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Alkyltin speciation in sea water with on-line hydride conversion and gas chromatography–atomic emission detection

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ABSTRACT

A method for the speciation of alkyltin pollutants in sea water combines solid phase extraction of organotin species from aqueous samples and an on-line hydride generation technique with gas chromatography, followed by element-specific detection for tin. Detection was performed using a microwave-induced plasma atomic emission detection system which provides a 0.5 pg detection limit for tin and a $3 \cdot 10^4$ tin-to-carbon selectivity ratio. Solid-phase extraction provides fast, simple analyte concentration while on-line hydride conversion improves chromatographic behavior and analyte recovery. This combination results in a method capable of measuring pg/ml levels of alkyltin species in the complex matrix of sea water.

INTRODUCTION

Alkyltin compounds have a wide range of uses and their consumption has grown to $50 \cdot 10^6$ kg world wide in 1986 [1]. The largest use of organotin compounds, such as di-*n*-octyl and di-*n*-butyl tins, is in the polymer industry, where there are used extensively as stabilizers in poly(vinyl chloride) (PVC), in products ranging from floor coverings and piping to food packaging.

Alkyltins are also used as herbicides, pesticides, and slimicides in cooling tower water and as antifoulants in boat paints [2]. The trialkyltins are most commonly used as biocides, tributyltin chloride being used as an additive in boat paints; this has led to aquatic environmental problems. These additives are toxic to barnacles and other aquatic species that accumulate on the bottoms of boats, but they are also toxic to other species, particularly shellfish. Levels of tributyltin of pg/ml have been shown to have detrimental effects on shellfish populations [3] and have been shown to be concentrated in oysters and mussels with concentration factors of 5000–50 000 [4]. The bioaccumulation of these toxic species could lead to their appearance in human food supply.

Tin toxicity varies widely, tetraalkyltins and trialkyltins being the most toxic and the inorganic forms less so. As the number of the alkyl substituents increases for alkyltins, the level of toxicity increases as illustrated below. To determine the level of toxic material in a sample containing tin, a total tin measurement is insufficient, and the levels of the different organic forms must be measured to gain an accurate assessment of toxicity.

Increasing toxicity of organotins from right to left: $R_4Sn = R_3SnX > R_2SnX_2 > RSnX_3 >>> SnX_4$.

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MEASUREMENT STRATEGIES

Extremely sensitive and selective methods of analysis are required to determine the highly toxic alkyltins present in complex environmental matrices. Atomic spectroscopic methods provide high degrees of sensitivity and selectivity, but cannot differentiate the organic bonded forms of a metal that may be present. Since such different forms of tin must be determined individually, analytical separation of the tin-containing components is needed. The microwave induced plasma (MIP) provides sensitive and selective spectroscopic detection of tin and when combined with gas chromatography allows speciation of volatile or derivatizable alkyltins in complex matrices [5,6].

Extraction/concentration techniques for alkyltin species in aqueous samples include purge and trap after hydride generation, liquid–liquid extraction and solid-phase extraction (SPE) [7–9]. The latter requires no reagent solutions, large extractant solvent quantities or tedious separation of phases; it is fast, simple and easily automated. The possibility of contamination of samples is also reduced. SPE readily provides analyte concentration factors of 1000 or more in situations in which the SPE extract can be concentrated by evaporation.

Conversion of the alkyltin chlorides to their analogous hydrides has several advantages. The former are difficult to gas chromatograph directly at trace levels because they react with chromatographic active sites, causing poor peak shape and analyte recovery. Hydride conversion provides more volatile derivatives, increases stability, improves peak shape and increases sensitivity. Utilized with GC–atomic emission detection (AED) methods, on-line hydride conversion gives "chemical reaction selectivity" in addition to chromatographic and spectroscopic selectivity. Analysis of the same sample with or without hydride conversion may aid confirmation and identification analytes. Craig *et al.* have developed an on-line hydride conversion technique [10,11] in which a small amount of solid sodium borohydride ($NaBH_4$) is placed inside the gas chromatograph injection port and extracted tin chlorides are injected through it. This paper presents a method which combines SPE, on-line hydride conversion and GC–AED, for alkyltin speciation in sea water samples. It provides the sensitivity and selectivity to measure pg/ml levels of organotin species with little sample preparation and few possibilities for sample contamination.

