APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. **13**, 881–889 (1999)

Determination of Organotins in Water by Micro Liquid Chromatography–Electrospray/Ion Trap Mass Spectrometry

Tammy L. Jones-Lepp[1]*, Katrina E. Varner[1], Mark McDaniel[2] and Lee Riddick[1]
[1]US Environmental Protection Agency, National Research Laboratory—Environmental Sciences Division, PO Box 93478, Las Vegas, NV 93478-3478, USA
[2]National Network for Environmental Management Studies Program, US EPA, Las Vegas, NV, USA

Due to the varying toxicity the species of organotins in their widespread applications, it is important for analytical methods to address their speciation. Traditional methods call for the hydrolysis and subsequent derivatization of the organotins before analysis. These methods can be time-consuming, derivatization can be incomplete and high levels of background interference produce difficulties in identification and quantification. The use is described of a non-derivatization and non-hydrolysis micro-liquid chromatography–electrospray/ion trap mass spectrometry for separation and detection of the organotins. Copyright © 1999 John Wiley & Sons, Ltd.

Keywords: organotins; ion trap mass spectrometry; electrospray

Received 9 October 1998; accepted 1 April 1999

INTRODUCTION

Organotins have widespread applications as fungicides, antifouling coatings for porous surfaces, herbicides, insecticides and as generic biocides.[1] Their release into the environment from these sources is likely to be in the cationic forms of dibutyltin (plastic stabilizers), triphenyltin (insecticides) and tributyltin (biocides). As health and safety measures have become increasingly stringent they have mandated a reduction, or restriction, of the use of heavy metals, and tin-based stabilizers have replaced lead-and cadmium-based materials. Therefore, the potential for exposure to organotins is increasing. The USA accounted for more than 40% of the world demand for organotins in 1993. The projected growth in world demand is approximately 14 000 t per year, from 1993 to 2005. By the year 2005, the total world demand for organotin stabilizers is projected to be around 222 000 t.[2,3] In the past 30 years, organotin compounds have developed into important industrial commodities. The largest markets for organotin stabilizers exist with rigid PVC, which is used for pipes, cables, windows, weather boarding and roofing in the construction industry, and in bottles and packaging materials in the food, beverage and household goods sectors. Therefore, tin probably has more organometallic applications than any other metal.

Organotins can elicit a wide range of endocrine and nervous-system effects, depending on the nature and number of alkyl groups bonded to the tin atom.[4] The organotins show a variety of adverse health effects in many species, including imposex in molluscs, neural degeneration in fetal rat cell cultures and induction of diabetes in hamsters.[5–7] Contamination by, and the fate of, organotin compounds in aquatic systems have been reported and linked with effects at different levels of biological organization. Butyl- and phenyl-tin compounds, particularly tri-substituted species, are known to be very toxic to marine organisms at very low concentrations.[8] Tributyltin (TBT) and its breakdown products, found at ppb (10^{-9}) levels in marine mammal tissues, are speculated to have led to the deaths of dolphins by suppressing their immune systems, making them more vulnerable to disease.[9,10] Fent and Stegeman indicated important biochemical effects of butyltins in fish and suggested that exposure to butyltins may alter both cytochrome P450-dependent metabolism and the induction response to other environmental pollutants.[11] The suspected origin of the organotins in the aquatic environment is mostly from the use of marine antifouling paints (containing tributyltin). It

* Correspondence to: Tammy L. Jones-Lepp, US Environmental Protection Agency, National Research Laboratory—Environmental Sciences Division, PO Box 93478, Las Vegas, NV 93478-3478, USA

