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REVIEW

The Use of High-performance Liquid Chromatography for the Speciation of Organotin Compounds

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This review of the use of high-performance liquid chromatography (HPLC) for the speciation of organotin compounds which are primarily of significance in the marine environment, is divided into sections on the basis of the different HPLC modes of separation. However, it should be noted that such a classification does not exist in reality. For instance, in an ion-pair reversed-phase system the separation mechanism for the ionic solutes may be ion-pair partitioning, or ion exchange, or both. The relevant practical information (e.g. column type, mobile phase, method of detection and detection limit) is presented in tabular form. A brief overview of the reported detection methods is included, because the delay in development of an easily interfaced, specific and sensitive detector has hindered the use of HPLC for organotin speciation studies. The literature reviewed covers publications from 1977, the year of the first application of HPLC to organotin speciation, to April 1995.

Keywords: review; high-performance liquid chromatography; HPLC; organotin; tributyltin; speciation; marine environment; antifouling paints; environmental pollution

ABBREVIATIONS

AAS	Atomic absorption spectroscopy
AES	Atomic emission spectroscopy
CT	Cryogenic trapping
DBT	Dibutyltin
DCP	Direct current plasma
DCyHT	Dicyclohexyltin
DET	Diethyltin
DIN	Direct injection nebulization
DMT	Dimethyltin
DOcT	Dioctyltin
DPhT	Diphenyltin
DPrT	Dipropyltin
FPD	Flame photometric detector
GF	Graphite furnace
HG	Hydride generation
ICP	Inductively coupled plasma
LC	Liquid chromatography
MBT	Monobutyltin
MET	Monoethyltin
MIP	Microwave induced plasma
MMT	Monomethyltin
MPhT	Monophenyltin
QF	Quartz furnace
RI	Refractive index
SDS	Sodium dodecylsulphate
SEC	Size exclusion chromatography
SFC	Supercritical fluid chromatography
STAT	Slotted tube atom trap
TBT	Tributyltin
TCyHT	Tricyclohexyltin
TeBT	Tetrabutyltin
TeET	Tetraethyltin
TeMT	Tetramethyltin
TeOcT	Tetraoctyltin
TePhT	Tetraphenyltin
TET	Triethyltin
THF	Tetrahydrofuran
TMT	Trimethyltin
TPhT	Triphenyltin
TPrT	Tripropyltin

INTRODUCTION

It is widely accepted that the determination of the total concentration of a metal in the environment reveals little about its toxicity, or its environ-

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mental occurrence, persistence and fate.[1,2] It is for this reason that a great deal of research has been carried out into developing analytical techniques that are able to determine quantitatively the chemical form of trace metals in a wide variety of sample matrices.

Tributyltin (TBT) has by far the greatest toxicity to aquatic organisms of all the organotin compounds found in the marine environment.[3] The most significant source of TBT contamination is from its use as the active ingredient in antifouling paints.[2,4] Leaching of TBT into the waters around harbours, docks and areas of high boating activity has been shown to adversely affect non-target organisms such as mussels, oysters and dog whelks.[5,6] Recognition of these effects has led directly to restriction of the use of antifouling coatings containing TBT in a number of countries [France, 1982; USA, 1986; UK, 1987; Canada and New Zealand, 1989; and Europe, 1991].[7]

The determination of the long-term cycling and toxicity of the organotin compounds present in the environment requires development of sensitive and specific speciation methods that can differentiate between the parent compound (such as TBT), and its degradation products (DBT and MBT). Commonly such methods have to be sensitive to the 0.1 ng g^{-1} level for sediments and 1.0 ng l^{-1} for water.[8] Techniques developed so far for the determination of organotin speciation combine a separation stage and a selective or specific detection method.[9]

A commonly used approach is the generation of organotin hydrides, followed by cyrogenic trapping (CT) and boiling point separation. Detection is usually by quartz furnace atomic absorption spectrometry (CT–QF–AAS)[10] or flame photometry (FPD).[11] Gas chromatography (GC) is also widely used, with detection by quartz furnace atomic absorption spectroscopy (QF–AAS),[12] flame photometric detection (FPD)[13] or mass spectrometry (MS).[14] For high-performance liquid chromatography (HPLC), detection by flame[15] or graphite furnace[16] atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP–MS)[17] or spectrofluorimetry[18] have been reported. Recently, articles describing the use of supercritical fluid chromatography (SFC) have been published.[19]

In some cases the generation of hydrides followed by sequential vaporization has resulted in a variation in sensitivity.[10] Use of GC to separate tin species necessitates the generation of hydrides[20] or the conversion of the relatively involatile organotin species to their corresponding methyl, ethyl or pentyl derivatives.[2] Both of these derivatization steps are subject to interference, and involve considerable sample manipulation that can lead to analyte losses. SFC has so far only been coupled to ICP–MS detection. HPLC has three main advantages; (i) derivatization of the organotin compounds is not necessary, (ii) minimum sample handling is required and (iii) stationary and mobile phases can be varied to obtain the best separation. However, interfacing to the detection system can be problematic.

Several reviews dealing with the use of chromatographic techniques in metal speciation studies have been published.[9,21–23] Some of these discuss the interfaces used between the HPLC and the detection system and specific papers on this subject are also available.[9,24] The use of HPLC for the speciation of organotin compounds has been reviewed[25] and so has the coupling of liquid chromatography to atomic spectroscopy for the determination of metals and organometallics.[9,26] The purpose of the present work is to review comprehensively all the HPLC methods developed to date for the determination of a number of organotin compounds, which are known to be important in the marine environment. Some of the work reviewed deals with inorganic tin and this is included only for completeness.

SEPARATION STRATEGIES IN ORGANOTIN SPECIATION STUDIES

Ion-exchange separation mode

Ion-exchange chromatography is carried out on ionizable analytes, by using column packing materials that possess charge bearing functional groups. In the case of organotin compounds the sample cations $[R_nSn]^{(4-n)+}$ compete with the mobile phase counter-ions Y^+ for the ionic sites X^- of the cation exchanger.[27–29] Currently just over one-third of the published HPLC methods separate the organotin species by use of the ion-exchange mode. The practical details of these methods are summarized in Table 1.

The stationary phase consists of a solid matrix bearing fixed negatively or positively charged functional groups, depending on whether it is designed for anion or cation exchange. The support material is usually either a styrene

Table 1 Summary of ion-exchange HPLC methods for the determination of organotin speciation

Species Sample matrix	Column type Dimensions Support Manufacturer	Mobile phase flow rate	Method of detection	Sample volume (µl)	Detection limits (ng as Sn)	Date	Ref.
TMT, TET, TBT, TPrT, TPhT, TCyHT, DBT Aqueous leachate	Partisil 10 SCX 25 cm×4.6 mm i.d., 10 µm Silica based Whatman	Methanol (50–70%) Diammonium citrate (0.005–0.12 M) Ammonium acetate (0.01–0.06 M) 1–2 ml min^{-1}	GF–AAS	50–200	16 TBT 5 TPhT 26 TCyHT	1981	16
TBT, TET, TMT, TPhT Standards	PXS cation exchange 25 cm×4.6 mm i.d., 10 µm NR Whatman	Methanol (60%) Ammonium acetate (0.042 M) pH 5.3 1 ml min^{-1}	Electrochemical (differential pulse detection)	NR	6 TBT 6 TET	1981	30
Sn(II), Sn(IV), TBT Marine waters	Partisil 10 SCX 25 cm×4.6 mm i.d., 10 µm Silica based Whatman	Methanol (70%) Ammonium acetate (0.1 M) 3 ml min^{-1}	Flame AAS (pulse nebulization and STAT)	200–2000	200 TBT	1985	15
DBT, TBT Animal tissue	Partisil SCX 25 cm×2.0 mm i.d., 10 µm Silica based NR	Methanol (90%) Ammonium acetate (0.1 M) 2 ml min^{-1}	GF–AAS	2.2–20	0.5 TBT	1986	31
Sn(IV), DBT, TBT Estuarine waters	Partisil SCX 25 cm×4.6 mm i.d., 10 µm Silica based Whatman	Methanol (80%) Ammonium acetate (0.15 M) 1 ml min^{-1}	Fluorescence [post-column reaction with Morin in a micellar solution (Triton X-100®); excitation 408 nm, detection 534 nm]	175	16 TBT	1987	18
TMT, TET, TPrT, TBT, TPhT Ground and estuarine waters	Partisil SCX 25 cm×4.6 mm id., 10 µm Silica based Whatman	Methanol (70%) Sodium acetate (10 mM) Benzyltrimethylammonium chloride (2 mM) pH 5.5 1 ml min^{-1}	Indirect photometric (UV) detection at 263 nm	100	820 TMT 140 TET 100 TPrT 210 TBT 4 TPhT	1988	32
DBT, TBT Wood preservatives	Partisil SCX 25 cm×2.0 mm i.d., 10 µm Silica based Whatman	Methanol (90%) Ammonium acetate (0.1 M) or Methanol (70%) Ammonium citrate (0.066 M) 0.2–0.3 ml min^{-1}	Continuously heated GF–AAS (1100 °C)	20	0.5 TBT	1988	33

Table 1 (continued)