EXPERIMENTAL

Instrumentation

A HP5921A GC–AED system (Hewlett-Packard, Avondale, PA, USA) [12–15] which features a thin walled, water cooled quartz discharge tube, a solvent venting system and a direct connection between the cavity and a nitrogen purged spectrometer, was used for all GC analyses. Wavelength dispersion in this system is with a fixed grating spectrometer with a flat focal plane, and detection is by a movable photodiode array. The photodiode array detector allows simultaneous multi-element monitoring within wavelength regions of about 20–30 nm, from 160 nm to 810 nm. Tin was detected at 303 nm, hydrogen and oxygen being added to the plasma gas as reagents. This system allows two different pre-set plasma make-up flow conditions, a "high" and "low" helium flow, the former providing better sensitivity and selectivity for tin.

A Model 5890 II gas chromatograph with a HP-7673A auto-injector and "Chem Station" (Hewlett-Packard) were used in combination with the AED system. GC columns used included: a 12 m × 0.32 mm I.D., 0.17 μm film thickness column HP-1 (Hewlett-Packard), a 25 m × 0.32 mm I.D., 0.25 μm film thickness DB-1 (J&W Scientific, Folsom, CA) and a 10 m × 0.53 mm I.D., 0.25 μm film thickness HP-1 (Hewlett-Packard).

Chemical and reagents

Tributyltin chloride and tripropyltin chloride (Aldrich, Milwaukee, WI, USA) standards were prepared in HPLC-grade dichloromethane or methanol (Fisher Scientific, Fair Lawn, NJ, USA). Sodium borohydride, consisting of crystalline material of mesh range *ca.* 40–100 was used as obtained (Fisher Scientific). Solid-phase

extractions were performed using C_{18} Sep-Paks (Millipore, Milford, MA, USA).

On-line hydride conversion

Hydrides were formed on-line by placing *ca.* 100 mg of solid sodium borohydride ($NaBH_4$) inside the injection port liner and injecting the extracted alkyltin chlorides through the solid material [10]. A cup-type liner was used, the distance from the septum to the top of the $NaBH_4$ bed was *ca.* 45 mm and the column end was *ca.* 13 mm below the bed. Hydrides which formed in the hot injection port were then carried by the carrier gas into the GC column. The optimum temperature for the complete conversion of chlorides to hydrides without redistribution reactions occurring was 240–250°C.

The solid $NaBH_4$ plug, which filled approximately 1 cm of the injection port liner, was held in place between plugs of glass wool. Initially the $NaBH_4$ was mixed in a ratio of approximately 4:1 with an inert material such as a molecular sieve in order to keep it from packing too tightly, thus causing non-uniform carrier gas glow patterns through the solid material. This procedure proved to be of small utility and was later eliminated for simplicity.

No problem was experienced with the $NaBH_4$ becoming inactive, even after 50–60 splitless injections, and so automated injections could proceed without interruption. Initially there was some concern that large splitless injections might dissolve some of the solid reagent and wash it onto the head of the column where it could precipitate and clog the column. This problem never occurred however, despite many 5-μl splitless injections.

Preparation of standards

Tripropyltin chloride and tributyltin chloride stock standards were prepared by dissolving the appropriate amount of the analyte in HPLC-grade methanol or dichloromethane. These stock solutions were stored under refrigeration in the dark, and were used to make fresh dilutions each day. Methanol stock solutions were used to spike water samples at the appropriate levels.

