrix effect must be evaluated and discussed in the context of method development before studying its performance characteristics and appropriate calibration technique compensating for these effects should be used.

ME was studied as described by Coscollà et al. [36]. In this way, two different sets of solutions were prepared (set A: standard solutions; set B: fortified surface and marine blank water) and determined using the optimised factor settings. The absence or presence of matrix effects on the quantification was evaluated by comparing the absolute peak areas of the two sets (ME%= $B/A \times 100$). Both A and B sets had concentrations of 3000 ng L^{-1}.

All compounds (MBT, DBT, TBT, MPhT, DPhT and TPhT) presented a strong matrix effect (ME ≫ 100%), showing a high signal enhancement in presence of both matrices (surface and marine water). Matrix-induced enhancement occurs mainly because matrix components mask active sites in the injector and column minimizing the adsorption and decomposition of organotins [37]. This indicates that is necessary to minimize the matrix effect using matrix-matched standard calibration and internal standard methodology. Internal standard TPhTd15 was used and added at the beginning of the sample preparation.

3.5. Analysis of water samples

To examine the applicability of the proposed method, 20 water samples collected in different sites mainly from the Valencia harbour, the Albufera lake and Cabanes irrigated ditch were analyzed.

Surface water samples and seawater samples were used to validate the developed methods. Surface water samples were collected at 6 irrigated ditches located close to the Valencia region coast (East of

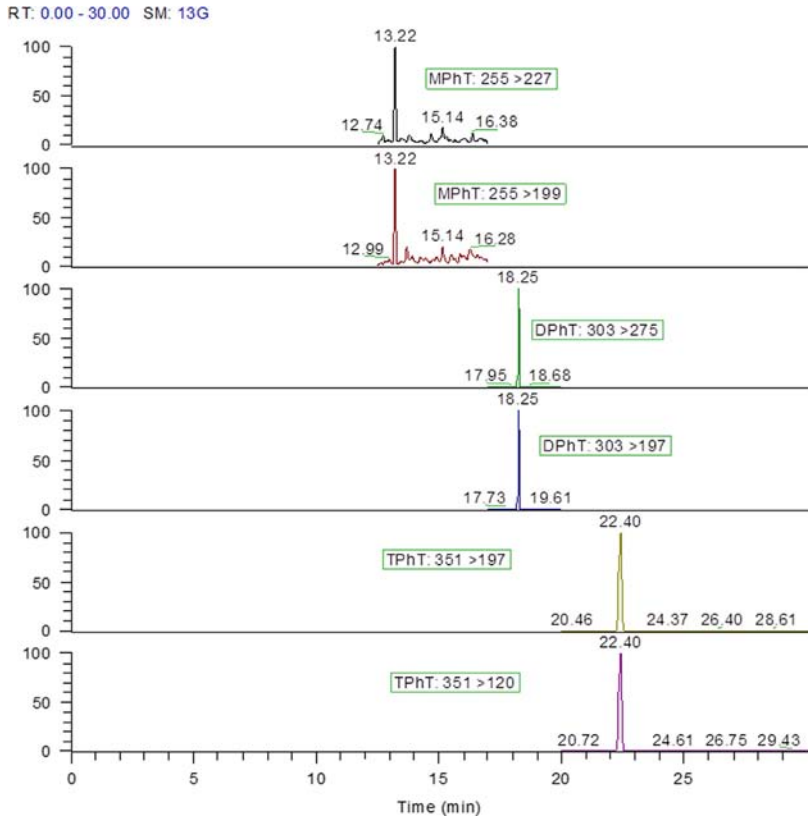

Fig. 2.2. Chromatogram of spiked surface water at 4000 ng L^{-1} for phenyltin compounds (MPhT, DPhT and TPhT) under optimum conditions.

Table 5
Organotin compounds recoveries from spiked surface and marine water using the optimized HS-SPME and GC-IT–MS/MS method.

