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Determination of triorganotin species in water samples by liquid chromatography–electrospray-mass spectrometry

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Abstract

Liquid chromatography coupled to electrospray–mass spectrometry (LC–ES-MS) with positive ion detection was evaluated for the determination of tributyltin and triphenyltin in water samples using tripropyltin as internal standard. The separation was performed in the isocratic mode on a silica-based C_{18} column with a mobile phase containing 0.02% trifluoroacetic acid in acetonitrile–water (50:50, v/v). The optimum LC–ES-MS conditions were established and quantification was performed on the basis of the $[M]^+$ ions. Limits of detection for standard solutions were 100 and 200 pg Sn injected for triphenyltin and tributyltin, respectively, and good reproducibility was observed. Solid-phase extraction was carried out on C_{18} cartridges to preconcentrate the analytes from natural water samples, with recoveries ranging from 80 to 110%. Limits of detection for SPE–LC–ES-MS were in the range of low ng l^{-1}, which demonstrates the suitability of the method for environmental samples.

Keywords: Solid-phase extraction; Water analysis; Tributyltin; Triphenyltin; Tripropyltin

1. Introduction

The pollution of freshwaters and coastal seawaters as a consequence of the widespread use of organotin compounds (OTs) in industry and agriculture has been of great concern. Among these compounds, the most prominent are the trisubstituted forms tributyltin (TBT) and triphenyltin (TPhT), which have been extensively used as biocides in antifouling paints for the past few decades [1,2]. Their severe effects on both aquatic organisms [3] and mammals [4,5], and their high bioaccumulation potential and persistence in sediments, have led to restrictions in their use in many countries [6]. Despite these regulations, TBT and TPhT, as well as their (bio)degradation products (mono- and di-organo-derivatives), are still found in natural waters and sediments at concentration levels that may exert sublethal and even lethal effects on aquatic organisms [7]. For instance, TBT has been identified as an endocrine disruptor, with effects even at ng l^{-1} level in water. Therefore, the monitoring of OTs in water at extremely low concentrations is an important issue, which requires highly sensitive analytical techniques. Most of the analytical methodologies developed for the speciation of OTs are based on gas chromatography (GC) [8,9] owing to its high resolution power and availability of sensitive detectors. In particular, the use of GC in combination with

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mass spectrometry (MS) is a powerful technique from the point of view of identification and confirmation of the compounds. However, GC needs a previous derivatization of the non-volatile compounds and this affects the duration and accuracy of the overall analytical procedure, especially for the analysis of complex natural matrices.

The use of liquid chromatographic (LC) methods is an alternative approach for OT speciation that does not require derivatization before analysis [10]. LC in conjunction with inductively coupled plasma-mass spectrometry (ICP-MS) [11–13] and fluorescence detection [14–17] has been successfully applied to the analysis of OTs in several environmental samples. All these methods provide good sensitivity but give no structural information about the measured analyte.

MS detection coupled to LC systems has been scarcely applied for OT monitoring [12,18,19]. It offers important advantages, since in addition to its sensitivity and the fact that no OT derivatization is required, it provides structural information that can be used for confirmatory purposes. Both electrospray (ES) and atmospheric pressure chemical ionisation (APCI) interfaces have been explored for OT analysis by LC–MS, and a few applications, such as water or sediment analysis have been reported [12,18,19]. The main limitation of LC–MS for quantification purposes seems to be related to the signal reproducibility, especially when dealing with complex matrices.

The aim of this work was to investigate the use of LC–ES-MS for the determination of TBT and TPhT. The study was not extended to the analysis of their degradation products, i.e. the di- and mono-OTs because of the poor compatibility of the mobile phase required for di- and mono-OTs (high acid content or complexing agents) [14,20] with the MS equipment used. The separation of tri-OTs in a C_{18} column with a suitable mobile phase was investigated, and the mass spectra were studied, optimising all the parameters influencing the ion formation. To overcome MS signal instability we propose the use of tripropyltin (TPrT) as internal standard. TPrT was selected according to its structural similarity with the target analytes and because it is not found in the environment. The proposed method was successfully applied to the analysis of TBT and TPhT in natural waters. Although the method does not provide information on di- and mono-OTs, it allows a simple and sensitive determination of TPhT and TBT, which are included in the list of prioritary pollutants of the EU.