Sample collection

Water samples were collected at two sites, the Thames River at the State Pier in New London, CT, USA, and the end of Long Wharf in Newport, RI, USA. The samples were collected by placing 1-l bottles just beneath the water surface and allowing them to be filled. Each of these sites was a marine environment where a high level of commercial boat traffic was known to occur.

RESULTS AND DISCUSSION

The linear dynamic range, limits of detection and selectivity for tin over carbon were determined as was the linear range of the hydride generation technique and its performance in the splitless injection mode.

Tin response factors for tetraethyltin, tripropyltin hydride and tributyltin hydride were 251, 193 and 203 area counts/ng tin injected, respectively. The slightly lower response factors for the hydrides may indicate a slight loss of the chlorides prior to conversion or that conversion to the hydrides was incomplete.

Each of these calibrations were made using the 12 m × 0.32 mm I.D. HP-1 column at a split ratio of 100:1. The 1-μl injections were made at 250°C for all three calibrations. Standards ranging in concentration from $0.12 \cdot 10^{-9}$ g/ml to $120 \cdot 10^{-9}$ g/ml were prepared in chloromethane. Standards above $8 \cdot 10^{-8}$ g/ml were found to extend beyond the linear range for tetraethyltin itself and when hydride generation was performed. A greater split ratio could be used to analyze these higher concentrations if required.

The limit of detection was calculated at 0.5 pg of tin absolute and selectivity over carbon was calculated to be greater than 30 000. A splitless 5-μl injection of $1 \cdot 10^{-9}$ g/ml provides 5 pg to the detector, this being well above the detection limit for tin. These figures of merit illustrate that GC–AED provides the sensitivity and selectivity required to detect ng/ml levels of tin in complex matrices. Also, as shown subsequently, the hydride conversion technique was shown to be quantitative, giving uniform conversion of the alkyltin chlorides to their hydrides.

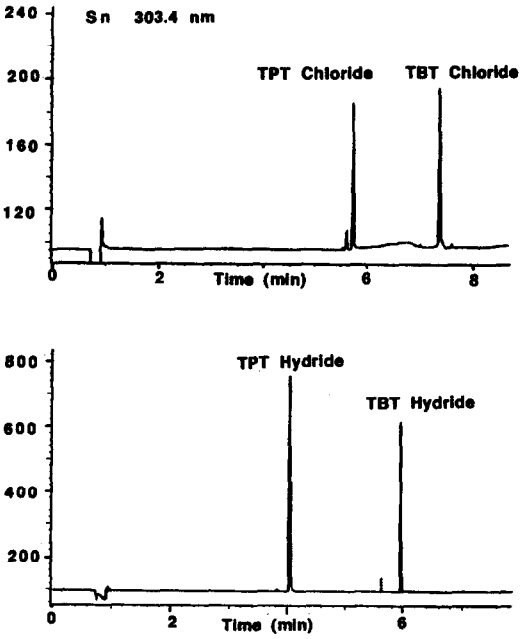

Fig. 1. Tin-specific chromatograms of (top) tripropyltin chloride (TPTCl) and tributyltin chloride (TBTCl) and (bottom) their corresponding hydrides (TPTH and TBTH) after hydride conversion with $NaBH_4$. Column 12 m × 0.32 mm I.D. HP-1, 250°C isothermal. Inlet split ratio 100:1.

The improvement in chromatography and recovery of injected material is illustrated in Fig. 1. In all GC–AED chromatograms, the *y* axis gives both indication of absolute response and also offset. A mixture of tripropyltin (TPT) and tributyltin (TBT) chlorides was injected with and without $NaBH_4$ in the injection port. The upper chromatogram shows the results when no $NaBH_4$ was used and the lower chromatogram shows the results with hydride conversion. The hydrides elute earlier than the corresponding chlorides and show less tailing. Increased response for the hydrides suggests better recovery of injected material.