 CCC 0268–2605/99/120881–09 $17.50

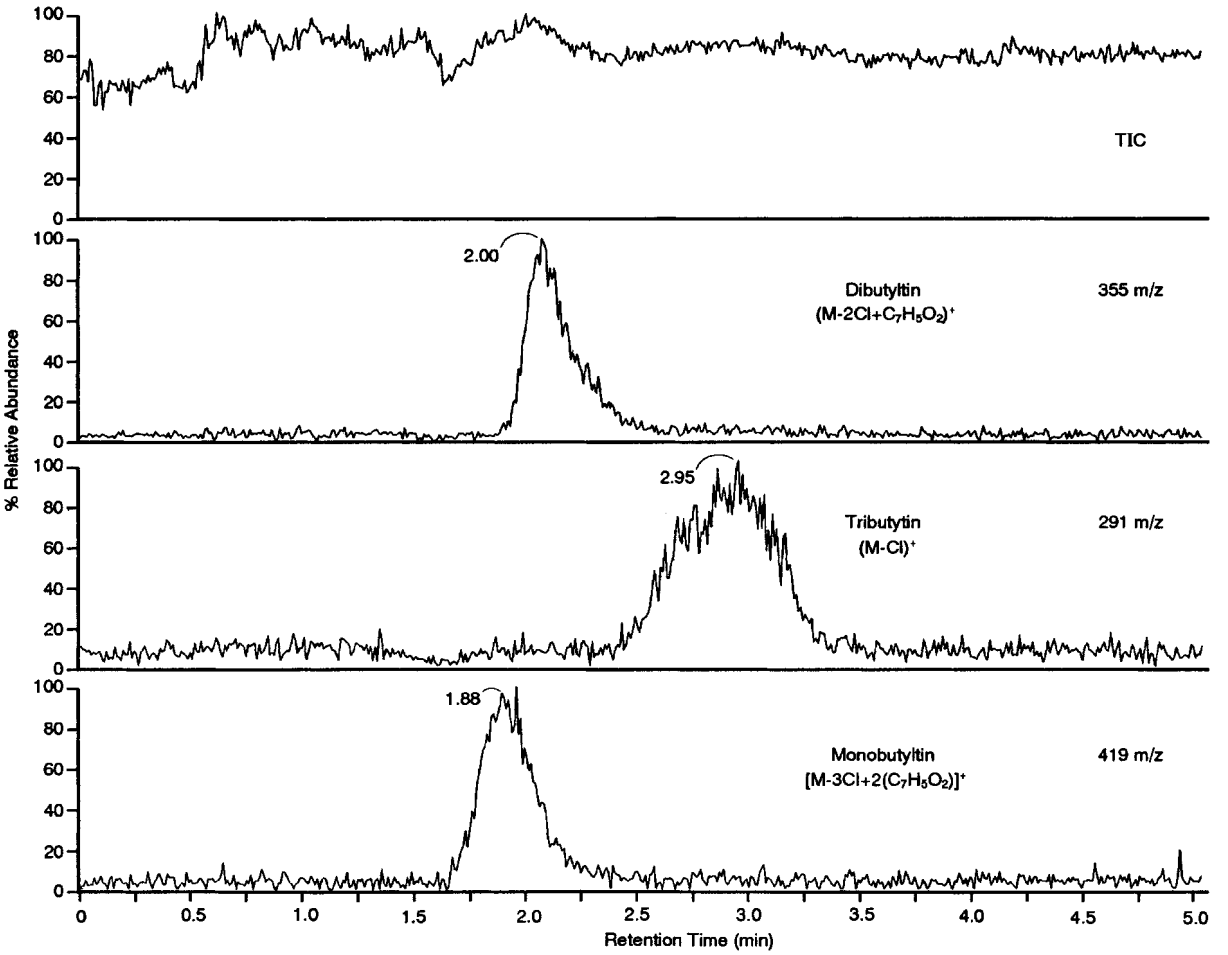

Figure 1 Mass chromatograms of tri-, di- and mono-butyltin.

has also been shown that leaching and normal weathering of PVC products contribute dibutyltin, and its decomposition products, to the aquatic and terrestrial ecosystems.[12] It is important that methods for the analysis of organotins distinguish among species of varying toxicity.

Traditional methods of detection of organotins call for the hydrolysis and subsequent derivatization of the organotins before analysis. These methods include gas chromatography with flame photometric detection and high-performance liquid chromatography–hydride generation–inductively coupled plasma atomic emission spectrometry (HPLC–ICP AES).[13,14] Other methods call for the complexing of the organotins before analysis by HPLC–ICP mass spectrometry (MS).[15] Recent sample preparation improvements call for the dissolution, extraction and derivatization in a focused microwave field.[16] Another new method involves rapid extraction using solid-phase extraction (SPE) disks, with a complexing agent, and then separation and detection using micellar electrokinetic chromatography (MEKC) with UV detection.[17] The use of complexing agents can produce high background interference and methods that use hydrolysis and derivatization can suffer from incomplete hydrolysis and derivatization, therefore with subsequently misleading results. Newer methodologies to overcome the use of derivatization and complexation include the use of capillary electrophoresis (CE) with indirect ultraviolet[18] and direct (UV)[19] absorbance detection, ICP MS with micellar liquid chromatography,[20] HPLC with ion-exchange chromatography coupled to ICP MS[21] and electrospray–mass spectrometry (ES–MS) (see Ref.[22], and references cited therein). Although ICP MS is a