2. Experimental

2.1. Chemicals

Triphenyltin chloride (TPhTCl) and tributyltin chloride (TBTCl) were purchased from Fluka (Buchs, Switzerland) purity >95%. Tripropyltin chloride (TPrTCl) was used as internal standard and was obtained from Merck (Darmstad, Germany). Stock standard solutions containing 1000 mg Sn l^{-1} were prepared in methanol and stored at 4 °C in dark glass bottles. Working standards (10 mg Sn l^{-1}) were prepared weekly by dilution in methanol. Further dilutions to prepare working solutions were made with the mobile phase.

Methanol and acetonitrile HPLC grade (Merck) and ultrapure water Milli-Q plus (Millipore, Molheim, France) 18.2 MΩ cm^{-1} were used throughout. Trifluoroacetic acid (TFA) (Uvsol) was also obtained from Merck.

Disposable solid-phase extraction (SPE) cartridges (average particle diameter 40 μm) containing 100 mg of C_{18} bonded silica (Bond Elut; Varian; Harbor City, CA, USA) were used for water analysis.

Glassware used for experiments was previously soaked in 10% nitric acid for 24 h and rinsed with doubly-deionized water.

2.2. Chromatographic conditions

For optimization of the mobile phase, LC with fluorimetric detection was carried out on a double piston pump (Model 525, Bio-Tek Kontron Instruments, Milan, Italy) and on an Aminco-Bowman Series 2 spectrofluorimeter (SLM Aminco, Rochester, NY, USA). For fluorimetric detection, tri-OTs were derivatized with a fisetin-based reagent in the post column mode [17].

Separations were performed using a Kromasil C_{18}

column (250×4.6 mm I.D., 5 μm, Phase Separations) with a guard column of the same material. Optimal separation was achieved in isocratic mode with a mobile phase containing 0.02% TFA in acetonitrile–water (50:50, v/v) at a flow-rate of 1 ml min^{-1}. The mobile phase was filtered through a 0.22-μm nylon membrane filter (MSI, Westboro, MA, USA) and the mixture was degassed for 10 min with a helium stream before use.

LC–MS was carried out using a Waters 2690 separation module (Milford, MA, USA) equipped with an automatic injector. A split system 1/50 was used to introduce the effluent into the ES source.

MS was performed using a VG Platform (Fisons Instruments, VG Biotech, Altrincham, UK) quadrupole mass spectrometer equipped with a standard pneumatically assisted ES ion source. The experimental conditions were the following: drying gas was heated to 90 °C and introduced to capillary region at a flow-rate of 400 l h^{-1}, the capillary voltage was hold at +3 kV and the extraction voltage was set at +40 V for TBT and TPrT, and at +70 V for TPhT. These values were optimized introducing a standard solution of each tri-OT using flow injection methodology.

For data acquisition in full scan mode, the mass spectrometer operated over a range of m/z 100.0–450.0 in the centroid mode, using a cycle time of 1 s and inter-scan time of 0.1 s. The selected-ion monitoring (SIM) mode was done on the basis of the $[M^+]$ ion for each organotin, with a dwell time of 100 ms and an inter-channel time of 1 ms. Three isotopic ions corresponding to ^{116}Sn, ^{118}Sn and ^{120}Sn, which are the most abundant of the tin natural isotopic distribution, were monitored for each molecular ion (Table 1). The instrument control and data processing were carried out by means of MASS LINX software.