Spiked water samples

Water samples were spiked at the 1 ng/ml and 0.1 ng/ml level with TPT and TBT chlorides. SPE of a 100-ml sample of 1 ng/ml solution and 500 ml of the 0.1 ng/ml solution were performed by pulling the samples through a Sep-Pak under a slight vacuum. Extracted components were eluted from the extraction cartridge with 25–30 ml of dichloromethane and concentrated by evaporation to 1 ml. The 1 ng/ml sample was chromatographed on the 25 m × 0.32 mm I.D. DB-1 column, and the 0.1 ng/ml sample was chromatographed on the 10 m × 0.53 mm I.D. (Megabore) HP-1 column. Splitless 1-μl injections were made with the 1 ng/ml standard, while 5-μl splitless injections were made with the 0.1 ng/ml sample. When splitless injections were made with the 0.32 mm I.D. column, the tripropyltin hydride showed poor peak shape due presumably to the injection volume exceeding the capacity of the column. Installation of a retention gap could remedy this problem, as could a modification in the initial column temperature.

A 0.53 mm I.D. column was then used in order to better evaluate the performance of the method for splitless injection. The peak shape of the tripropyltin hydride was improved, due to the greater column capacity and its ability to operate at higher carrier gas flow rates (Fig. 2). The higher carrier gas flow allows the large vapor cloud that forms in the injection port when splitless injections are made, to enter the column more quickly with less dispersion. This reduces peak tailing of early eluting components. The peak shape of the tributyltin hydride remained

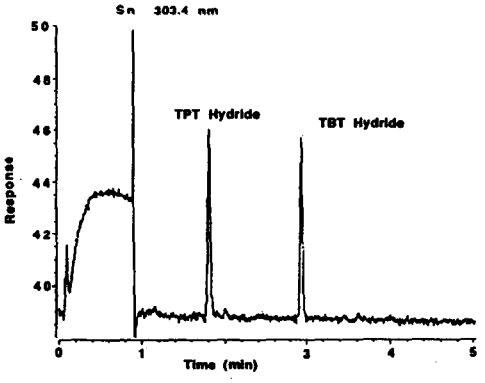

Fig. 2. Tin-specific chromatogram from 0.1 pg/ml aqueous trialkyltin chloride solution, extracted (Sep-Pak) and hydride converted to TPTH and TBTH. Column 10 m × 0.53 mm I.D. HP-1, 250°C isothermal, splitless injection.

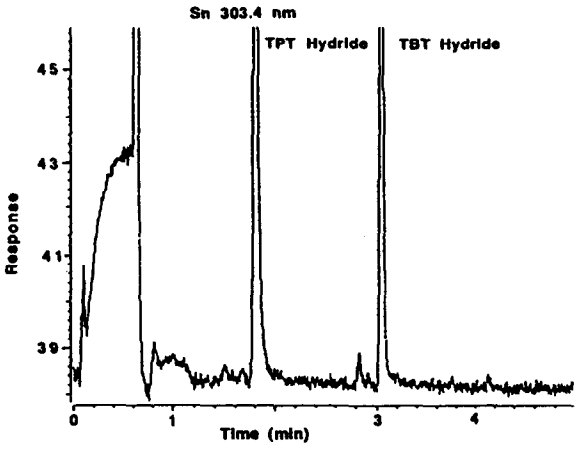

Fig. 3. Tin-specific chromatogram of converted trialkyltin chloride (as hydrides) standards. Column and conditions as in Fig. 2, 5-μl splitless injection, 200 pg tin levels on column.