Species Sample matrix	Column type Dimensions Support Manufacturer	Mobile phase flow rate	Method of detection	Sample volume (μl)	Detection limits (ng as Sn)	Date	Ref.
TET, TPrT, TBT Sediment	Partisil SCX NR, 10 μm Silica based Whatman	Methanol (75%) Ammonium acetate (0.05 M) pH 5.1 2 ml min^{-1}	Laser-enhanced ionization spectroscopy (flame as an atom reservoir)	20	0.06 TBT	1988	34
DBT, TBT Estuarine waters	Partisil SCX 25 cm×4.6 mm i.d., 10 μm Silica based Whatman	Methanol (80%) Ammonium acetate (0.1 M) 3 ml min^{-1}	Flame AAS (pulsed nebulization and STAT)	2000	40 000 TBT	1988	35
DBT, TBT, TPhT Standards	Nucleosil 5 SA 25 cm×4.0 mm i.d., NR NR NR	Methanol (85%) Ammonium acetate (0.15 M) 1 ml min^{-1}	Fluorescence [post-column reaction with Morin in a micellar solution (Triton X-100®); excitation 392 nm, detection 555 nm]	NR	0.93 TBT 1.49 TPhT 25.3 DBT	1989	36
TBT, TPhT, inorganic Water	Partisil 10 SCX 25 cm×4.6 mm i.d., 10 μm Silica based Whatman	Methanol (80%) Ammonium acetate (0.1 M) 1.5 ml min^{-1}	Flame AAS (pulsed nebulization and STAT) ICP–MS	1000 175	200 TBT 1.6 TBT	1989	37
TMT, TBT, TPhT Standards	Adsorbosphere SCX 25 cm×4.6 mm i.d., 5 μm NR Alltech Assoc.	Methanol (85%) Ammonium acetate (0.1 M) 1 ml min^{-1}	ICP–AES ICP–MS	200 200	450 TMT 1500 TPhT 800 TBT 0.4 TMT 1.0 TPhT 0.8 TBT	1989	38
DBT, TBT Harbour sediment (PACS-1 SRM)	Partisil SCX 25 cm×4.6 mm i.d., 10 μm Silica based Whatman	Methanol (60%) Ammonium citrate (0.18 M) pH 6 1 ml min^{-1}	ICP–MS	100 or 200	0.02 TBT 0.04 TBT	1990	17
Sn(IV), TBT Harbour water	Partisil SCX 25 cm×4.6 mm i.d., 10 μm Silica based Whatman	Methanol (80%) Ammonium acetate (0.1 M) NR	Conversion to Sn(IV) by in-line UV photolysis, then hydride generation–flame AAS	1000 or 100	2 TBT	1991	39

Table 1 (continued)

Species Sample matrix	Column type Dimensions Support Manufacturer	Mobile phase flow rate	Method of detection	Sample volume (µl)	Detection limits (ng as Sn)	Date	Ref.
TMT, TET, TBT Standards	SCX NR NR NR	Methanol (80%) Ammonium acetate (0.2 M) pH 4 4 ml min^{-1}	Laser-excited atomic fluorescence spectrometry (LEAFS) with a flame as an atom reservoir	20	0.7 TBT	1991	40
MBT, DBT, TBT Aqueous solutions	Zorbax SCX 25 cm×4.6 mm i.d., 10 µm Silica based NR	Methanol (30%) Acetic acid (5%) Ammonium citrate (0.05 M) 1 ml min^{-1}	ICP–MS Fluorescence [after post-column reaction with Morin in a micellar solution (Brij-35®) at pH 3.5; excitation 408 nm, detection 534 nm] ICP–AES	100 100 NR	0.2 TBT 1.5 TBT NR	1993	41
MBT, DBT, TBT Vegetal sponge Harbour sediment (PACS-1 SRM)	Spherisorb SCX 25 cm×4.6 mm i.d., 5 µm Silica based NR	Methanol (60%) Diammonium citrate (0.18 M) pH 6.5 to pH 4.0 after 28 min 1 ml min^{-1}	GF–AAS	100	0.5 MBT 1.1 DBT 0.8 TBT	1993	42
Sn(IV), MBT, DBT, TBT Natural water	PRP X-200 25 cm×4.1 mm i.d., NR Resin based Hamilton	Methanol (100%) Citric acid (50 mM) Oxalic acid (4 mM) Lithium hydroxide (50 mM) 1 ml min^{-1}	HG–QF–AAS	100	0.55 MBT 0.93 DBT 0.78 TBT	1994	43
TPhT Marine water	Partisil SCX 25 cm×4.6 mm i.d., 10 µm Silica based Whatman	Methanol (80%) Ammonium acetate (0.15 M) 1 ml min^{-1}	Fluorescence [after post-column reaction with 3-hydroxyflavone in a micellar solution (Triton X-100®) at pH 6.0]	200	0.02 TPhT	1995	44

Abbreviations: NR, not reported; SCX, strong cation exhange; SRM, standard reference material.

divinylbenzene resin or silica. The resin ion-exchangers suffer from swelling effects with aqueous mobile phases, which result in their compressibility at high pressure. This drawback can be overcome to some extent by cross-linking, but this in turn leads to an unfavourable decrease in mass transfer processes. Silica based ion-exchangers overcome these problems to some degree, because they are mechanically stable and thus allow for high-pressure, fast separations. However, silica bonded phase columns are chemically unstable and can only be used in the restricted pH range of 2 to 8. It can be seen in Table 1 that the majority of organotin speciation studies employing ion-exchange chromatography are carried out with silica based columns.

To reduce the possibility of tailing, the mobile phase must be buffered. This ensures that the proportions of the neutral and ionized forms of the solute do not change throughout the chromatographic separation. In all of the systems that use silica based columns, the mobile phase consists of a certain percentage of methanol (50–90%, v/v) and a salt, which is usually ammonium acetate or citrate (0.005–0.2 M) (see Table 1). The use of a lower percentage of methanol (30%) necessitates the inclusion of a small amount of acetic acid to elute the strongly adsorbed monobutyltin species from the column.[41]

Jewett and Brinckman[16] were the first to develop the separation of organotin compounds on silica bonded ion-exchange columns and most of the subsequent work using these columns has been based on their findings. A number of column parameters were optimized, prior to the analysis of organotin compounds, $R_nSn^{(4-n)+}$, differing in their degree of substitution (n) and functionality (R). This work also instigated the development of element specific detection, to compensate for the absence of chromophores in most organotin compounds (the exception being the phenyl-substituted compounds) and the low detection limits associated with conventional HPLC detectors.

The separation mechanism they suggested for the organotin species was based on the three main characteristics of the column, namely, cation exchange due to the sulphonate groups, reversed phase due to the bonded phase and adsorption arising from exposed silanol sites. The scheme used to qualitatively describe the separation involved equilibrium reactions between an organotin cation, a singly charged anionic ligand and a substrate. The equilibrium constants described were measures of the dominant ion exchange and the less important partition and adsorption processes.

A considerable amount of research into the separation and determination of organotin compounds has been carried out by Ebdon and co-workers. Most of their work in this area involves the use of silica based cation-exchange columns and an eluent consisting of methanol and ammonium acetate. By using this system, coupled to a variety of detectors, they were able to determine Sn(II) and TBT in harbour water,[15] TBT in estuarine water,[18,35] inorganic tin, phenyltin and TBT acetate in spiked water samples,[37] inorganic tin and TBT in harbour water,[39] and inorganic tin and different butyltin and phenyltin species in aqueous solution.[41]

McLaren *et al.*[17] analysed an extract of a sediment standard reference material (PACS-1) by using a silica based cation-exchange column coupled to ICP–MS. A step gradient with 0.3 M ammonium citrate in 60% (v/v) methanol, with a pH change from 6 to 3 after 1 min, resulted in the elution of MBT over a large background peak due to inorganic tin. Because the extraction procedure they used was not effective for MBT, they limited their analysis to DBT and TBT and used isocratic elution employing 0.18 M ammonium citrate in 60% (v/v) methanol.

The above conditions were used by Pannier *et al.*[42] as the starting point for their investigation of the effects of pH, ionic strength and modifier concentration on the separation mechanism of the butyltin compounds. This work showed that the retention mechanism of these compounds could not be solely due to ion exchange, but must involve some degree of reversed phase interaction and adsorption, as suggested by the earliest work in this area.[16] This study also suggests that the butyltin cations may form stable complexes with the oxygen containing citrate ligands present in the eluent.

Pannier *et al.*[42] considered that DBT was retained via an ionic mechanism, that TBT was affected by adsorption to silanol groups and that MBT was totally dependent on adsorption for retention. All three butyltin compounds in PACS-1 were successfully determined by using 0.18 M diammonium citrate and 60% (v/v) methanol at pH 6.5, with a step change to pH 4 after 28 min.

To date, there is only one report of the use of resin based ion-exchange columns for organotin speciation.[43] The work utilized a sulphonated poly(styrene–divinylbenzene) column, an eluent

composed of 50 mM citric acid, 50 mM lithium hydroxide and 4 mM oxalic acid in 100% (v/v) methanol and detection via post-column hydride generation followed by electrically heated QF-AAS. With these conditions all three butyltin compounds and Sn(IV) were resolved in less than 10 min using isocratic elution.

Reversed-phase separation mode

The reversed phase mode involves the use of a polar eluent with a non-polar stationary phase and is particularly useful for the chromatography of polar molecules.[27–29] The bonded stationary phase usually consists of an alkyl moiety, which is chemcially bound to a silica support material. The eluent is usually water, containing a proportion of organic modifier such as methanol. The eluting power or 'strength' of the mobile phase dramatically increases with the proportion of organic solvent present. Separation of different organotin compounds has been achieved by a number of workers using this approach and a complete list of the practical details are given in Table 2.