Table 1
Monitored ions in the SIM mode

Time window (min)	Compound	M	Ions monitored (m/z)	ES cone (V)
0–4.5	TPrT	249	245, 247, 249	40
4.5–6.5	TPhT	351	347, 349, 351	70
6.5–10	TBT	291	287, 289, 291	40

2.3. Sample preparation

Water samples were collected in 2.5-l glass amber bottles, filtered through a 2-μm glass microfiber filter (Whatman, Maidstone, UK), acidified to pH 2 with hydrochloric acid and stored at 4 °C.

The disposable C_{18} cartridge was conditioned by rinsing with 5 ml of methanol, followed by 10 ml of water at pH 2. Afterwards, a 250-ml aliquot of water sample was passed through the cartridge at a flow-rate of 5 ml min^{-1}. After retention, the column was washed with 10 ml of water and dried by pumping air through the cartridge for about 2 min. Elution was performed with 2 ml of 0.02% TFA in acetonitrile–water (50:50, v/v) i.e. the mobile phase of the chromatographic method, at a flow-rate of 0.5 ml min^{-1} in the back-flush mode. Then, the appropriate volume of a standard solution of TPrT was added to the extract. The eluate was filtered through a 22-μm nylon membrane and injected into the chromatographic system using a 100-μl loop.

3. Results

3.1. Optimization of the chromatographic conditions

The LC separation of TPhT, TBT and TPrT, selected as internal standard for quantitation, was carried out in the reversed-phase mode on a C_{18} column due to its compatibility with MS detection.

In order to optimise the chromatographic separation, several mobile phases consisting of methanol–water or acetonitrile–water mixtures containing TFA were assayed. Methanol-based mobile phases proved to be satisfactory for the separation of TBT and TPhT, but resulted in coelution of TPhT and TPrT. In contrast, acetonitrile–water mixtures provided good separation of the three OTs, and thus they were used in further work. The effect of acetonitrile and TFA concentration on the separation was studied over the ranges 45–75% and 0.01–0.04%, respectively (Fig. 1). Regardless of the mobile phase composition the elution order was TPrT, TPhT and TBT. As expected, a decrease in the acetonitrile percentage led to an increase in retention times, but also in resolution. An increase in TFA

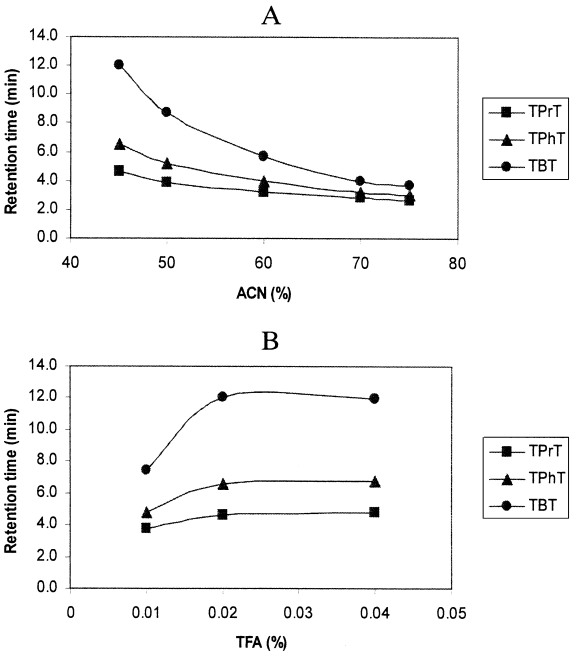

Fig. 1. Effect of (A) acetonitrile content (0.02% TFA) and (B) trifluoroacetic acid (TFA) concentration (50% ACN) on OT retention times. Column: Kromasil C_{18}. Mobile phase: acetonitrile–trifluoroacetic acid–water.

concentration also affected retention times but, in the assayed range, had little effect on resolution between TPrT and TPhT peaks. Since a 0.02% TFA in acetonitrile–water (50:50, v/v) provided a good baseline separation between the three OTs in less than 10 min, this composition was chosen. A typical chromatogram from a standard solution of triorganotin compounds using MS detection in the full-scan mode is shown in Fig. 2.