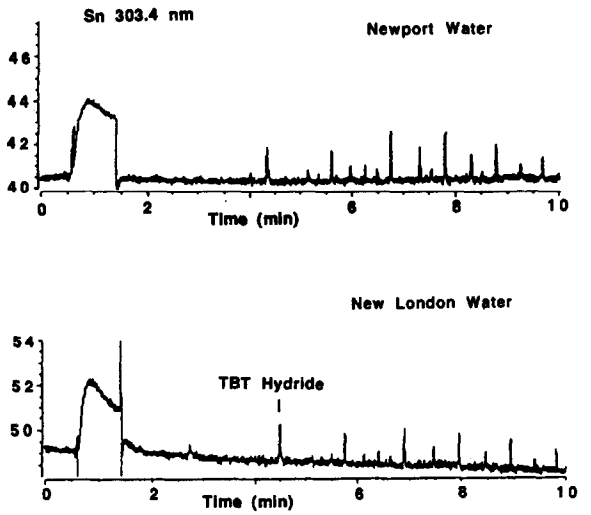

Fig. 4. Tin-specific chromatograms of Newport, RI, USA (top) and New London, CT, USA (bottom) water extracts (500-fold concentration). Column and conditions as in Fig. 2, 5-μl splitless injection.

sharp and symmetric. Fig. 3 illustrates the peak shapes obtained for a more concentrated sample, the response axis having been extended to emphasize the peak symmetry. The 0.53 mm I.D. column was clearly preferred, especially when splitless injections must be made.

Despite the different conditions under which the 1 ng/ml and 0.1 ng/ml samples were analyzed, the relative responses were very close to those predicted. After accounting for the different volumes of water extracted and the different injection volumes, the peak areas per unit mass of alkyltin for each of these samples should be similar. The peak areas for the tripropyltin hydride were 170 and 180 for the 0.1 ng/ml solution and the 1 ng/ml solution respectively, and those for the tributyltin hydride were 180 and 200 for the 0.1 ng/ml and 1 ng/ml solutions respectively. In each case recoveries were approximately 90%.

Sea water samples from New London, CT, USA and Newport, RI, USA were collected and solid phase extraction of 500-ml volumes was carried out as described earlier. Splitless 5-μl injections of the extracts were made, representative chromatograms being shown in Fig. 4, tributyltin hydride being expected at a retention time of approximately 4.5 min. Both samples show response at the expected retention time and based on the calibration for tributyl tin hydride, each was found to contain approximately 30 pg/ml tributyltin. In addition to the response seen at 4.5 min, several other peaks appear later in both chromatograms.

The responses seen for the analyte of interest were near the detection limit as were those for unexpected peaks. Therefore, confirmation of the identity of the analyte peak was necessary and the identification of the additional presumed tin containing peaks was also of interest. The source and removal of the additional peaks is discussed subsequently.

The combination of the photodiode array detector and software allows three-dimensional chromatograms to be plotted (retention time *versus* wavelength against emission intensity). These plots allow spectral regions to be viewed during a chromatographic peak and aid confirmation of the presence of an element in that peak. Tin shows characteristic atomic emission in the MIP at 301 nm and 303.4 nm. An indication of these lines can be seen in Fig. 5 which depicts the peak at 4.4 min in the New London water sample. However, the low level of the organotin species present, and the large amounts of inter-

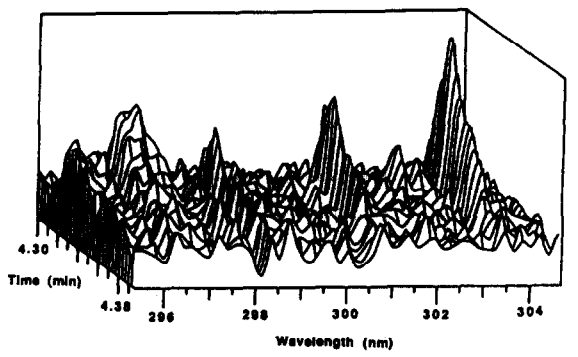

Fig. 5. Three-dimensional spectral–chromatographic snapshot of the peak at 4.4 min in the New London water extract chromatogram.

fering carbon species, make even this powerful feature of the GC-AED system inconclusive.