The earliest reported separation of organotin compounds by HPLC was in 1977 by Brinckman *et al.*,[45] who analysed mixtures of triphenyltin, tributyltin and tripropyltin. These workers employed C_2 and C_{18} bonded phase columns, a mobile phase containing 100% (v/v) methanol and a GF–AAS system for detection. The authors proposed a retention mechanism based on a number of competing equilibria, involving different mobile and stationary phase ligands.

Burns *et al.*[46] compared the separation of different methyl- and ethyl-tin chlorides by GC and HPLC. They used a C_{18} column with an acetone/pentane mobile phase for both groups of analytes (the relative proportions were different in each case). They concuded that HPLC was better suited to the analysis of the methyltin chlorides, since redistribution reactions occurred with GC, but that for the ethyltin chlorides both techniques were satisfactory. The detection limits they achieved in this work using a refractive index detector were not low enough for environmental work, being of the order of 1 μg tin. In a later paper[48] the same authors employed identical separation conditions but detection was by QF–AAS, both directly and after hydride generation (HG). The detection limits were of the order of 10 ng tin for the direct flame method and 1 pg tin for the HG approach. This shows the enhancement in detection limit afforded by more specific detection methods, and hydride generation in particular.

The first successful use of the reversed-phase mode for the speciation of a large number of organotin compounds differing in both the type (e.g. methyl, ethyl, butyl etc.) and the number (e.g. mono-, di-, tri- etc.) of substituents was the work of Kadokami *et al.*[54] They established that aqueous, methanol or tetrahydrofuran eluents were unsuitable because the peak shapes of the di- and tri-substituted compounds were not symmetrical, and that the mono-substituted compounds could not be eluted from the column. In an effort to overcome these problems they added tropolone (2-hydroxy-2,4,6-cycloheptatrienone) or oxine (8-hydroxyquinoline) to the mobile phase. The idea for this came from the use of these reagents as complexing ligands in liquid/liquid extractions and also because oxine had been previously used to overcome adsorption interactions in the reversed-phase separation of other organotin compounds.[51] The inclusion of 0.2% (m/v) tropolone with tetrahydrofuran (54%, v/v), water (38%, v/v) and acetic acid (8%, v/v) allowed resolution of eight organotin compounds, including TBT, DBT and MBT, within 10 min. The detection limits using flame AAS adapted with a long absorption tube were 5 ng as tin for all eight organotin compounds. The same authors went on to demonstrate the use of this technique for the analysis of TBT in seawater.[54]

This separation system was further investigated by Dauchy *et al.*[57] by using ICP–MS as the method of detection. They found that the use of THF in the mobile phase produced a decrease in plasma stability, which they overcame by using methanol (80%, v/v), water (14%, v/v) and acetic acid (6%, v/v) containing 0.1% (m/v) tropolone. The limit of detection was of the order of 0.2 ng as tin. Further work[58] demonstrated the application of this HPLC–ICP–MS protocol to the determination of TBT, DBT and MBT in two marine sediment reference materials (PACS-1 and CRM 462 EEC).

Normal phase separation mode

This approach involves the use of stationary phases that have a higher polarity than that of the eluent.[27–29] The bonded phase columns used are made by covalent attachment of a polar organic moiety to the surfce of the microparticulate silica gel support. Non-polar organic solvents are usually employed as the eluent, although chloroform, ethanol or aqueous acetonitrile have

Table 2 Summary of reversed phase HPLC methods for organotin speciation

Species Sample matrix	Column type Dimensions Support Manufacturer	Mobile phase Flow rate	Method of detection	Sample volume (μl)	Detection limits (ng as Sn)	Date	Ref.
TPhT Saline solution	Lichrosorb C_{18} 25 cm×4.6 mm i.d., 10 μm Silica based NR	Methanol (100%) 1.5 ml min^{-1}	UV GF–AAS	20 20	166 TPhT[a] NR	1977	45
TPhT, TBT, TPrT Saline solution	Lichrosorb C_2 25 cm×4.6 mm i.d., 10 μm Silica based NR	Methanol (100%) 1.5 ml min^{-1}	UV GF–AAS	20 20	NR NR	1977	45
MMT, DMT, TMT, TeMT Standards	Spherisorb S5W 25 cm×3.0 mm i.d., NR NR Phase separations	Acetone (60%) Pentane (40%) NR	RI	50	100 000 MMT 90 000 DMT 80 000 TMT 50 000 TeMT	1980	46
MET, DET, TMT, TeET Standards	Spherisorb S5W 25 cm×3.0 mm i.d., NR NR Phase Separations	Acetone (70%) Pentane (30%) NR	RI	50	100 000 MET 90 000 DET 80 000 TET 50 000 TeET	1980	46
Sn(IV), TePhT, DBT, TMT, DMT, DPrT, DCyHT, TPhT Standards	Lichrosorb C_{18} 25 cm × 4.6 mm i.d., 10 μm NR NR	Methanol (97.5%) 0.1 ml min^{-1}	GF–AAS (curvette modified with zirconium in some instances)	NR	NR	1980	47
MMT, DMT, TMT, TeMT Standards	Spherisorb S5W 25 cm×3.0 mm i.d., NR NR Phase Separations	Acetone (60%) Pentane (40%) 1 ml min^{-1}	Flame AAS	50	19 MMT 17 DMT 16 TMT 11 TeMT	1981	48
			HG–QF–AAS (electrothermal atomization)	50	0.008 MMT 0.009 DMT 0.010 TMT 0.010 TeMT		
MET, DET, TMT, TeET Standards	Spherisorb S5W 25 cm×3.0 mm i.d., NR NR Phase separations	Acetone (70%) Pentane (30%) 1.2 ml min^{-1}	Flame AAS (nitrous oxide–acetylene)	50	19 MET 17 DET 16 TET 11 TeET	1981	48
			HG–QF–AAS (electrothermal atomization)	50	0.010 MET 0.012 DET 0.014 TET 0.014 TeET		

[a] Based on arsenic speciation calibration

Table 2 (continued)

Species Sample matrix	Column type Dimensions Support Manufacturer	Mobile phase Flow rate	Method of detection	Sample volume (µl)	Detection limits (ng as Sn)	Date	Ref.
TMT, TET, TPrT, TBT, TPhT Standards	Nucleosil C_{18} 10 cm×4.6 mm i.d., 5 µm NR Interchim	Methanol (80%) Ammonium acetate (0.1 M) pH 4.9 1.2 ml min^{-1}	UV Flame AAS	20 20	30 TET NR	1983	49
TPrT, TBT, TPhT Standards	Nucleosil C_{18} 10 cm×4.6 mm i.d., 5 µm NR Interchim	Tetrahydrofuran (50%) Ammonium acetate (0.1 M) pH 4.9 1.2 ml min^{-1}	UV Flame AAS	20 20	NR NR	1983	49
TBT, TPhT Anti-fouling coatings	Nucleosil C_{18} 10 cm × 4.6 mm i.d., 5 µm NR Interchim	Tetrahydrofuran (60%) Tetrahydrofuran (10%) Ammonium acetate (0.1 M) pH 4.9 1.2 ml min^{-1}	UV Flame AAS	20 20	NR NR	1983	49
TBT, TPhT Standards	Micropack MCH-5-N-Cap NR	Methanol (100%) 1 ml min^{-1}	GF–AAS	250	25 TBT	1984	50
TET, TBT, TPhT Standards	NR NR	Methanol (100%) 0.5 ml min^{-1}	GF–AAS	10	25 TBT		
DMT, DBT, DOcT Standards	Spherisorb S 5 ODS 15 cm × 3.2 mm i.d., NR NR Phase Separations	Methanol (85%) Water (15%) Oxine (1%) 1 ml min^{-1}	UV (380 nm) [pre-column reaction with chromophore (oxine); post-column decomposition of TBT and TeBT via UV radiation]	NR	20 DMT 40 DBT 400 DOcT	1984	51
TBT, TCyHT TPhT Standards	Spherisorb S 5 ODS 15 cm×3.2 mm i.d., NR NR Phase Separations	Acetonitrile (81%) Water (14%) Acetic acid Oxine (0.025%) In some cases LiCl was added (0.5–0.75%) 1 ml min^{-1}	UV (380 nm) [pre-column reaction with chromophore (oxine); post-column decomposition of TBT and TeBT via UV radiation)	NR	500 TBT 500 TCyHT 100 TPhT	1984	51

Table 2 (continued)