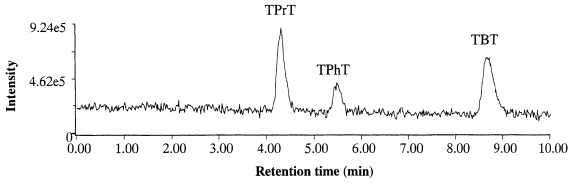

Fig. 2. Chromatogram obtained by ES in full-scan and positive mode at 50 V for a standard solution containing 1 mg Sn l^{-1} of tri-OTs in the selected chromatographic conditions.

3.2. Optimization of the MS parameters

In order to optimize the ES-MS parameters under the LC conditions described, flow injection methodology was used to introduce the analytes (20 μl of a 1 mg Sn l^{-1} individual standard solution) into the mass spectrometer. Typical mass spectra of TPhT, TBT and TPrT obtained in full scan mode are shown in Fig. 3. For each ion very characteristic peak clusters, consistent with the distribution of the ten stable tin isotopes were observed. The signals corresponding to ^{116}Sn, ^{118}Sn and ^{120}Sn isotopes were the most prominent in the spectra. In fact, to simplify discussion, m/z values have been assigned on the basis of ^{120}Sn, the most abundant tin isotope.

Optimization of the ES-MS parameters resulted in the operational conditions given in Section 2.2. Among these parameters, the extraction voltage was the most important, since it greatly affected both the appearance of the spectra and the response intensity. Thus, in order to establish the optimum conditions

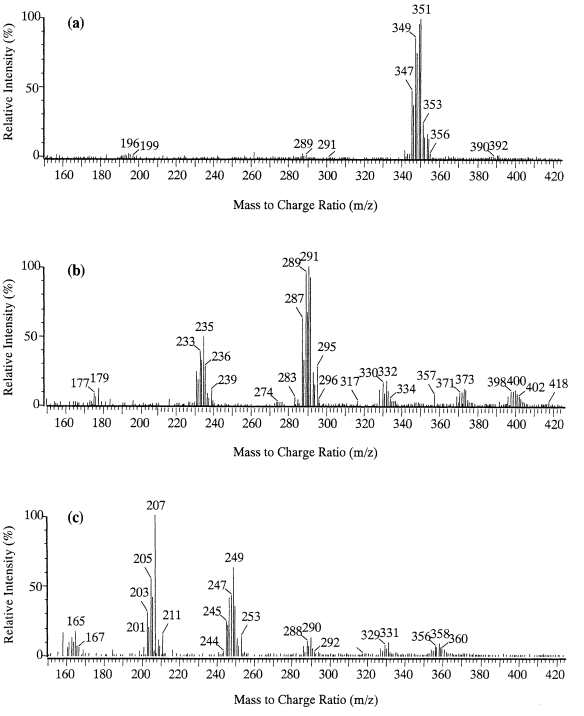

Fig. 3. Typical mass spectra of (a) TPhT, (b) TBT and (c) TPrT obtained by FIA analysis at 50 V.

for the analysis of all the compounds, standard solutions were injected at extraction voltages from +20 to +90 V and the mass spectra were recorded in full scan mode. Fig. 4 shows the normalized absolute abundances of the fragment ions of each compound vs. the extraction voltage.

At voltage values below 50 V the mass spectra obtained for the three compounds exhibited intense $[M]^+$ ion cluster, besides the formation of the $[M+MeCN]^+$ adduct. In the case of TPhT, the $[M+MeCN]^+$ ion was the most prominent in the spectra up to 30 V, whereas at higher voltages the $[M]^+$ ion was the base peak. TPhT was poorly fragmented in the ES interface, and began to fragment only at 70 V to yield *m/z* 197 and 123, which correspond to the loss of two and three phenyl groups. In contrast, TBT and TPrT, which followed the same fragmentation pattern, showed a high level of fragmentation at voltages higher than 40 V. The spectra of TBT showed *m/z* 235 and 179, which are attributed to the loss of one and two butyl groups $[M-Bu+H]^+$ and $[M-2Bu+2H]^+$, respectively. The spectra obtained for TPrT showed *m/z* 207 and 165, corresponding to $[M-Pr+H]^+$ and $[M-2Pr+2H]^+$. At cone voltages >70 V a greater fragmentation was observed for both TBT and TPrT, and the peak base changed to be *m/z* 123 which corresponds to $[Sn+3H]^+$. The main ions obtained at two extraction voltages with their corresponding tentative assignations and relative abundances are given in Table 2.