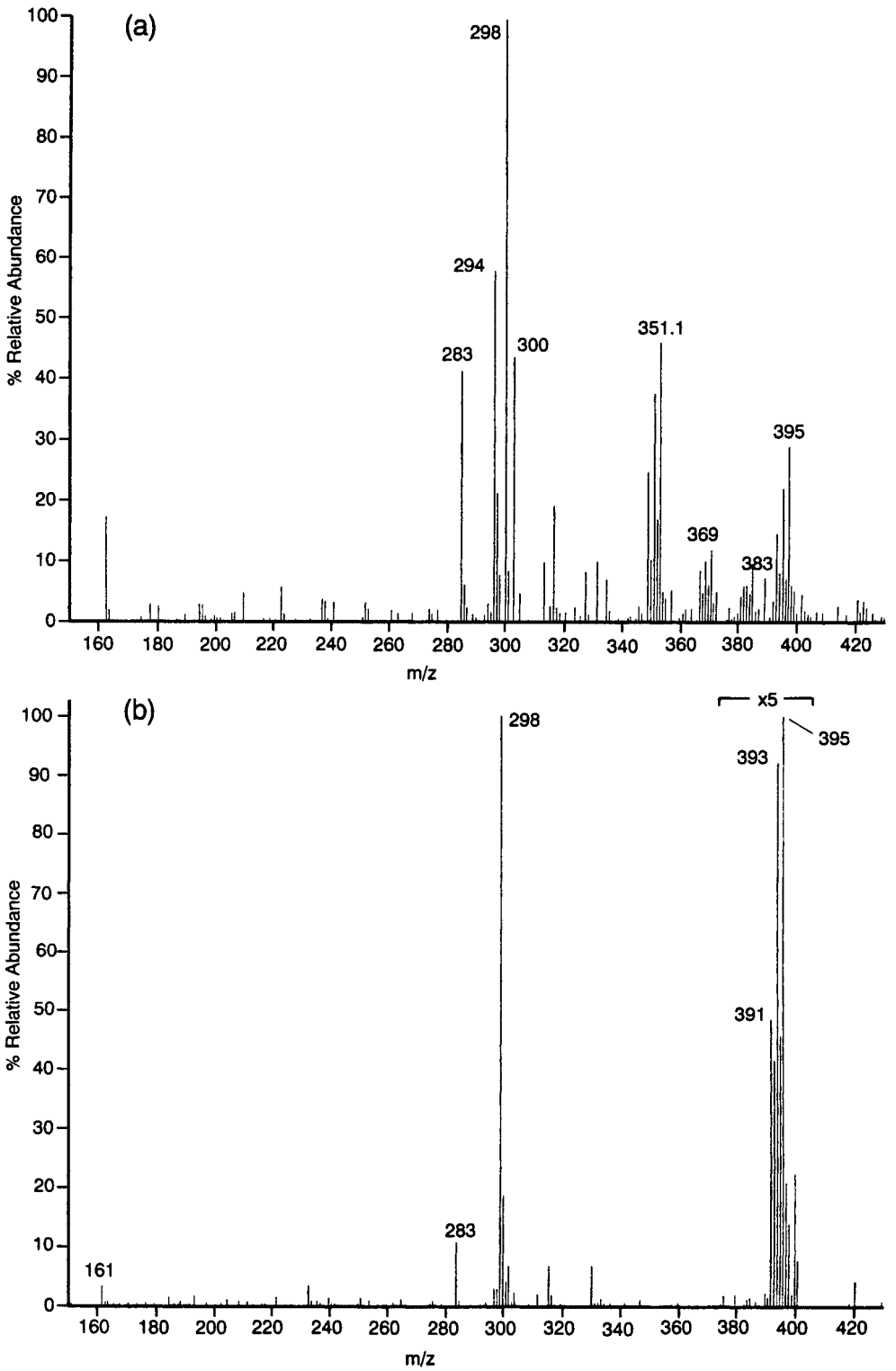

Figure 2 Mass spectra of (a) triphenyltin; (b) diphenyltin.