Species Sample matrix	Column type Dimensions Support Manufacturer	Mobile phase Flow rate	Method of detection	Sample volume (µl)	Detection limits (ng as Sn)	Date	Ref.
TeBT, TePhT, TeOcT Standards	Spherisorb S 5 ODS 15 cm×3.2 mm i.d., NR NR Phase Separations	Methanol (90%) Water (10%) Oxine (0.01%) for 2 min then Methanol (75%) 2-Propanol (25%) Acetic acid (0.025%) Oxine (0.01%) 1 ml min^{-1}	UV (380 nm) [pre-column reaction with chromophore (oxine); post-column decomposition of TBT and TeBT via UV radiation]	NR	800 TeBT 150 TePhT 5000 TeOcT	1984	51
TBT, TPhT Antifouling coatings	Nucleosil C_{18} 10 cm×4.6 mm i.d., 5 µm NR NR	Methanol (60%) Tetrahydrofuran (10%) Ammonium acetate (0.1 M) pH 4.9 NR	Flame AAS	20	25 TPhT	1986	52
DMT, DET, DPrT, DBT Textiles	RS-Pak DE-613 15 cm×6.0 mm i.d., NR NR Shodex	Methanol (70%) Phosphate buffer (0.01 M) pH 2.3 1 ml min^{-1}	Fluorescence	NR	NR	1987	53
DBT, DOcT Textiles	Asahipak GS 310H 25 cm×7.6 mm i.d., NR NR NR	Methanol (70%) Potassium dihydrogen phosphate (0.01 M) pH 2.0 0.5 ml min^{-1}	Fluorescence	NR	NR	1987	53
MMT, DMT, TMT, MBT, DBT, TBT, DPhT, TPhT Seawater	TSK gel ODS-80TM 25 cm×4.6 mm i.d., NR NR Toyo Soda	Tetrahydrofuran (54%) Water (38%) Acetic acid (8%) Tropolone (0.2%) 1 ml min^{-1}	Long-tube flame AAS	200	5 for all of the compounds	1988	54
DBT, TBT Water SRM	Nucleosil CH 15 cm×4.6 mm i.d., 5 µm NR NR	Toluene (100%) Tropolone (1 µg ml^{-1}) NR	GF–AAS (post-column modifier 0.01% picric acid in toluene)	NR	NR	1990	55

Table 2 (continued)

Species Sample matrix	Column type Dimensions Support Manufacturer	Mobile phase Flow rate	Method of detection	Sample volume (µl)	Detection limits (ng as Sn)	Date	Ref.
DBT, TBT Marine bivalves, shells	µBondapak C_{18} 30 cm× 3.9 mm i.d., 10 µm NR Waters Assoc.	Tetrahydrofuran (98%) Acetone (2%) Acetic acid (2%) NR	GF–AAS (post-column modifier 5 µl of 20 ppm palladium in 2% citric acid) Thermospray mass spectrometry (post-column modifier 0.2% trifluoroacetic acid)	50 10	NR NR	1990	56
MBT, DBT, TBT Standards	TSK gel ODS-80 TM 25 cm×4.6 mm i.d., NR NR Toso Haas	Methanol (80%) Water (14%) Acetic acid (6%) Tropolone (0.1%) 0.9 ml min^{-1}	ICP–MS	100	0.24 MBT 0.24 DBT 0.15 TBT	1993	57
MBT, DBT, TBT Marine sediment (PACS-1 and CRM 462 SRMs)	TSK gel ODS-80 TM 25 cm×4.6 mm i.d., NR NR Toso Haas	Methanol (80%) Water (14%) Acetic acid (6%) Tropolone (0.1%) 0.9 ml min^{-1}	ICP–MS	100	NR	1994	58

Abbreviations: NR, not reported; ODS, octadecyl silica; SRM, standard reference material.

Table 3 Summary of normal phase HPLC methods for organotin speciation

Species Sample matrix	Column type Dimensions Support Manufacturer	Mobile phase Flow rate	Method of detection	Sample volume (μl)	Detection limits (ng as Sn)	Date	Ref.
DMT, DET, DPrT, DBT, DOcT Animal tissue	Unisil QCN 25 cm×4.0 mm i.d., NR Cyanopropyl-bonded silica Gasukuro Kogyo	n-Hexane (50–95%) Ethyl acetate (5–50%) containing Acetic acid (5%) 1.2 ml min^{-1}	Fluorescence (post-column complex formation with morin in ethanol; excitation 420 nm, emission 500 nm)	NR	0.1–1 depending on species	1983	59
MMT, MET, MBT MBT, DBT Standards	Stainless steel 25 cm×4.6 mm i.d., 5 μm MPLC cartridge 10 cm×4.6 mm i.d., 5 μm Cyanopropyl-bonded silica Brownlee Labs	Toluene (85%) Acetic acid (5%) Methanol (2%) Acetonitrile (8%) Morin (5 μM) 1 ml min^{-1}	Fluorescence (pre-column complex formation with morin; excitation 420 nm, emission 500 nm)	10	NR	1984	60
TMT, TET, TBT, DBT TMT, TET, TPrT, TBT, TPhT Standards	Stainless steel 25 cm×4.6 mm i.d., 5 μm MPLC cartridge 10 cm×4.6 mm i.d., 5 μm Cyanopropyl-bonded silica Brownlee Labs	Hexane (98%) Acetic acid (1%) Ethyl acetate (1%) 1 ml min^{-1}	RI	NR	NR	1984	60
MBT, MOcT, DMT, DBT Standards	LiChrosorb Si 60 15 cm×3.2 mm i.d., 5 μm NR NR	Di-isopropyl ether (96%) Formic acid (4%) Oxine (0.25 M) 1.5 ml min^{-1}	UV (380 nm) (post-column decomposition via UV radiation)	NR	600 MBT 800 MOcT 400 DMT 200 DBT 100 DOcT	1984	51
DMT, DET, DPrT, DBT, DOcT, DPhT Standards	Stainless steel 25 cm×4.6 mm i.d., 5 μm MPLC cartridge 10 cm×4.6 mm i.d., 5 μm Cyanopropyl-bonded silica Brownlee Labs	Toluene Acetic acid (1–5%) Ethanol or methanol (1–5%) Morin (0.0015%) 1 ml min^{-1}	Fluorescence (pre-column reaction with morin; excitation 420 nm, emission 500 nm)	10	0.001 DPhT 0.004 DMT	1984	61
DBT, TBT Marine sediment	Nucleosil 15 cm×4.0 mm i.d., 5 μm Cyanopropyl-bonded silica NR	Toluene (100%) Tropolone (0.001%) 1 ml min^{-1}	GF–AAS (post-column modifier 0.5% picric acid in toluene)	250	5 TBT	1989	62

Table 3 (continued)

Species Sample matrix	Column type Dimensions Support Manufacturer	Mobile phase Flow rate	Method of detection	Sample volume (μl)	Detection limits (ng as Sn)	Date	Ref.
DMT, DET, DBT, DPhT, TMT, TET, TBT, TPhT Standards and tungsten complexes	RoSil CN 15 cm×4.6 mm i.d., 3 μm Cyanopropyl-bonded silica (silanol activity deactivated with Iodine Chloride) RSL, Eke, Belgium	Hexane (90%) Tetrahydrofuran (10%) Acetonitrile (in some cases) 1 ml min^{-1}	UV (220 nm)	10	NR	1990	63
MBT, DBT, TBT Water and sediment	Nucleosil 25 cm×4.0 mm i.d., 5 μm Cyanopropyl-bonded silica NR	Toluene (100%) Tropolone (0.005%) 1 ml min^{-1}	GF–AAS (post-column modifier 0.5% picric acid in toluene)	NR	NR	1992	64

Abbreviations: NR, not reported.

been used in some instances.[28] The stationary phases used are classified according to the degree of polarity of the functional groups at the surface. For organotin speciation the majority of the columns employed cyanopropyl bonded phases (see Table 3), which are considered to be of medium polarity.

Most studies that employ reversed- or normal-phase separation modes encounter problems associated with adsorption of the organotin compounds onto unreacted silanol groups. A number of methods are available to overcome this unwanted interaction, including the use of a chelating agent such as morin (2′,3,4′,5,7-pentahydroxyflavone) or tropolone,[54,64] or the inclusion of acetic acid, or other reagents,[41,61] to block interactions with silanol groups.

The use of a cyanopropyl bonded stationary phase to separate diphenyltin and dialkyltin compounds was reported by Langseth.[60] The mobile phase he used consisted of toluene, acetic acid, methanol or ethanol and morin. The organotin compounds were thought to form stale complexes with the morin, and this helped to reduce tailing due to adsorption by residual silanol groups present on the column. The high fluorescence intensity of the morin complexes (excitation at 420 nm and emission at 500 nm) gave good selectivity, sensitivity and detection limits of 1.0 pg for diphenyltin to 4.0 pg for dimethyltin. Further work[61] using these conditions led to the simultaneous determination of the butyltin, ethyltin and methyltin trichlorides as well as dibutyltin, monobutyltin and monomethyltin chlorides.

Various different stationary phases were evaluated for the liquid chromatography of organotin compounds by Praet *et al.*[63] Most of the phases evaluated were not suitable because of low efficiency [poly(styrene–divinylbenzene)], adsorption from residual silanol groups (octadecyl silica gel) or reaction with the stationary phase (aminopropyl silica gel). However, separation of some tetraalkyl and dialkyl organotin compounds was achieved by using a cyanopropyl column which had been treated with iodine chloride to mask its silanol activity.

Astruc *et al.*[64] used 0.005% (m/v) tropolone in toluene with a 0–5% (v/v) gradient of methanol to separate monobutyltin, dibutyltin, tributyltin and tetrabutyltin. This method could not be used routinely because it slowly degraded the column. With isocratic conditions using tropolone in toluene as eluent, TBT and TeBT co-eluted, whereas DBT was resolved but MBT was strongly adsorbed on the column. The HPLC system was interfaced to a GF–AAS system and the method used to determine the DBT and TBT concentrations in river water and sediment (it was assumed that no TeBT was present in the samples).