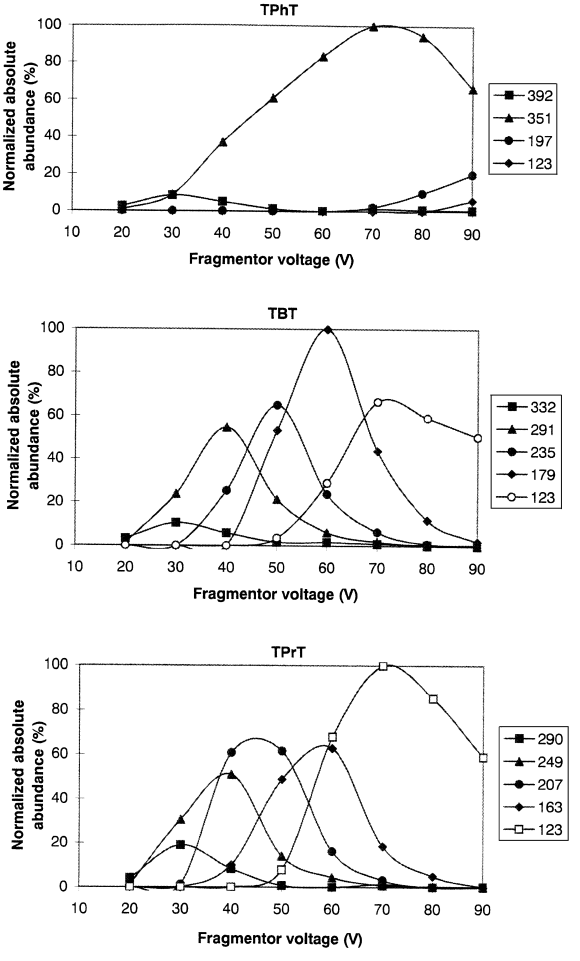

Fig. 4. Variation of the normalized absolute abundances (%) of the fragments ions vs. the extraction voltage for (a) TPhT, (b) TBT and (c) TPrT.

When selecting the optimum extraction voltage, conflicting interests arise. There is, on the one hand, the need to achieve maximum sensitivity, which requires low fragmentation and, on the other hand, the need to obtain the maximum structural information, which demands high fragmentation. However, in the case of OTs, due to the characteristic tin isotopic distribution, confirmation by MS can be fulfilled with low fragmentation on the basis of tin isotopic ratios of just one selected ion for each species, e.g. the molecular ion. The combination of LC retention time, *m/z* of the M^+ ion, and isotopic ratios is therefore a powerful tool for OT speciation.

In this work the selection of the optimum voltages was made on the basis of the maximum signal for the molecular ion of the analytes. The molecular ions of TBT and TPrT showed their maximum at +40 V, whereas TPhT showed a maximum at +70 V (Fig. 4), and hence these were the selected extraction voltages.

3.3. LC–ES-MS

The quality parameters of the LC–ES-MS system were studied from spectra obtained in SIM acquisition mode, and the mass spectrometer was programmed to switch between three sets of SIM masses during the course of each run (Table 1). Data were calculated on the basis of the ^{120}Sn ion, which provided a higher signal than the other isotopes.