Compound (ng L^{-1})	Surface water											
	MBT		DBT		TBT		MPhT		DPhT		TPhT	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
1000					70–130	25	70–130	25	78		72–85	12
2000	105–111	4	74–110	22	90–105	11	85–96	6	83–106	12	76–109	18
3000	97–117	8	82–109	15	80–101	16	79–107	13	95–99	2	95–119	11
4000	76–79	3	73–108	25	77–87	9	80–87	4	79–106	15	75–115	23
5000	78–111	14	98–109	4	77–93	9	87–114	12	81–107	11	89–105	6
6000	99–107	5	91–99	4	88–118	12	106–114	5			96–119	11
Marine water												
1000			74–120	23	80–120	25	70–130	25	87–120	25	91–120	14
2000	92–114	15	71		99–110	7	87–96	7	77–108	23	80–99	15
3000	92–120	10	95–119	9	106–120	7	100–115	7	82–100	8	87–114	10
4000	83–120	18	84–109	18			91–111	11	79–91	10	81–105	13
5000	83–117	12	88–117	14	84–107	10	75–101	20	73–102	13	91–108	7
6000	83–84	1	84–112	14	97–107	7	92–120	13	79–113	18	75–100	13

$n=5$; RSD: relative standard deviation.

Spain). Seawater samples were collected at 5 different geographical sites situated along the Valencia region coast. Samples were introduced into amber bottles and kept in the fridge at 4 °C till the analysis.

Real sample analysis was carried out with 5 mL of water sample in a 22 mL vial. Then, 5 mL of Milli-Q water, 5 mL of acetate buffer, 66 μL of the internal standard and 0.3 mL of sodium tetraethylborate were added into the vial (total volume of 11 mL using Milli-Q water) and finally the sample was extracted in headspace for 45 min with constant stirring.

All samples presented concentration lower than their limit of quantification.

4. Conclusions

Statistical design of experiments (DOE) was successfully used for the optimization of a method for the simultaneous determination of six organotin compounds in water samples, using headspace-solid phase microextraction and gas chromatography coupled to mass spectrometry in tandem (MS/MS). The advantage of headspace SPME was combined with GC–MS/MS in order to minimize matrix interferences and provide a selective method for simultaneous determination of MBT, DBT, TBT, MPhT, DPhT and TPhT in surface and marine water.

The proposed optimized methodology can be applied to high polluted areas like situations of spilling accidents in harbors, areas with high shipping activities or occasionally in effluents from industrial plants,

The optimized conditions in the HS-SPME were as follows: incubation temperature, 80 °C; buffer pH, 5.3 and derivatization concentration, 1%. The best conditions for the GC-IT–MS/MS were working at the optimized excitation voltage and excitation time for each compound. The fixed conditions for all compounds were electron energy of 70 eV, ion source temperature of 200 °C and isolation time of 20 s.

Good average percentage recoveries and repeatability in spiked surface and marine waters were obtained for all studied compounds. The optimized methodology was applied to 20 real aqueous samples in surface and marine water from the coast of Valencia region. All samples presented concentration lower than their limit of quantification.

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.2013.11.052.