Size exclusion chromatography

This mode of separation, which has also been referred to as gel-permeation chromatography, is used for resolution of molecules on the basis of molecular size. More simply, molecules too large to enter the pores of the stationary phase remain in the eluent, whilst the smaller molecules which can permeate the phase are retained. By the use of polymers of accurately known molecular weight as calibrants and a well controlled flow rate, the molecular weight of an unknown solute can be estimated. Table 4 shows the practical details of the size-exclusion methods that have been used for organotin speciation studies.

Two types of packing material are commonly used: inorganic packings based on silica gel or glass, and cross linked polystyrene gels. The latter are compatible with a wide range of organic eluents, whereas the inorganic packings are suitable for both aqueous and organic mobile phases. The pore size of the packing material is important and, where a large range of solute molecular size exists, a number of SEC columns of different pore sizes will be arranged in series.

The use of size-exclusion chromatography (SEC) for the separation of organotin species has generally focused on the tin containing polymers used in antifouling paint formulations. It can be seen from Table 4 that, in every case, the columns were packed with cross-linked poly(styrene–divinylbenzene).

Parks *et al.*[65] employed two columns of nominal pore sizes 10^2 and 10^3 Å in series and a mobile phase of THF, to separate samples of organometallic polymers used as antifouling coatings. Using GF–AAS in combination with UV and RI detection, they were able to discern two different molecular weight fractions. In further work[68] using a single 10^3 Å column, a third peak was observed when acetic acid (0.5–5.0 μl) was injected 1–2 min after injection of the polymer formulation. The first two peaks were assigned to the organometallic polymer and tributyltin methacrylate, whereas the last peak was thought to be due to TBT^+, eluted by acetic acid as TBT acetate.

Several styrene–divinylbenzene polymer columns of different pore sizes have been used to

Table 4 Summary of size exclusion HPLC methods for organotin speciation

Species Sample matrix	Column type Dimensions Pore size Support Manufacturer	Mobile phase Flow rate	Method of detection	Sample volume (μl)	Detection limits (ng as Sn)	Date	Ref.
Organometallic polymers used as antifouling coatings (e.g. poly(tri-n-butyltin methacrylate–methyl methacrylate)	Styragel 30 cm×7.8 mm i.d., 10 μm Two columns of nominal pore size 10^3 and 10^2 Å were used in series Poly(styrene–divinylbenzene) Waters Assoc.	Tetrahydrofuran (100%) Tetrahydrofuran (95%) Acetonitrile (5%) 1 ml min^{-1}	RI UV (254 nm) GF–AAS	50 or 100	NR NR NR	1979	65
Sn(IV), MMT, DMT, TMT, TeMT Purification of organotin halides	Styragel 50 cm×7.8 mm i.d., <37 μm Three separate columns with pore sizes 60, 100 and 500 Å were used Poly(styrene–divinylbenzene) Waters Assoc.	Carbon tetrachloride (100%) 1–2 ml min^{-1}	RI	25	NR	1979	66
Tin containing organometallic compounds	μStyragel NR Pore size 100 Å Poly(styrene–divinylbenzene) Waters Assoc.	Toluene (100%) 0.3 ml min^{-1}	ICP–AES	50	NR	1981	67
Organometallic polymers of tributyltin methacrylate and methyl methacrylate	μStyragel 30 cm×7.8 mm o.d., 10 μm Nominal pore size 10^2 Å Poly(styrene–divinylbenzene) Waters Assoc.	Tetrahydrofuran (100%) (followed by injection of trace amounts of 0.5–50.0 μl acetic acid)	RI UV (254 nm) GF–AAS	100	NR NR NR	1983	68

Abbreviation: NR, not reported.

separate and purify the methylated tin halides.[66] The best results were obtained using a 60 Å pore-size column, with carbon tetrachloride as eluent. However, it was apparent from the elution order that the separation mechanism was not purely one of exclusion, but also involved adsorption.

Other separation modes

Micelle- and vesicle-mediated chromatography

Both of these separation modes have been used for organotin speciation (see Table 5) and involve the inclusion of a surfactant in the mobile phase. The use of micelles in the eluent for liquid chromatography separations was first studied by Armstrong and co-workers,[69,70] whereas vesicle-mediated separations have received little attention in the literature. Surfactant-based organized media, such as micelles and vesicles, assist in dissolving solutes that are not easily solubilized in aqueous eluents. The efficiencies obtained with these types of mobile phase can be comparable with those of hydro-organic eluents.[71,72]

Surfactants have a non-polar tail and a polar head group.[74] Upon reaching a critical concentration they form micelles, with the polar head groups in contact with the aqueous solution and the tails directed into a central, non-polar core. If the surfactant has two or more hydrophobic tails it can form into a bilayer, which upon sonication forms a doughnut-shaped vesicle. The important difference between these two configurations is the number of compartments that the solute molecule can occupy. With the micelle there are five possible positions and with the vesicle nine available sites. In principle, vesicles offer a greater variety of interactions with the solute molecule than do micelles.

Three different surfactants were tested for the separation of organotin compounds in the micellar mode.[73] Preliminary studies using a reversed-phase column and ICP–AES detection indicated that the only surfactant to facilitate separation was positively charged sodium dodecylsulphate (SDS). Separation of TMT-Cl, TET-Cl and TPrT-Cl was achieved with a concentration of 0.1 M SDS, whereas TBT-Cl eluted as a broad peak, with a large retention time. Further investigations indicated that the capacity factors decreased with increasing micelle concentration and column efficiencies increased with increasing acetic acid concentration. The separation of the mono-, di- and tri-methyltin chlorides required a reduced concentration of SDS (0.02 M) and the inclusion of potassium fluoride, which improved the peak shape of MMT.

Once the separation had been developed, the detector was changed to ICP–MS, which afforded lower detection limits. Some adjustments had to be made to the torch injector and mobile phase to avoid clogging. The detection limits with this approach were lower than those achieved by using ion exchange or ion-pair separations with a hydro-organic eluent.[38]

The use of mobile phases containing vesicles has not been as successful for the separation of organotin compounds as for other species such as arsenic, mercury and selenium.[74] This is primarily due to the high degree of hydrophobicity exhibited by compounds such as the butyltins. A reversed phase C_{18} column and an eluent containing ammonium citrate (0.1 M), acetic acid (5%, v/v) and vesicles of dihexadecyl phosphate (10^{-5} M) at pH 4.5, with a methanol gradient of 50–90% (v/v), was used to separate mono-, di- and tri-butyltin. To obtain an acceptable capacity factor and peak shape for TBT, at least 60% methanol was necessary. The retention time of all the butyltin species increased with surfactant concentration, which was considered to be typical of ion-pair surfactant chromatography where no micellar aggregates form.

Ion-pair chromatography

This mode is particularly useful for the separation of ionized or ionizable compounds and has been used for the separation of organotin compounds (see Table 5). The 'ion-pair' is formed between the solute ion and a counter ion of opposite charge, and has a low net charge and polarity. The technique has increased in popularity because of the limitations of the ion-exchange mode already mentioned.

Ion-pair separations can be carried out both in the normal phase and in reversed phase, but in the case of organotin compounds only the reversed-phase mode has been used. The mechanism by which separation takes place in reversed-phase ion-pair chromatography is not fully understood, but three models have been developed to explain it. These are the ion-pair, the dynamic ion-exchange, and the ion-interaction models.[29,78] Further discussion of these models is beyond the scope of this work, but is available in the literature.[79,80]

Only a few reports using the reversed-phase ion-pair approach for organotin speciation have appeared in the literature and these have focused on the determination of mono-, di- and tri-

Table 5 Summary of miscellaneous HPLC methods for organotin speciation

Mode of separation Species Matrix	Column type Dimensions Support Manufacturer	Mobile phase Flow rate	Method of detection	Sample volume (μl)	Detection limits (ng as Sn)[a]	Date	Ref.
Micellar	Spherisorb ODS-2	Sodium dodecyl sulphate	ICP–AES	100	NR		
MMT, DMT, TMT	5 cm×4.6 mm i.d., 5 μm	(0.1 M)	ICP–MS	100	0.027 TMT		
Standards	Silica based	Acetic acid (3%)			0.051 TET		
	Phase Separations	Propanol (3%)			0.110 TPrT		
		1 ml min^{-1}					
TMT, TET, TPrT		Sodium dodecyl sulphate	ICP–MS	100	0.046 MMT		
Standards		(0.02 M)			0.026 DMT		
		Acetic acid (3%)			0.126 TMT		
		Propanol (3%)					
		Potassium fluoride (0.01 M)					
		1 ml min^{-1}					
Vesicle-mediated	Spherisorb C_{18}	Gradient elution with 50–90% (B)	HG–ICP–AES	500	NR	1994	74
MBT, DBT, TBT	25 cm×4.6 mm NR, 10 μm	(A) Ammonium citrate					
Standards	Silica based	(0.1 M)					
	Phase Separations	Acetic acid (5%)					
		Dihexadecyl phosphate vesicles					
		pH 4.5					
		(B) Methanol					
Ion-pair	PRP-1	Sulphuric acid (0.01 M)	DCP emission	200	250 ppb MMT	1985	75
MMT, DMT, TMT	15 cm × 4.1 mm i.d., 10 μm	1-Hexanesulphonic acid	spectroscopy (303.4 nm)		250 ppb DMT		
	or	(0.003 M)			2000 ppb		
	25 cm×4.1 mm i.d., 10 μm	Potassium fluoride (0.003 M)			MMT		
Spiked food and waters	Poly(styrene–divinylbenzene)	Acetic acid (2.5%)	HG–DCP emission	NR	25 ppb MMT		
	Hamilton	2.5 ml min^{-1}	spectroscopy		250 ppb DMT		
					150 ppb TMT		

[a] Unless otherwise indicated.