The added dimension of chemical selectivity provided by the on-line hydride conversion gave additional confirmation of the analyte peak. Fig. 6 compares the chromatograms obtained with and without hydride conversion. The sample injected with sodium borohydride shows the expected analyte peak at approximately 4.4 min in addition to the unidentified later eluting peaks. The sample injected without hydride conversion does not show response at 4.4 min but does show response for the other unidentified peaks. Also, in this sample at approximately 5.5 min there is a slight shift in the baseline, which is considered to correspond to tributyltin chloride which would elute in this region and show poor peak shape at this low level. The fact that this ill defined peak is not seen in the reacted sample chromatogram in which the hydride peak occurs, provides further evidence for the validity of the conversion reaction.

It is interesting to note that the interfering peaks did not change in retention time or peak shape in the presence or absence of the hydride conversion reaction. This suggests that if these were tin containing species, they are probably tetraalkyl species. Although each of these peaks showed some indication of the two tin emission lines expected (303 nm and 303.4 nm), its presence in these peaks could not be absolutely confirmed, as discussed above.

Tin-containing interferences

Initially it was thought that the additional peaks appearing in the tin chromatogram were due to non-selective response of carbon containing species. However, Fig. 7 shows that the major carbon response does not correspond with the pattern of peaks seen in the tin chromatogram. Most of the carbon response occurs later in the chromatogram and the unidentified peaks in the tin chromatogram were not due to carbon interference. Silicon, sulfur, phosphorus and

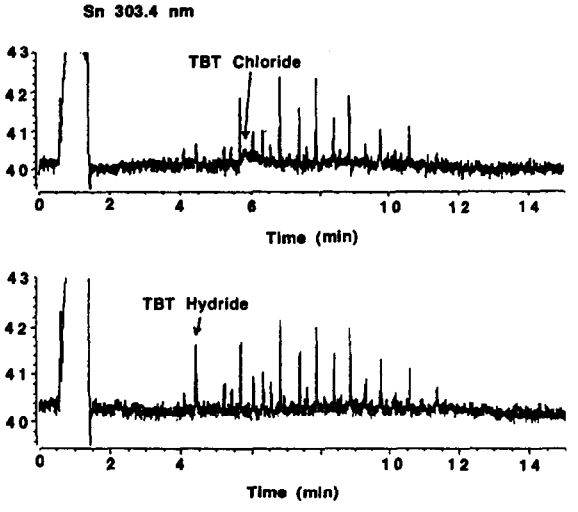

Fig. 6. Tin-specific chromatograms of the Newport, RI water extract with (bottom) and without (top) $NaBH_4$ conversion. Column and conditions as in Fig. 2, 5-μl splitless injection.

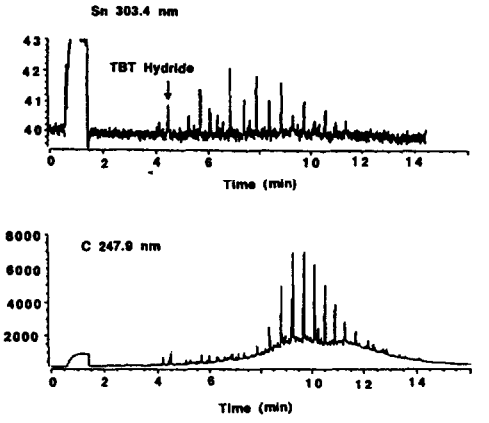

Fig. 7. Tin-specific (top) and carbon-specific (bottom) chromatograms of the Newport, RI water extract. Column and conditions as in Fig. 2, 5-μl splitless injection.

chlorine specific detection was performed, and none of these element specific chromatograms showed the same pattern of responses seen in the tin chromatogram. This indicates that these elements were not responsible for the unidentified peaks in the tin chromatograms.

Identification of the source and removal of interferences

Since the New London and Newport water extracts showed the same pattern of interfering species, it was hypothesized that they were being introduced during the sample preparation procedure. Each step of the procedure was thus examined.