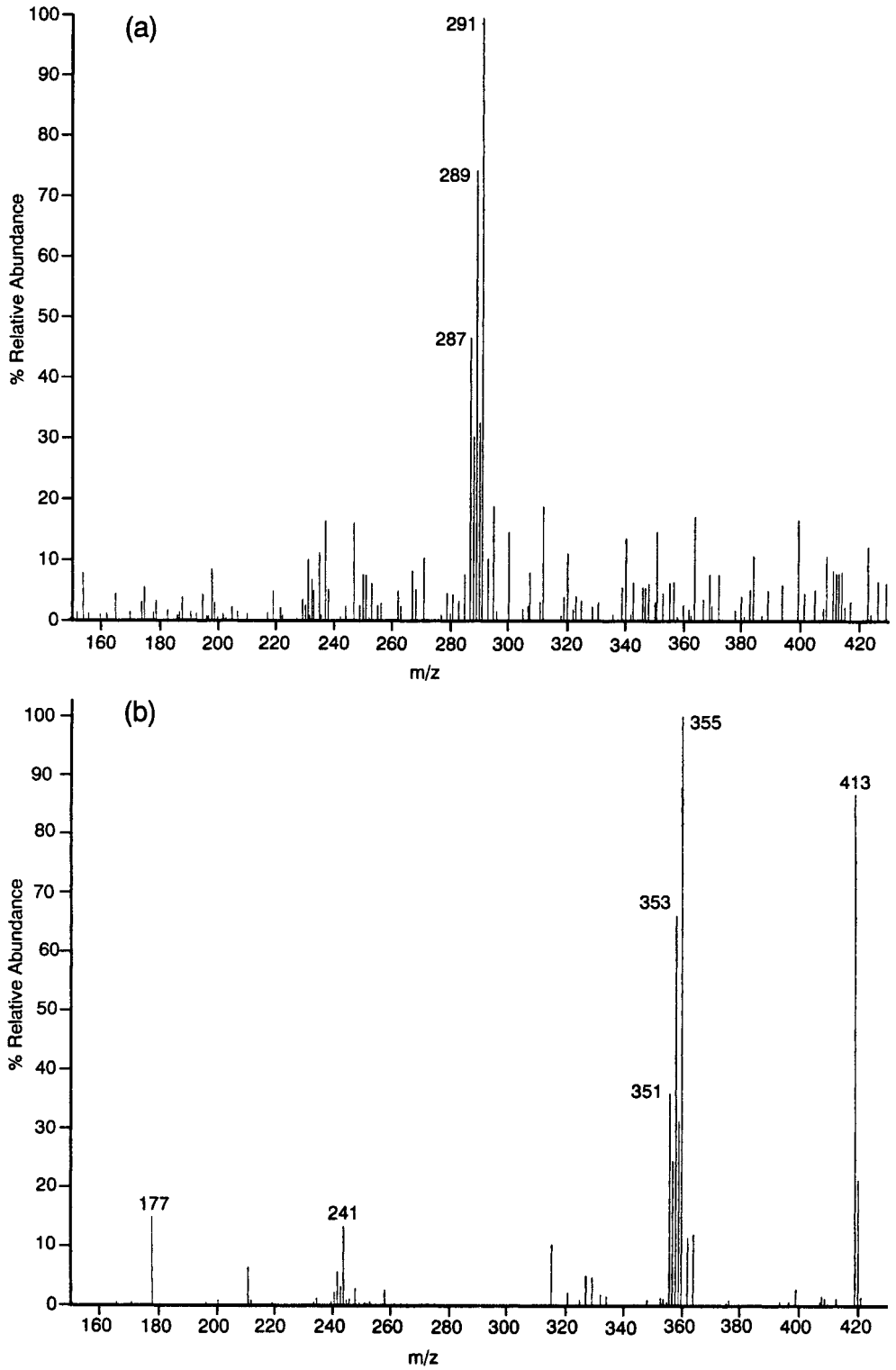

Figure 3 Mass spectra of (a) tributyltin; (b) dibutyltin.

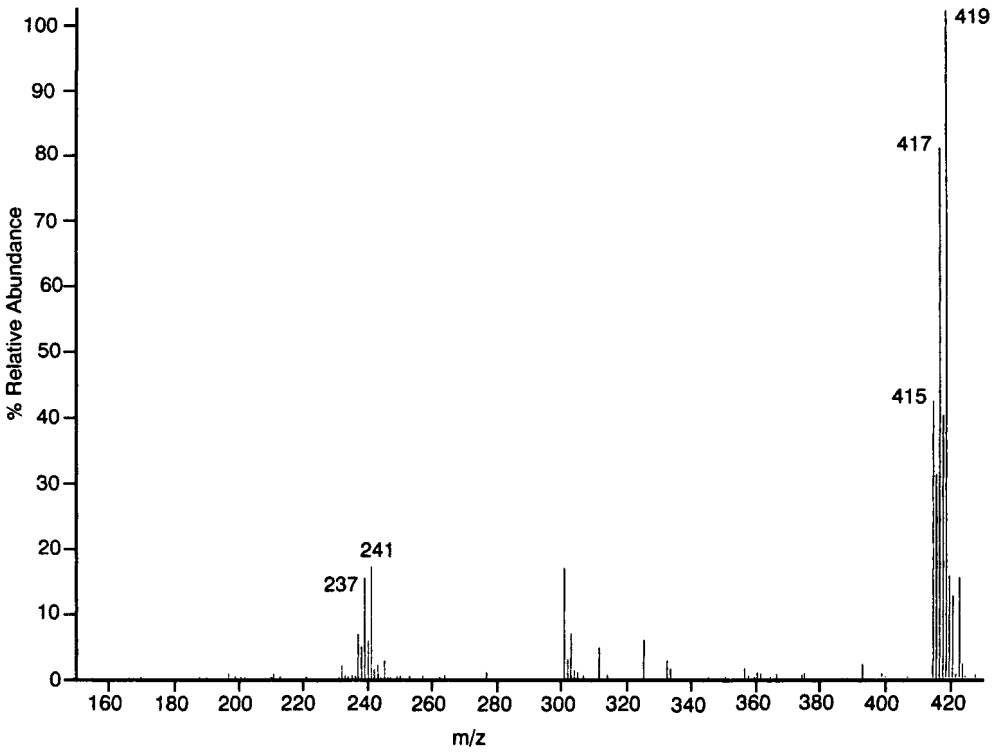

Figure 4 Mass spectrum of monobutyltin.

very sensitive mass-spectrometric method, still it is not totally definitive. The mass ions produced by ICP MS are from the individual tin isotopes, but are not indicative of the organic ligand(s). The goal of the work presented in this paper was to develop a method for extracting, separating and detecting the organotins, from a water matrix, without derivatization or hydrolysis, that would provide rapid (in less than 7 min) and definitive identification of the organotins (through the use of mass assignment by electrospray/ion trap mass spectrometry), and use very little solvent (in both the extraction and separation method).

EXPERIMENTAL

Materials

The organotin standards, 96% tributyltin chloride (TBT), 96% dibutyltin dichloride (DBT), 95% monobutyltin trichloride (MBT) and 96% diphenyltin (DPT), were obtained from Aldrich (Milwaukee, WI, USA). Triphenyltin (TPT) was obtained from ChemService (West Chester, PA, USA).

Stock solutions (approx. 500 ng μl^{-1}) were prepared in HPLC-grade methanol (Burdick & Jackson, Muskegon, MI, USA) and stored in darkness at 4 °C.

The solvents used for both the extractions and the mobile phase for micro-HPLC were HPLC-grade methanol (Burdick & Jackson, Muskegon, MI, USA), glacial acetic acid (J.T. Baker, Phillipsburg, NJ, USA), deionized water (NANOpure™, Barnstead, Dubuque, IA, USA), and tropolone (Aldrich, Milwaukee, WI, USA).