Table 5 (continued)

Mode of separation Species Matrix	Column type Dimensions Support Manufacturer	Mobile phase Flow rate	Method of detection	Sample volume (µl)	Detection limits (ng as Sn)[a]	Date	Ref.
Ion-pair TMT, TBT, TPhT Standards	Spherisorb ODS-2 25 cm×4.6 mm i.d., 5 µm Silica based Phase Separations	Methanol (80%) Water (19%) Acetic acid (1%) Sodium pentanesulphonate (0.004 M) pH 3.0 (1 M H_2SO_4) 1 ml min^{-1}	ICP–AES ICP–MS	200 NR	200 TMT 1700 TBT 1300 TPht 0.4 TMT 0.7 TBT 1.0 TPhT	1989	38
Ion-pair MMT, DMT, DET, TMT Standards	Inertsil ODS-2 10 cm×1 mm i.d., 5 µm (metal-free glass-lined microcolumn) Silica based Scientific Glass Engineering	Methanol (25%) Ammonium heptanesulphonate (0.005 M) pH 3.1 30 µl min^{-1}	ICP–MS (with DIN)	0.5	0.010 MMT 0.008 DMT 0.010 DET 0.009 TMT	1992	76
Ion-pair TMT, TBT, TPhT Fish tissue	PRP-1 15 cm×4.1 mm i.d., 5 µm Poly(styrene–divinylbenzene) Anspec	Methanol (94%) Water (5%) Acetate buffer (1%) (composition of sodium acetate and acetic acid dependent on pH) Sodium pentanesulphonate (0.004 M) 1 ml min^{-1}	ICP–MS	200	0.002 TMT 0.002 TBT 0.002 TPhT	1993	77

[a] Unless otherwise indicated.
Abbreviation: NR, not reported.

methyltin chlorides[75,76] and of the tri-substituted methyl-, phenyl- and butyl-tin chlorides.[38,77] No work has been reported on the separation of the more environmentally significant butyltin chlorides using this approach.

Krull and Panaro[75] used a mobile phase containing 1-hexanesulphonic acid (0.003 M), potassium fluoride (0.003 M) and acetic acid (2.5%, v/v) made up in sulphuric acid (0.01 M) and a poly(styrene–divinylbenzene) column to separate the mono-, di- and tri-substituted methyltin chlorides. The addition of potassium fluoride improved peak shape and resolution, whereas the presence of a strong acid was necessary to protonate the organotin species and allow formation of the ion-pairs. The initial method of detection used was direct-current plasma emission spectroscopy (DCP), but this did not afford sufficiently low detection limits. To overcome the problem, post-column hydride formation was used, prior to detection by DCP emission spectroscopy. This extra step improved the detection limits by one order of magnitude. No interference between the composition of the mobile phase and the formation of hydrides was apparent.

Although ICP–AES does not give sufficiently low detection limits it has been successfully used to optimize separation prior to analysis by ICP–MS.[38] In this study the tri-substituted methyl-, butyl- and phenyl-organotin compounds were resolved using a C_{18} column and a methanol (80%, v/v), water (19%, v/v) and acetic acid (1%, v/v) mobile phase, containing sodium pentanesulphonate as the ion-pair reagent at pH 3. The detection limit using ICP–MS was three times lower than with ICP–AES. No problems due to the high methanol content of the eluent were reported, but a high background signal was apparent. It was thought that the background signal was due to tin compounds accumulating on the column and slowly eluting off over time.

Further studies[77] were carried out to investigate the accumulation of tin on the stationary phase and what effect this had on the separation of the tri-substituted methyl-, butyl- and phenyl-tin chlorides. A silica bonded reversed phase column and a polymer based reversed phase column were compared to determine the different characteristics of each. The results showed less retention of inorganic tin on the resin based column compared with the silica column, which had a high background signal after only a few injections.

Most of the ion-pair HPLC–ICP–MS studies reported have employed conventional analytical size HPLC columns (25 cm × 4.6 mm i.d.). However, a microbore column (25 cm × 1 mm i.d.) packed with Inertisil ODS-2 has been used to separate the mono-, di- and tri-methyltin chlorides and diethyltin dichloride[76] using an ion-pair approach. Direct injection nebulization (DIN) was used since it overcomes the band broadening associated with the standard nebulizers used in ICP. With this method the detection limits were about 10 pg tin for each compound, which is an improvement by a factor of 5–150 over previous reports,[38,73] but inferior to values obtained with SFC–ICP–MS.[81]

Separation by supercritical fluid chromatography (SFC)

The term 'supercritical fluid' is usually applied to fluids with critical temperatures (temperature above which the fluid cannot be liquefied) below 200 °C and with densities on the order of 0.1–1 g l^{-1} at pressures of 1000–6000 psi.[82] Carbon dioxide is probably the single most common SFC mobile phase. On a practical level, SFC can be thought of as a form of gas chromatography using a liquefied gas as mobile phase. It overcomes many of the limitations of GC and HPLC, because it can be used to separate thermally labile, non-volatile and high molecular weight compounds, as well as having higher efficiency nebulization into the detector, associated with gaseous samples.[81] The practical applications of SFC to the speciation of organotin compounds is shown in Table 6.

The speciation of organotin compounds via SFC was first investigated by Caruso and co-workers.[81] Using carbon dioxide as the eluent and a pressure programme consisting of 100 atm held for 1 min followed by a pressure ramp of 80 atm min^{-1} to a final pressure of 200 atm, separation of TBT and TePhT was achieved. The detection limits for both these compounds were very low, being in the range 0.035–0.045 pg. However, they were unable to separate TBT, DBT or TeMT with any of the conditions they tried. It was concluded that the approach showed potential for simultaneously determining complex mixtures of organometallics containing arsenic, mercury, lead and tin.

Further work by this research group[83] resulted in an SFC–ICP–MS system capable of separating TBT, TeBT, TPhT and TePhT with absolute detection limits ranging between 0.2 and 0.8 pg. A number of operating parameters such as the

Table 6 Summary of supercritical fluid chromatography (SFC) methods for organotin speciation

Species Sample matrix	Column type Dimensions Support Manufacturer	Mobile phase Flow rate	Method of detection	Sample volume (nl)[a]	Detection limits (Pg as Sn)	Date	Ref.
TBT, TeBT Standards	SB-Octyl-50 capillary column 0.25 μm film thickness, 50 μm i.d., 195 μm o.d., 2.5 m long Lee Scientific	Carbon dioxide NR	ICP–MS	10	TBT 0.043 TeBT 0.035	1991	81
TBT, TeBT, TPhT, TePhT Standards	SB-Biphenyl-30 capillary column 0.25 μm film thickness, 50 μm i.d., 375 μm o.d., 2.5 m and 10 m long Lee Scientific	Carbon dioxide (methanol modifier in some cases) 1.5 cm s^{-1}	ICP–MS	0.05 mm^3	TBT 0.80 TeBT 0.26 TPhT 0.57 TePhT 0.20	1992	83
TBT, TeBT Standards	SB-Biphenyl-30 capillary column 0.25 μm film thickness, diameter NR, 2 m long Lee Scientific	Carbon dioxide NR	ICP–MS	67	TBT 0.025 TeBT 0.035	1994	19

Abbreviation: NR, not reported.
[a] Unless otherwise reported

interface temperature, the oven temperature, carbon dioxide pressure programme, mobile phase composition and column length were evaluated to determine their effect on the separation and detection of these organotin compounds.

Modification of the sample introduction system to produce a more easily used interface between the SFC system and the ICP detector led to the determination of TBT and TeBT with detection limits of 0.025 and 0.035 pg respectively.[19] A number of other parameters, including the restrictor temperature, were also studied.

DETECTORS FOR HPLC SPECIATION STUDIES

It was realized from the outset of work using HPLC for organotin speciation studies that sensitive, element-specific detectors would be required.[45] The intention in this section is not to review all the detectors that have been used, but merely to highlight the more comon techniques in use.

The conventional HPLC detection methods that have been used include fluorescence,[18, 36, 41, 53, 59–61] ultraviolet,[32, 45, 49, 51, 63, 65, 68] electrochemical[30] and refractive index.[46, 60, 65, 66, 68] The majority of organotin species contain no ultraviolet or fluorescence chromophore (the exception being the phenyl-substituted compounds); consequently the commonly used photometric detection methods have to be modified to study these compounds.

In the case of fluorescence detection this is facilitated by reaction of the organotin compounds with a suitable reagent such as morin either pre-column[60, 61] or post-column.[18, 36, 41, 59] Direct UV detection at 254 nm has been used to determine TPhT[45] and different tin containing organometallic polymers used as antifouling coatings.[65, 68] The lack of a suitable UV chromophore on the alkyl-substituted organotin compounds has been overcome in a number of ways, including the use of a photometrically active counter-ion (benzyltrimethylammonium cation) in the mobile phase with indirect photometric detection of the organotins,[32] and on-column complexation of mono- and di-butyltin with oxine (8-hydroxyquinoline) after conversion of TBT and TeBT to those species by photochemical decomposition and detection at 380 nm.[51] Electrochemical detection methods[30] have not been widely reported for organotin speciation work, probably because of their lack of selectivity. The use of refractive index detection has been reported,[46, 60, 65, 66, 68] but for the methyl ethyl- and butyl-substituted organotin compounds it showed a marked lack of sensitivity.