Table 2
Assigment of typical fragments ions and relative abundances of triorganotin compounds using 0.02% TFA in acetonitrile–water (50:50, v/v) as LC eluent at two different extraction voltages under ES-MS conditions described in Section 2.2

Compound	M_r	m/z	Tentative assignation	Relative intensity (%)	
				+ 40 V	+ 70 V
TPhT	351 + Cl	392	$[M+MeCN]^+$	18	2
		351	$[M]^+$	100	100
		197	$Sn^{II}Ph^+$	2	3
		123	$[Sn+3H]^+$	–	1
TBT	291 + Cl	332	$[M+MeCN]^+$	16	1
		291	$[M]^+$	100	2
		235	$[M-Bu+H]^+$	46	5
		179	$[M-2Bu+2H]^+$	12	37
		123	$[Sn+3H]^+$	–	100
TPrT	249 + Cl	290	$[M+MeCN]^+$	12	1
		249	$[M]^+$	64	1
		207	$[M-Pr+H]^+$	100	4
		165	$[M-2Pr+2H]^+$	12	22
		123	$[Sn+3H]^+$	–	100

Linear calibration graphs ($r^2>0.99$) were obtained from 5 to 1000 μg l^{-1} and from 10 to 1000 μg l^{-1}, for TPhT and TBT, respectively, using TPrT as I.S. To determine the run-to-run precision, five replicate determinations on the same day of a standard solution containing 50 μg l^{-1} of TPhT and TBT were carried out. Relative standard deviations (RSDs) based on S_A/S_{IS} ranged between 5 and 6%. Values obtained for peak area were comparable to those obtained from peak height, and therefore the former was chosen for quantitation. The limits of detection (LODs) and the limits of quantitation (LOQs) were calculated using a signal-to-noise ratio of 3 and 10, respectively. The mass detection limits, for a 100-μl injection volume, were 100 and 200 pg injected, for TPhT and TBT, respectively, and the LOQ values were 400 and 800 pg, all amounts referring to tin.

3.4. Application to natural water samples

The proposed LC–ES-MS method was applied to the quantitative analysis of TBT and TPhT in seawater samples from a trading port (S1), a marina (S2), and an urban area (S3) and a river water sample from an agriculture area (R1), all of them located on the NW Mediterranean coast. Determination of OTs at environmental concentration levels requires an enrichment step, to preconcentrate the analytes, prior the chromatographic run. In this study preconcentration was achieved by SPE on C_{18} cartridges, which has been reported as a suitable bonded phase for OT species [15]. Direct analysis of the natural samples (Table 3) proved that whereas none of them contained measurable amounts of TPhT, TBT was detected at the sub-μg l^{-1} level in S1 and S2 samples. This was confirmed from the isotopic ratio of the $[M]^+$ ion, since differences between theoretical and experimental isotopic ratios were about 5%. To evaluate the accuracy of the method proposed, samples were spiked with TBT and TPhT at concentration levels between 0.16 and 0.19 μg Sn l^{-1}. The analysis of spiked samples shows (Table 3) that, in spite of the low TPhT and TBT levels measured, good recoveries (80–110%) and precisions (8–12%) were obtained. Chromatograms from water extracts were clean, even those corresponding to samples of ports with large commercial activities, with no interfering peaks with tri-OT quantitation (Fig. 5). The detection limits of the whole procedure depend on the volume of sample processed. If a 250-ml sample is preconcentrated, i.e. a preconcentration factor of 125, the LODs of the SPE–LC–MS method are 16 and 8 ng l^{-1} for TBT and TPhT,

Table 3
Determination of tributyltin and triphenyltin in natural waters

Compound	Sample	Sampling area	Added (ng Sn l^{-1})	Found[a] (ng Sn l^{-1})	Recovery (%)
TPhT	S1	Trading port	–	N.d.	
			194	186±17	96±9
	S2	Sport harbour	–	N.d.	
			194	175±19	90±11
	S3	Urban	160	164±13	103±8
	R1	Agricultural	160	155±14	97±9
TBT	S1	Trading port	–	98±10	
			190	300±16	106±10
	S2	Sport harbour	–	93±7	
			190	234±13	80±10
	S3	Urban	165	182±19	110±12
	R1	Agricultural	165	175±18	106±11

[a] Mean value±standard deviation ($n=3$); n.d., not detected.