Instrumentation and operating conditions

Extractions

Extractions were done using a CPI International (Santa Rosa, CA, USA) Accuprep 7000™ manifold and either CPI nu-phase™ fiber C_{18} solid-phase extraction (SPE) disks or 3M Empore™ C_{18} SPE disks (3M Company, Roseville, MN, USA). The disks were prepared according to the manufacturers' instructions. The spiked waters (the organotins were spiked at approximate 7 to 12 ppb) were adjusted to approximately pH 2.5 with HCl,

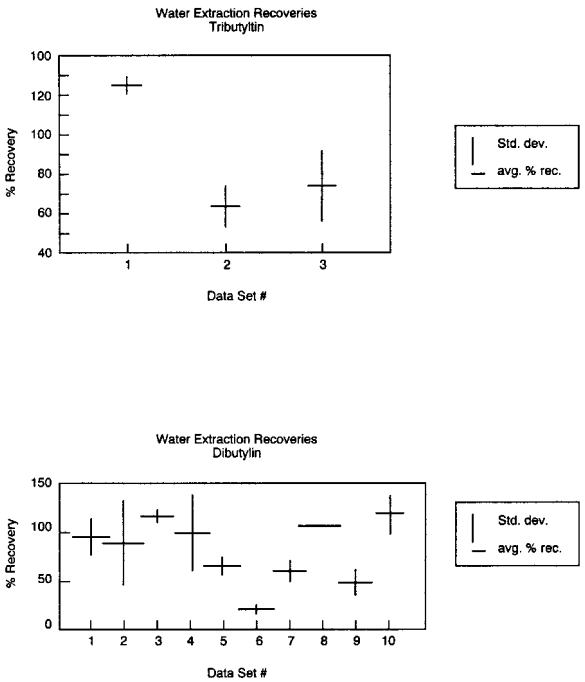

Figure 5 Recoveries from water extractions: (a) tributyltin (three duplicate sets); (b) dibutyltin (ten duplicate sets).

then allowed to gravity-extract through the prepared SPE disks for 30–40 min. The organotins were eluted from the disks with three 10-ml volumes of 99% methanol/1% acetic acid. The extracts were evaporated to 0.5 ml.

Analysis

The extracts were analyzed for the organotins by μ-liquid chromatography–electrospray/ion trap mass spectrometry (μ-LC–ES/IT MS).

Chromatography

The micro-capillary columns were prepared in-house. The 160-μm i.d. (360-μm o.d.) fused silica columns (Polymicro Technologies, Phoenix, AZ, USA) were packed with approximately 10–12 cm of 5-μm ODS-Hypersil (Shandon, Astmoor, UK), as described elsewhere by Moseley *et al.*[23]

An isocratic mobile phase of 80% methanol, 14% water, 6% acetic acid and 0.1% tropolone (w/v) was used.[24]

Mass spectrometry

A Finnigan LCQ™, configured with an electrospray (ES) ion source, was used to detect the organotins. The LCQ™ is an ion-trap mass spectrometer (IT MS) detector that performs real-time mass analyses of liquid chromatograph (LC) eluents over a mass-to-charge ratio range of 50–2000:1. The LCQ was run in the positive ion mode. The ES needle was run at approximately 4.5 to 5.2 kV, and the IT MS was scanned from 150 to 430 amu (full-scan mode) in 3 μscans with an ion injection of 200 ms.

RESULTS AND DISCUSSION

Micro-liquid chromatography

As discussed in the Experimental section, a micro-bore (160 μm i.d. $\times$ 10 cm packed material) C_{18} column was used for separation. An isocratic mobile phase of 80% methanol, 14% water, 6% acetic acid and 0.1% tropolone (w/v), with a flow rate of 4 μl min^{-1}, was used. This method was adapted in-house from an LC method developed by Dauchy *et al.*[24]

The use of tropolone as an additive in the mobile phase improved the chromatography and helped to stabilize the predominant ions of the organotins formed during the electrospray process. Without the use of tropolone, the organotins either do not elute at all, or elute in the void volume (no separation occurring), depending upon the species. Also, without the use of tropolone, for dibutyltin and diphenyltin, an oscillation occurred between the ions being formed during the electrospray process. For example, during one injection of dibutyltin, there would be more of the ion at *m/z* 179 $[M - 2\,Cl - C_4H_9 + H)^+]$, and then during the next injection the ion at *m/z* 293 $[M - 2\,Cl + CH_3CO_2)^+]$ would predominate. The addition of tropolone stopped this oscillation from occurring and stabilized the predominant ion of dibutyltin, at *m/z* 355 $[(M - 2Cl + C_7H_5O_2)^+]$, the dibutyltin–tropolone complex ion.

The three butyltins were adequately separated on the column. Monobutyltin elutes first, at about 1.9 min, followed by dibutyltin at 2.1 min and tributyltin at 3 min. By traditional chromatography (e.g. UV/Vis) this would not be an adequate separation but, by using IT MS and the masses as an indicator of separation, adequate resolution is obtained (Fig. 1). For the phenyltins, diphenyl tin elutes around 1.2 min, followed by triphenyltin at 2.8 min.