The most commonly used detection systems for metal speciation work are atomic spectrometric methods,[1] such as atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES). These have been reviewed,[1, 9, 21, 22, 26] as have the interfaces used.[9, 24] Both of these techniques are metal-specific; this eliminates many of the problems associated with the conventional HPLC detectors previously mentioned. The general consensus is that the method of atomization (flame, furnace or plasma) must be able to handle large volumes of mobile phase, solvent flow rates in the range 0.1–2.0 ml min^{-1}, and eluents that may be non-aqueous in nature.[26]

Flame atomic absorption spectroscopy readily accepts liquid samples, but does not provide low enough detection limits to determine organotin levels in environmental samples. Three approaches have been used to overcome this problem, including the use of the slotted tube atom trap,[15, 35] the quartz furnace[48] or the generation of hydrides.[39, 43, 48] The first two approaches effectively increase the residence time of the analyte in the flame, whereas the hydride generation method overcomes the low nebulization efficiency encountered with aspiration of liquid samples. For some species the limit of detection can be lowered by a factor of 1000 by using hydride generation (HG–QT–AAS), and this approach also eliminates interferences from compounds that do not readily form hydrides.[48]

Electrothermal atomization offers higher sensitivity than the use of flames,[9] but because of the temperature cycle involving drying, ashing and atomization steps, analysis is usually off-line and discontinuous in nature. The interfaces used to couple HPLC to ET–AAS have been reviewed[9] and are based upon those developed by Brinckman *et al.*[45] which comprised some form of fraction collector with autosampler for injection into the graphite cuvette. With organotin studies some form of modifier, such as palladium,[56] is usually added to reduce the formation of refractory tin carbide compounds. The use of a continuously heated electrothermal system has been reported[33] but not widely adopted. The system involved separation on a microbore (2 mm i.d.) column at a low flow rate (0.2 ml min^{-1}).

The column was connected via a fused silica capillary to a heated interface (110 °C) and a cuvette continuously heated at 1100 °C. The cuvettes were side-heated and had a lifetime of 60 h of heating time.

Atomic emission spectroscopy has the advantage of long linear calibration ranges and simutaneous on-line determination of a number of elements. For these reasons it comes closest to meeting the requirements necessary for a universal HPLC detector for the determination of organometalllic species. However, the low temperature of flame atomic emission spectroscopy does not offer detection limits low enough for environmental work and therefore has not been used for organotin speciation. The use of a plasma as the method of atomization for AES has been reviewed[9, 26, 84] and found to be more sensitive than the various flames used, because of the greater atomization efficiency.

The three principal plasma sources that have been used in analytical studies include inductively coupled argon plasma (ICP), a direct-current argon plasma jet (DCP) and microwave-induced helium plasma (MIP). However, only the first two (ICP and DCP) have been coupled to HPLC for organotin determinations. Both of these are able to accommodate large aqueous or organic flows, whereas MIP is unable to tolerate aerosol introduction without destabilization or extinction of the plasma. For this reason MIP has been more widely used as a GC detector.

The high efficiency with which singly charged positive ions are produced by ICP mean that it is a very effective ionization source for mass spectrometry. Compared with plasma AES, the use of ICP–MS offers increased detection limits of two to three orders of magnitude (sub-picogram levels) with the additional capability of isotopic analysis. For these reasons, as well as its ability to readily accept eluent flow rates normally used in HPLC (0.2–1 ml min^{-1}), it is the most successful detection system for organometallic speciation studies. The use of plasma mass spectrometry for such work has increased over recent years (see Tables 1–6) and several reviews have been published.[9, 84–88]

The most important problems normally encountered when coupling HPLC to ICP-MS result from the composition of the eluent. High buffer concentrations can block the sampling and skimmer oirifices, adversely affecting the detection limits. The use of organic solvents in the eluent decreases the sensitivity, because of plasma instability and carbon deposition on the sampling and skimmer cones. Both of these effects can be overcome by various methods, including use of mixed gas plasmas, cooling the spray chamber, increasing the radio frequency power or using an acid wash between runs.[9, 84–88] Another approach has involved the replacement of the usual hydro-organic eluents used in some separation modes, with mobile phases containing micelles. Suyani *et al.*[73] obtained sub-ppb detection limits and relative standard deviations in the range 1–2% for the separation of a number of alkyltin compounds, when using sodium dodecylsulphate, acetic acid, propanol and potassium fluoride as the eluent.

A siginficant drawback of the ICP–MS systems commonly in use is the inefficiency of the nebulizers. As noted for flame AAS, conventional nebulization is only 2–5% efficient, so very little of the sample reaches the plasma and thus the maximum detection limit is not realized. Methods used to overcome this problem involve the formation of hydrides and the use of other nebulizers, such as the direct injection nebulizer. The use of this kind of nebulizer minimizes band broadening effects that are often apparent with larger volume nebulizers and thus allows for the use of microbore and capillary HPLC separations.[76]

The use of HPLC coupled to other mass spectrometric techniques for environmental analysis has been reviewed.[85–87] The combination of these analytical methods can provide sensitivity together with important structural information, thus allowing for comprehensive identification of unknown species. However, very few reports on the use of LC–MS for organotin speciation studies have appeared in the literature; this is probably due to problems encountered in interfacing the two techniques.

Cullen *et al*[56] used thermospray mass spectrometry to determine the presence of butyl- and cyclohexyl-tin compounds in oyster material, after a reversed-phase separation on a C_{18} column. This type of interface has also been used to determine di- and tri-butyltin as well as the antifouling agent triphenyltin acetate.[88] Siu *et al.*[89] used ion-spray mass spectrometry to determine the concentration of TBT in the certified reference material PACS-1 with greater accuracy and precision than are furnished by the commonly used techniques of GC–FPD and HPLC–ICP–MS. Sample introduction was by flow injection and thus interfacing problems were reduced, but no separation of different species was available.

CONCLUSIONS

HPLC has become an important technique for the analysis of organotin compounds, since it does not require the derivatization of analytes prior to analysis and therefore it reduces the number of sample manipulation steps and possible losses. The use of tin specific detectors coupled to the HPLC separation provides the detection limits necessary for the analysis of environmental samples and also reduces the need for time-consuming sample clean-up to remove interferences.

Of increasing interest is the development of more efficient interfaces for HPLC and ICP–MS, which would improve detection limits. This would also allow for the coupling of microbore (1–2 mm i.d.) and capillary (< 0.5 mm i.d) separations to ICP–MS. Such an approach would decrease solvent consumption and improve detection limits. Element specific detection systems that allow for the simultaneous determination of a number of different organometals and organometalloids after HPLC separation are also feasible and would simplify the hazard assessment of environmental samples.