References

[1] D.B. Hibbert, J. Chromatogr. B 910 (2012) 1–12.
[2] R.De Carvalho Oliveira, R.E. Santelli, Talanta 82 (2010) 9–24.
[3] E. Beceiro-González, a Guimaraes, M.F. Alpendurada, J. Chromatogr. A 1216 (2009) 5563–5569.
[4] European_Commission, Off. J. Eur. Union C513 (2000). Directive 2000/60/EC.
[5] European_Commission, Off. J. Eur. Union L348/84 (2008). Directive 2000/60/EC.
[6] S. Mishra, R.M. Tripathi, S. Bhalke, V.K. Shukla, V.D. Puranik, Anal. Chim. Acta 551 (2005) 192–198.
[7] A. Delgado, A. Usobiaga, A. Prieto, O. Zuloaga, A. de Diego, J.M. Madariaga, J. Sep. Sci. 31 (2008) 768–774.
[8] A. Ugarte, N. Unceta, M.C. Sampedro, M.A. Goicolea, A. Gómez-Caballero, R.J. Barrio, J. Anal. At. Spectrom. 24 (2009) 571–574.
[9] D.-R. Parkinson, I. Bruheim, I. Christ, J. Pawliszyn, J. Chromatogr. A 1025 (2004) 77–84.
[10] Z. Mester, R. Sturgeon, Spectrochim. Acta, Part B 60 (2005) 1243–1269.
[11] G. Centineo, E.B. González, A. Sanz-Medel, J. Chromatogr. A 1034 (2004) 191–197.
[12] Q. Zhou, G. Jiang, J. Liu, Sci. World J. 2 (2002) 655–659.
[13] M. Le Gac, G. Lespes, J. Chromatogr. A 999 (2003) 123–134.
[14] S. Aguerre, C. Pécheyran, G. Lespes, Anal. Bioanal. Chem. 376 (2003) 226–235.
[15] J. Muñoz, M. Gallego, M. Valcárcel, Anal. Chem. 77 (2005) 5389–5395.
[16] S. Aguerre, C. Pécheyran, G. Lespes, At. Spectrom. 16 (2001) 1429–1433.
[17] P.N. Carvalho, L.F. Pinto, M.C.P. Basto, M.T.S.D. Vasconcelos, Microchem. J. 87 (2007) 147–153.
[18] C. Naert, S. De Saeger, C. Van Peteghem, Rapid Commun. Mass Spectrom. 18 (2004) 2317–2322.
[19] M. Mandalakis, M. Tsapakis, E.G. Stephanou, J. Chromatogr. A 925 (2001) 183–196.
[20] V. Flotron, J. Houessou, A. Bosio, C. Delteil, A. Bermond, J. Chromatogr. A 999 (2003) 175–184.
[21] D. Massart, C. Vandeginste, B.G.M. Buydens, S. De Jong, P. Lewi, J. Smeyers-Vrbeke, Handb. Chemom. Qualim. Part A (1997).
[22] L.S. Riter, O. Vitek, K.M. Gooding, B.D. Hodge, R.K. Julian, J. Mass Spectrom. 40 (2005) 565–579.
[23] V. Yusà, O. Pardo, A. Pastor, M. De La Guardia, Anal. Chim. Acta 557 (2006) 304–313.
[24] C. Devos, M. Vliegen, B. Willaert, F. David, L. Moens, P. Sandra, J. Chromatogr. A 1079 (2005) 408–414.
[25] L. Segovia-Martínez, a. Bouzas-Blanco, P. Campíns-Falcó, a. Seco-Torrecillas, Talanta 80 (2010) 1888–1893.
[26] E. Millán, J. Pawliszyn, J. Chromatogr. A 873 (2000) 63–71.
[27] C.-C. Chou, M.-R. Lee, J. Chromatogr. A 1064 (2005) 1–8.
[28] M. Bravo, G. Lespes, I. De Gregori, H. Pinochet, M.P. Gautier, Anal. Bioanal. Chem. 383 (2005) 1082–1089.
[29] N.M. Tzollas, G.A. Zachariadis, J. Sep. Sci. 33 (2010) 1610–1616.
[30] H. Tao, R.B. Rajendran, C.R. Quetel, T. Nakazato, M. Tominaga, A. Miyazaki, Anal. Chem. 71 (1999) 4208–4215.
[31] B.R. Ramaswamy, H. Tao, M. Hojo, Anal. Sci.: Int. J. Jpn. Soc. Anal. Chem. 20 (2004) 45–53.
[32] M. Ikonomou, M. Fernandez, T. He, D. Cullon, J. Chromatogr. A 975 (2002) 319–333.
[33] S. Tsunoi, T. Matoba, H. Shioji, L.T.H. Giang, H. Harino, M. Tanaka, J. Chromatogr. A 962 (2002) 197–206.
[34] F. Bianchi, M. Careri, M. Maffini, A. Mangia, C. Mucchino, J. Anal. At. Spectrom. 21 (2006) 970.
[35] H. Shioji, S. Tsunoi, H. Harino, M. Tanaka, J. Chromatogr. A 1048 (2004) 81–88.
[36] C. Coscollà, V. Yusà, P. Martí, A. Pastor, J. Chromatogr. A 1200 (2008) 100–107.
[37] C. Coscolla, M. Castillo, A. Pastor, V. Yusa, Anal. Chim. Acta 693 (2011) 72–81.