Electrospray/ion trap mass spectrometry

Electrospray ionization is considered a 'soft'

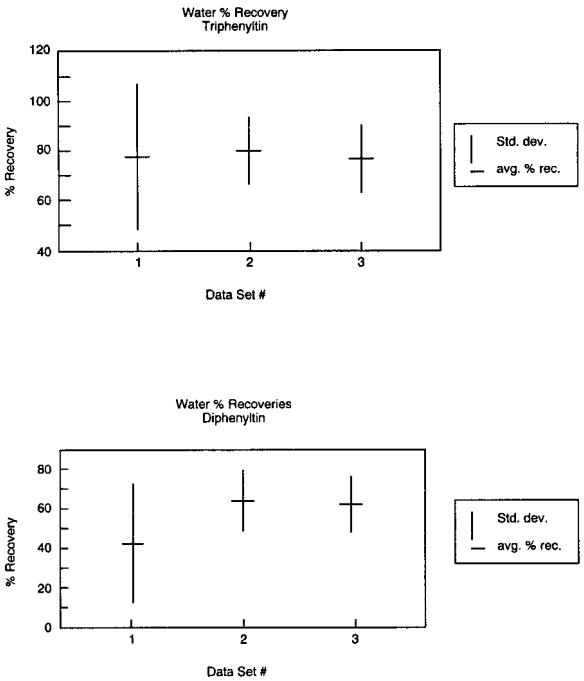

Figure 6 Recoveries from water extractions, (three duplicate sets): (a) triphenyltin (b) diphenyltin.

ionization technique. Consequently, few ions are produced, usually the molecular ion plus some adduct ion from the mobile phase solutions.[25,26] In the discussion of the mass spectra that follows, only the most abundant ion of the isotope cluster, based on ^{120}Sn (tin has ten isotopes), will be mentioned.

Due to their suspected presence in the environment from anthropomorphic sources dibutyltin, tributyltin and triphenyltin were the three main organotins chosen for this study. From physical studies these organotins are known to cycle and breakdown into other organotins (e.g. tributyltin to dibutyltin and monobutyltin, and triphenyltin to diphenyltin).[27] Therefore, the other related organotins (e.g. monobutyltin and diphenyltin) were added to this study, so that their definitive identification in environmental samples would be possible.

The LCQ was run in the full-scan (150–430 amu) positive ionization mode. Although the negative ionization mode was tried, it was not as sensitive as the positive ionization mode under the experimental conditions used in this study.

Excluding interference ions from the mobile phase (e.g. masses 298, 294, 300, 283), the base ion for triphenyltin chloride (mol.wt = 386 Da) is *m/z* 351: see Fig. 2(a). This is attributable to the loss of one chlorine atom from triphenyltin chloride, $(M - Cl)^+$ (triphenyltin cation is an even-electron ion). Other ions present in the spectrum at 62%, 31% and 22% (based on 100% relative abundance of mass 351), respectively, are *m/z* 395 $(M - Cl - C_6H_5 + C_7H_5O_2)^+$, the loss of one phenyl group and one chlorine, and the addition of one tropolone; *m/z* 369 $(M - Cl + H_2O)^+$, the loss of one chlorine and the addition of water; and *m/z* 383, $(M - Cl + CH_3OH)^+$, the loss of one chlorine and the addition of methanol. The formation of these ions can best be viewed as a nucleophilic attack of the non-bonding electrons of the oxygen on the very stable triphenyltin cation with formation of a tin–oxygen bond and with the positive charge residing on the resulting oxonium ion (oxygen bonded to three groups, as to tin and the two hydrogen atoms for water, or to a hydrogen and a CH_3 for methanol and also in the case of $C_7H_5O_2$). All of these species are prevalent in this electrospray ionization (ESI) system and so a bimolecular reaction is reasonable. The driving force is the stability of the tin–oxygen bond, suggested by the bond strength in diatomic molecules D°_{298} of 131 ± 5 kcal mol^{-1} (548 ± 21 kJ mol^{-1}).[28] and the case of accommodation of the positive charge on the oxygen of an oxonium ion.

In spectra averaged from before and after the TPT chromatographic peak, the predominant ions have masses 298, 294, 300 and 283, and it is therefore concluded that these ions are background ions attributable to the components of the mobile phase. Some of these ions have been investigated by MS/MS and are attributable to methanol, water, acetic acid and tropolone ion clusters. It is also observed that the background ions are not present in the spectra of TBT, DBT and MBT (see Figs 3(a), 3(b), and 4). This is due to the fact that the background ions were better subtracted (using the LCQ™ software) from the butyltin analyte spectra than from the phenyltin spectra. One explanation of this is that the ionization efficiency is better, more ions are in solution and they are more completely dissociated with the butyltins than with the phenyltins. The butyltins produce a stronger analyte ion signal than the background ion signal, and therefore are more easily extracted from the background (using the LCQ™ software programs) ion signal.

The spectrum for diphenyltin dichloride (mol.wt = 344 Da) is easily identified, with only mass 395 present (from DPT) (Fig. 2b). The presence of mass 395 is from the loss of two chlorine atoms and the addition of the tropolonium ion, $(M - 2Cl + C_7H_5O_2)^+$.

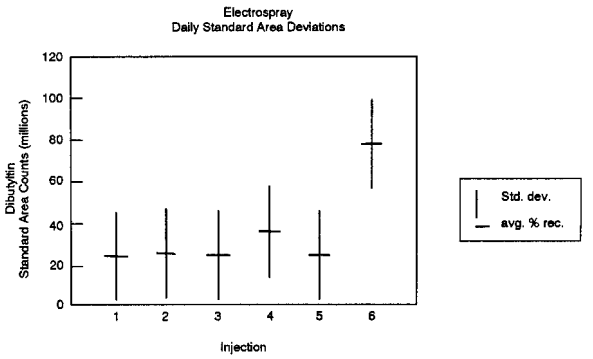

Figure 7 Electrospray daily standard area deviations.