REFERENCES

1. Y. K. Chau and P. T. S. Wong, *Fresenius' Z. Anal. Chem.* **339**, 640 (1991).
2. J. A. J. Thompson, M. G. Sheffer, R. C. Pierce, Y. K. Chau, J. J. Cooney, W. R. Cullen and R. J. Maguire, NRCC No. 22494, National Research Council of Canada, Ottawa, Canada, 1985.
3. R. J. Maguire, *Appl. Organomet. Chem.* **1**, 475 (1987).
4. P. H. Dowson, D. Pershke, J. M. Bubb and J. M. Lester, *Environ. Poll.* **76**, 259 (1992).
5. R. J. Huggett, M. A. Unger, P. F. Seligman and A. O. Valkirs, *Environ. Sci. Technol.* **26**, 232 (1992).
6. Oceans, 86, *Proc. Int. Organotin Symp., Washington DC*, Vol. 4 1986. Institute of Electrical and Electronics Engineers, New York.
7. C. Stewart and J. A. J. Thompson, *Marine Poll. Bull.* **10**, 601 (1994).
8. W. M. R. Dirkx, R. Lobinski and F. C. Adams, *Anal. Chim. Acta* **286**, 309 (1994).
9. R. M. Harrison and S. Rapsomanikis (eds) *Environmental Analysis Using Chromatography Interfaced with Atomic Spectroscopy*, Ellis Horwood Series in Analytical Chemistry, Ellis Horwood Ltd, Chichester, 1989.
10. V. F. Hodge, S. L. Seidel and E. D. Goldberg, *Anal. Chem.* **51**, 1256 (1979).
11. R. S. Braman and M. A. Tompkins, *Anal. Chem.* **51**, 12 (1979).
12. W. M. R. Dirkx, M. B. de la Calle, M. Ceulemans and F. C. Adams, *J. Chromatogr.* **683**, 51 (1994).
13. M. D. Muller, *Anal. Chem.* **59**, 617 (1987).
14. J. R Ashby and P. J. Craig, *Appl. Organomet. Chem.* **5**, 173 (1991).
15. L. Ebdon, S. J. Hill and P. Jones, *Analyst (London)* **110**, 515 (1985).
16. K. L. Jewett and F. W. Brinckman, *J. Chromatogr. Sci.* **19**, 583 (1981).
17. J. W. McLaren, K. W. M. Siu, J. W. Lam, S. N. Willie, P. S. Maxwell, A. Palepu, M. Koether and S. S. Berman, *Fresenius' Z. Anal. Chem.* **337**, 721 (1990).
18. L. Ebdon and J. I. Gardia-Alonso, *Analyst (London)* **112**, 1551 (1987).
19. E. Blake, M. W. Raynor and D. J. Cornell, *J. Chromatogr.* **683**, 223 (1994).
20. S. Clark and P. J. Craig, *Appl. Organomet. Chem.* **2**, 33 (1988).
21. J. C. Van Loon and R. R. Barefoot, *Analyst (London)* **117**, 563 (1992).
22. Y. K. Chau, *Analyst (London)* **117**, 571 (1992).
23. J. C. Van Loon, *Anal. Chem.* **51**, 1139A (1979).
24. S. Hill, L. Ebdon and P. Jones, *Anal. Proc.* **23**, 6 (1986).
25. X. Dauchy, A. Astruc, M. Borsier and M. Astruc, *Analusis* **20**, 41 (1992).
26. L. Ebdon, S. Hill and R. W. Ward, *Analyst (London)* **112**, 1 (1987).
27. C. F. Simpson (ed.), *Techniques in Liquid Chromatography*, John Wiley and Sons, Toronto, 1982.
28. J. H. Knox, J. N. Done, A. F. Fell, M. T. Gibert, A. Pryde and R. A. Wall, *High Performance Liquid Chromatography*, Edinburgh University Press, Edinburgh, 1978.
29. L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd edn, John Wiley and Sons, New York.
30. W. A. MacCrehan, *Anal. Chem.* **53**, 74 (1981).
31. D. K. Orren, W. M. Braswell and P. J. Mushak, *J. Anal. Toxicol.* **10**, 93 (1986).
32. C. W. Whang and L.-L. Yang, *Analyst (London)* **113**, 1393 (1988).
33. O. Nygren, C. A. Nilsson and W. Frech, *Anal. Chem.* **60**, 2204 (1988).
34. K. S. Epler, T. C. O'Haver, G. C. Turk and W. A. MacCrehan, *Anal. Chem.* **60**, 2063 (1988).
35. L. Ebdon, K. Evans and S. Hill, *Sci. Tot. Environ.* **68**, 207 (1988).
36. W. Kleibohmer and K. F. Cammann, *Fresenius' Z. Anal. Chem.* **335**, 780 (1989).
37. S. Branch, L. Ebdon, S. J. Hill and P. O'Neill, *Anal. Proc.* **26**, 401 (1989).
38. H. Suyani, J. Creed, T. Davidson and J. A. Caruso, *J. Chromatogr. Sci.* **27**, 139 (1989).
39. L. Ebdon, S. J. Hill and P. Jones, *Talanta* **6**, 607 (1991).
40. A. P. Walton, G-T Wei, Z. Liang, G. Michel and J. B. Morris, *Anal. Chem.* **63**, 232 (1991).
41. J. I. Garcia-Alonso, A. Sanz-Medel and L. Ebdon, *Anal. Chim. Acta* **283**, 261 (1993).

42. F. Pannier, X. Dauchy, M. Poutin-Gautier, A. Astruc and M. J. Astruc, *Appl. Organomet. Chem.* **7**, 213 (1993).
43. G. Schulze and C. Lehmann, *Anal. Chim. Acta* **288**, 215 (1994).
44. R. Compano, M. Granados, C. Leal and M. D. Prat, *Anal. Chim. Acta* **302**, 185 (1995).
45. F. E. Brinckman, W. R. Blair, K. L. Jewett and W. P. Iverson, *J. Chromatogr. Sci.* **15**, 493 (1977).
46. D. T. Burns, F. Glocking and M. Harriott, *J. Chromatogr.* **200**, 305 (1980).
47. T. M. Vickrey, H. E. Howell, G. V. Harrison and G. J. Ramelow, *Anal. Chem.* **52**, 1743 (1980).
48. D. T. Burns, F. Glocking and M. Harriott, *Analyst (London)* **106**, 921 (1981).
49. C. Lattard and J. L. Rocca, *Analusis* **9**, 457 (1993).
50. R. Pinel, M. Z. Benabdallah, A. Astruc, M. Potin-Gautier and M. Astruc, *Analusis* **7**, 344 (1984).
51. W. G. Lakata, E. P. Lankmayr and K. F. Muller, *Fresenius' Z. Anal. Chem.* **319**, 563 (1984).
52. C. Basset and J. L. Rocca, *Double Liaison—Chim. Peint.* **33**, 23 (1986).
53. H. Nakashima, S. Hori, S. Iwagami, H. Nakazaua and M. Fujita, *Bunseki Kagaku* **36**, 867 (1987).
54. K. Kadokami, T. Uehiro, M. Morita and K. Fuwa, *J. Anal. At. Spectrom.* **3**, 187 (1988).
55. A. Astruc, R. Pinel and M. Astruc, *Anal. Chim. Acta* **228**, 129 (1990).
56. W. R. Cullen, G. K. Eigendorf, B. U. Nwata and A. Takatsu, *Appl. Organomet. Chem.* **4**, 581 (1990).
57. X. Dauchy, R. Cottier, A. Batel, R. Jeannot, M. Borsier, A. Astruc and M. Astruc, *J. Chromatogr. Sci.* **31**, 416 (1993).
58. X. Dauchy, R. Cottier, A. Batel, R. Jeannot, M. Borsier, A. Astruc and M. Astruc, *Environ. Technol.* **15**, 569 (1994).
59. T.-H. Yu and Y. Arakawa, *J. Chromatogr.* **258**, 189 (1983).
60. W. Langseth, *Talanta* **31**, 975 (1984).
61. W. Langseth, *J. Chromatogr.* **315**, 351 (1984).
62. A. Astruc, R. Lavigne, V. Desauziers, R. Pinel and M. Astruc, *Appl. Organomet. Chem.* **3**, 267 (1989).
63. A. Praet, C. Dewaele, L. Verdonck and G. P. Van der Kelen, *J. Chromatogr.* **507**, 427 (1990).
64. A. Astruc, M. Astruc, R. Pinel and M. Potin-Gautier, *Appl. Organomet. Chem.* **6**, 39 (1992).
65. E. J. Parks, F. E. Brinckman and W. R. Blair, *J. Chromatogr.* **185**, 563 (1979).
66. E. B. Jessen, K. Taugbol and T. Greibrokk, *J. Chromatogr.* **168**, 139 (1979).
67. D. W. Hausler and L. T. Taylor, *Anal. Chem.* **53**, 1223 (1981).
68. E. J. Parks, R. B. Johannesen and F. E. Brinckman, *J. Chromatogr.* **255**, 439 (1983).
69. D. W. Armstrong and J. H. Fendler, *Biochim. Biophys. Acta* **478**, 75 (1977).
70. D. W. Armstrong and S. J. Henry, *J. Liq. Chromatogr.* **3**, 657 (1980).
71. J. G. Dorsey, M. T. DeEchegaray and J. S. Landy, *Anal. Chem.* **55**, 924 (1983).
72. J. S. Landy and J. G. Dorsey, *Anal. Chim. Acta* **178**, 179 (1985).
73. H. Suyani, D. Heitkemper, J. Creed and J. A. Caruso, *Appl. Spectrosc.* **43**, 962 (1989).
74. A. Sanz-Medel, B. Aizpun, J. M. Marchante, E. Segovia, M. L. Fernandez and E. Blanco, *J. Chromatogr.* **683**, 233 (1994).
75. I. S. Krull and K. W. Panaro, *Appl. Spectrosc.* **39**, 960 (1985).
76. S. C. K. Shum, R. Neddersen and R. S. Houk, *Analyst (London)* **117**, 577 (1992).
77. U. T. Kumar, J. G. Dorsey, E. H. Evans and J. A. Caruso, *J. Chromatogr.* **654**, 261 (1993).
78. S. Ahuja, *Selectivity and Detectability Optimization in HPLC*, Winefordner, J. D. (ed.), John Wiley and Sons, New York, 1989.
79. H. Liu and F. F. Cantwell, *Anal. Chem.* **63**, 2032 (1991).
80. M. T. W. Hearn (ed.), *Ion Pair Chromatography: Theory and Biological and Pharmaceutical Applications*, Marcel Dekker, New York 1985.
81. W-L Shen, N. P. Vela, B. S. Sheppard and J. A. Caruso, *Anal. Chem.* **63**, 1491 (1991).
82. *Focus—Supercritical Fluid Chromatography*, Technical Marketing Assoc. Ltd, 1987.
83. N. P. Vela and J. A. Caruso, *J. Anal. At. Spectrosc.* **7**, 971 (1992).
84. P. C. Uden (ed.) *Element Specific Chromatographic Detection by Atomic Emission Spectroscopy*, ACS Symp. Ser. No. 479, American Chemical Society, Washington, DC, 1992.
85. M. Linscheid, *Int. J. Environ. Anal. Chem.* **49**, 1 (1992).
86. R. D. Voyksner, *Environ. Sci. Technol.* **28**, 118A (1994).
87. T. R. Covey, E. D. Lee, A. P. Bruins and J. D. Henion, *Anal. Chem.* **58**, 1451A (1986).
88. I. Tolosa, J. M. Bayona, J. Albaiges, L. F. Alencastro and J. Tarradellas, *Fresenius' Z. Anal. Chem.* **339**, 646 (1991).
89. K. W. M. Siu, G. J. Gardner and S. S. Berman, *Anal. Chem.* **61**, 2320 (1989).