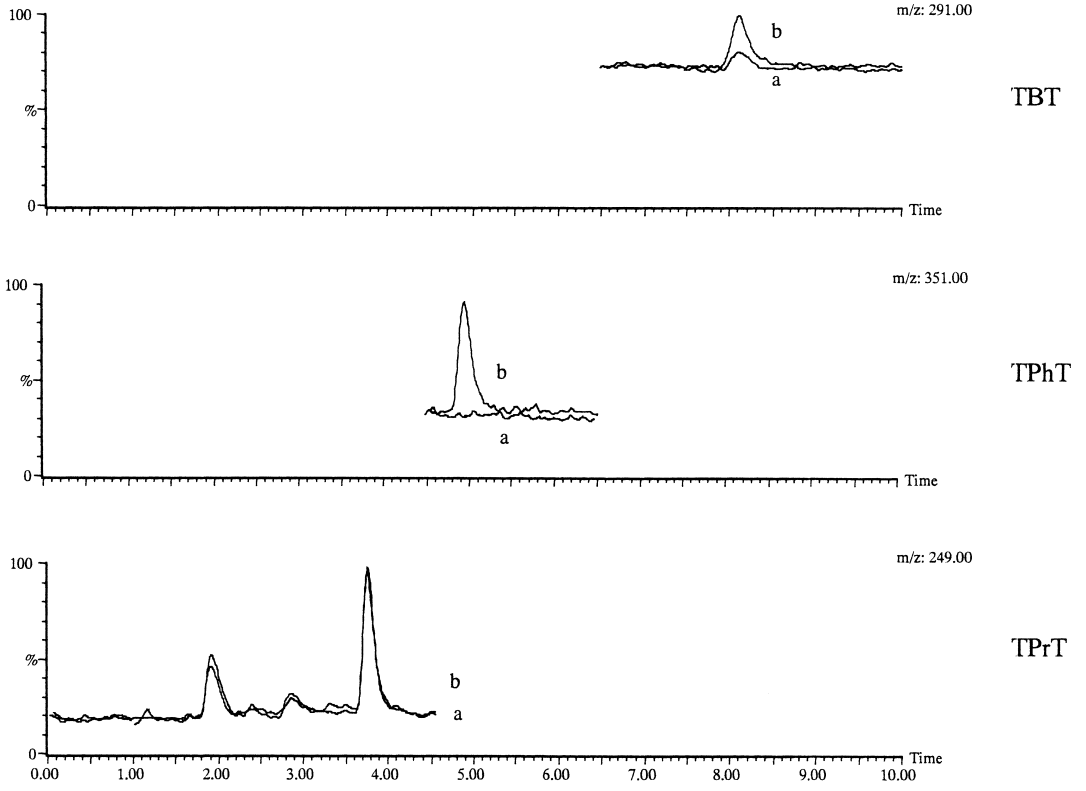

Fig. 5. Chromatograms obtained by SPE–LC–ESI-MS under the positive ion mode and time-schedule SIM of (a) 250 ml of seawater from a commercial harbour and (b) the same sample spiked with 194 ng Sn l^{-1} of TPhT and 190 ng Sn l^{-1} of TBT. Eluates were spiked with 50 μg Sn l^{-1} of the internal standard.

respectively, which values are suitable for environmental analysis. These LOD values were confirmed by injecting real sample extracts, prepared from sample S3 spiked at concentration levels around the LODs.

4. Conclusions

LC–ES-MS has proved to be a suitable technique for tributyltin and triphenyltin analysis in environmental samples. Problems reported about signal stability were overcome by the use of TPrT as internal standard, resulting in good reproducibility. The method is sensitive enough to make it suitable for monitoring tri-OTs in natural waters at the low ng l^{-1} level. Although LC–ICP-MS methods are more sensitive than the proposed technique, LC–ES-MS provides molecular information of the intact OT species, which allows confirmation of the identity of the analytes.

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