Tributyltin (mol.wt = 326 D) presented some problems during the analyses. Its detection and subsequent spectra were sensitive to variable electrospray conditions, such as nitrogen flow, capillary tube temperature and the voltage difference between the tube lens and the capillary tube. Under ion source conditions optimized for tributyltin, there was only one ion present in the tributyltin spectra, *m/z* 291, due to the loss of one chlorine atom, $(M - Cl)^+$ (Fig. 3a). However, as ion source conditions change, more breakdown ions can be produced, such as *m/z* 235 from the loss of one chlorine atom and one butyl group with the addition of one hydrogen, $(M - Cl - C_4H_9 + H)^+$; and *m/z* 179, which is from the loss of one chlorine atom and two butyl groups and the addition of two hydrogens, $(M - Cl - 2C_4H_9 + 2H)^+$. Eventually, ion source conditions can deteriorate to the point where tributyltin is no longer detected. The spectrum of dibutyltin dichloride (mol.wt = 304 - Da) has only two ions present (attributable to dibutyltin); see Fig. 3(b). At *m/z* 355 is the base ion, from the loss of two chlorine ions and the addition of one tropolonium ion, $(M - 2Cl + C_7H_5O_2)^+$. The other ion present at 15% is *m/z* 241, from Sn(II) plus one tropolonium ion, $(Sn(II) + C_7H_5O_2)^+$. The presence of Sn(II) ions is not unexpected and was seen by one of the authors in previous work the ion at *m/z*.[22] Also, 413 (Fig. 3b) is a background ion, attributable to ion clusters formed from the mobile-phase constituents. The monobutyltin (mol.wt = 282 Da) spectra was relatively simple, with only two ions present (Fig. 4). The base peak (100%) was *m/z* 419, from the loss of three chlorine atoms and the addition of two tropolonium ions, $[M - 3Cl + 2(C_7H_5O_2)]^+$. The other ion present at 18% was *m/z* 241, from Sn(II) plus one tropolonium ion, $(Sn(II) + C_7H_5O_2)^+$.

The limit of detection (LOD) was determined for each of the three butyltin compounds. The LOD is defined as the lowest concentration of an analyte that an analytical process can detect and is located at 3σ (σ = standard deviation) above the gross blank signal.[29] By regression analysis of the data obtained from analyzing each of the three butyltin compounds at four or five different concentrations (ranging from 750 pg to 50 ng), using full-scan mode, the LODs were calculated as follows: tributyltin 780 pg; dibutyltin 970 pg; and monobutyltin 1 ng (the R^2 values were 0.99, 0.99, and 0.84, respectively). These results are approximately five times less sensitive than those reported by Dauchy *et al.*,[24] whose data, however, were given as nanograms of tin only. The values reported in this work are for the intact organometallic analyte.

Extraction recoveries

Deionized water was spiked between 7 to 10 ppb for dibutyltin and tri- and di-phenyltin, while tributyltin was spiked at 20 ppb. The extraction recoveries of tri- and di-butyltin from water are shown in Fig. 5(a) and (b), respectively. The average recovery of tributyltin was 86% and that of dibutyltin was 82%, with relative standard deviations (%RSDs) of 43% and 37%, respectively. The average recovery of triphenyltin and diphenyltin, from water, shown in Fig. 6(a) and (b), was 78% and 56%, respectively, with %RSDs of 21% and 36%. Overall the %RSDs for the organotin recoveries are large. This can be explained by the daily variations exhibited in the electrospray process and by the unstable nature of the organotins under electrospray conditions (see earlier discussion of spectra). The use of tropolone to stabilize the organotins (e.g. dibutyltin and diphenyltin) in solution corrects for the instability of the organotin ions, but not for the electrospray process. By its very nature electrospray is not a very 'rugged' technique. For example, slight variations in liquid pressure, mobile-phase flow rates, sheath-gas flow rates, voltage changes, buildup of surface contaminants on the capillary inlet and cone, and the ionization potential of the analyte, can all cause fluctuations in the flow of ions to the trap and subsequently in the signal from the electron multiplier. Figure 7 shows the daily variance from the electrospray from six dibutyltin standard (25-ng) injections over the course of 6 h. Due to just one standard area being greater, the %RSD is 68%. If that data point is removed from the data set then the %RSD drops to 18%. During the daily operation of

the electrospray these spurious fluctuations sometimes occur and were therefore averaged into the data. From our observation of the data some variation is observed from the extraction process, but most if not all of the contribution to the large %RSDs comes from the fluctuations of the electrospray process. This is a reflection of a short-term instability problem with the electrospray technique, and can be overcome through the use of statistical analysis and the re-analysis of those data that are out of statistical range. Another method to minimize this instability would be through the choice of an internal standard that would mimic the organotins, and yet not be environmentally available (e.g. deuterated organotins); the fluctuations would then be divided out, thereby minimizing the %RSD.

CONCLUSIONS

This new technique, combining SPE discs for extraction with μ-LC-ES/IT MS, for separation and detection of organotins, proved to be a sensitive method. Although there are stability problems with the electrospray process, they can be overcome. Future experiments will include improvements in the ruggedness of the detection method with the use of internal standards to correct for the electrospray variations. The new method has the advantage of low solvent usage, no interference from derivatization or hydrolysis by-products, and adequate (e.g. low-ppb) sensitivity necessary to detect organotins in environmental samples.

Acknowledgements One of us (T.L.J.-L) thanks Dr Spiros Pergantis (former NRC Post-doctoral Fellow at the EPA/NERL-ESD, Las Vegas, NV, USA) for his adaptation of Dauchy *et al*'s.[24] HPLC separation method for the butyltins to a micro-HPLC column. We also thank our colleagues Dr Wayne Sovocool and Dr L. Don Betowski for their help in clarifying the mechanistic aspects of the organotin spectra.

The US Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded and performed the research described. This manuscript has been subjected to the EPA's peer and administrative review and has been approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA for use.

REFERENCES

1. S. J. Blunden and A. Chapman, Organotin compounds. In: *Organometallic Compounds in the Environment*, Craig, P. J., (ed.), John Wiley, New York 1986.
2. *Synthetic Organic Chemicals — United States Production and Sales, 1991*, 75th edn, Publication No. 2607, US International Trade Commission, 1993, Chapter 15–10.
3. *The Economics of Tin*, 7th edn, Roskill Information Services, London, 1995, pp. 242–255.
4. K. Attar, *Appl. Organomet. Chem.* **10**, 317 (1996).
5. R. J. Huggett, M. A. Unger, P. F. Seligman and A. O. Valkirs, *Environ. Sci. Technol.* **26**, 232 (1992).
6. F. Monnet-Tschudi, M.-G. Zurich, B. M. Riederer and P. Honegger, *Neurotoxicology* **16**, 97 (1995).
7. S. Ohhira and H. Matsui, *Toxicol. Lett.* **85**, 3 (1996).
8. P. J. Craig (ed.), *Organometallic Compounds in the Environment*, Longman, Harlow, 1986.
9. H. Iwata, S. Tanabe, T. Mizuno and R. Tatsukawa, *Appl. Organomet. Chem.* **11**, 257 (1997).
10. K. Kannan, K. Senthilkumar, B. G. Loganathan, S. Takahashi, D. K. Odell and S. Tanabe, *Environ. Sci. Technol.* **31**, 296 (1997).
11. K. Fent and J. Stegeman, *Aquat. Toxicol.* **24**, 21 (1993).
12. Ph. Quevauviller, A. Bruchet and O. F. X. Donard, *Appl. Organomet. Chem.* **5**, 125 (1991).
13. M. A. Unger, W. G. MacIntyre, J. Greaves and R. J. Huggett, *Chemosphere* **15**, 461 (1986).
14. P. Rivaro, L. Zaratin, R. Frache and A. Mazzucotelli, *Analyst (London)* **120**, 1937 (1995).
15. H.-J. Yang, S.-J. Jiang, Y.-J. Yang and C.-J. Hwang, *Anal. Chim. Acta* **312**, 141 (1995).
16. I. R. Pereiro, V. O. Schmitt, J. Szpunar, O. F. X. Donard and R. Lobinski, *Anal. Chem.* **68**, 4135 (1996).
17. K. Li, S. F. Y. Li and H. K. Lee, *J. Liq. Chromatogr.* **18**, 1325 (1995).
18. F. Han, J. L. Fasching and P. R. Brown, *J. Chromatogr. Sect. B: Biomed. Appl.* **669**, 103 (1995).
19. T. L. Jones and L. Riddick, *J. Cap. Elec.* **4**, 33 (1997)
20. Y. Inoue, K. Kawabata and Y. Suzuki, *J. Anal. At. Spectrosc.* **10**, 363 (1995).
21. C. Rivas, L. Ebdon, E. H. Evans and S. J. Hill, *Appl. Organomet. Chem.* **10**, 61 (1996).
22. T. L. Jones and L. D. Betowski, *Rapid Commun. Mass Spectrom.* **7**, 1003 (1993).
23. M. A. Moseley, L. J. Deterding, K. B. Tomer and J. W. Jorgenson, *Anal. Chem.* **63**, 1467 (1991).
24. X. Dauchy, R. Cottier, A. Batel, R. Jeannot, M, Borsier, A. Astruc and M. Astruc, *J. Chromatogr. Sci.* **31**, 416 (1993).
25. M. Yamashita and J. B. Fenn, *J. Phys. Chem.* **88**, 4451 (1984).
26. M. Mann, *Organic Mass Spectrom.* **25**, 575 (1990).
27. E. Pelletier, Environmental organometallic chemistry of mercury, tin and lead: present status and perspectives. In: *Metal Speciation and Bioavailability in Aquatic Systems*, Tessier, A. and Turner, D. R. (eds.), John Wiley & Sons, Chichester (1995).
28. *CRC Handbook of Chemistry and Physics*, 64th edn, R. C. Woast, M. J. Astle, W. H. Beyer (eds.), CRC, Boca Raton, FL, (1983–1984)
29. D. MacDougall, W. B. Crummett, et al., *Anal. Chem.* **52**, 2242 (1980).