Dichloromethane which had not been in contact with any of the sample preparation materials was injected and none of the interfering peaks were observed. Each piece of glassware and the syringes used in the sample preparation were washed with dichloromethane and these washes were injected, none giving response on the tin channel. Finally, a Sep-Pak that had not been used to extract any samples was washed with 15 ml of dichloromethane. This extract was condensed to 1 ml as for the sample extracts. Injection of this Sep-Pak wash showed the same response pattern for the interfering species seen in the previous water extracts but did not show response at the retention time expected for the TBT hydride (Fig. 8, top). This indicated that the interferents were being washed from the Sep-Pak. Extraction of a second aliquot of the New London water with the now "clean" Sep-Pak showed response for the analyte of interest, but none of the interfering species were observed (Fig. 8, bottom). Subsequent extractions were performed after washing each Sep-Pak with approximately 15 ml of dichloromethane.

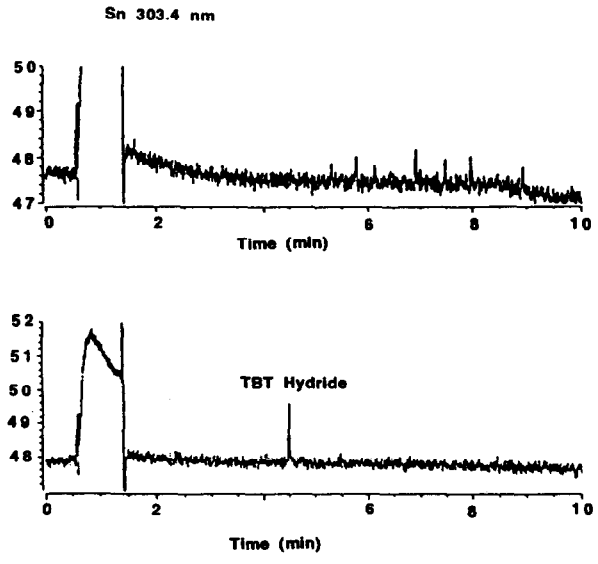

Fig. 8. Tin-specific chromatograms of Sep-Pak blank and the New London, CT water extracted with the "clean" Sep-Pak. Column and conditions as in Fig. 2, 5-μl splitless injection.

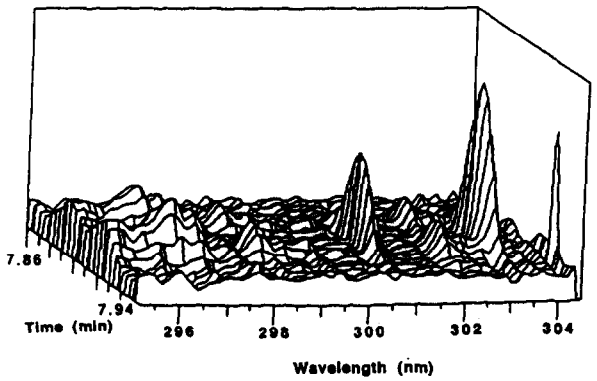

Fig. 9. Three-dimensional spectral-chromatographic snapshot of the peak at 7.84 min in the chromatogram of the five combined Sep-Pak washes.

In order to confirm that the interfering compounds were being washed from the Sep-Paks and that they did contain tin, five unused cartridges were washed with dichloromethane. These washes were then combined and condensed to 1 ml. Injection of the condensate showed the same pattern of response on the tin channel as was seen for the sea water extracts except for tributyltin. Three-dimensional plots for each of the major peaks showed characteristic emission of tin at 303 nm and 303.4 nm (Fig. 9). It was evident that the interferents were being washed from the Sep-Pak cartridges, and did contain tin. Pre-washing the Sep-Pak with 10–15 ml of dichloromethane before the extraction of samples removed these contaminants.

CONCLUSIONS

The use of toxic alkyltin compounds in boat paints has led to their appearance in aquatic

environments where extremely sensitive and selective methods of analysis are required for trace detection and analyte speciation.

The microwave-induced plasma atomic emission detector for GC provides a low detection limit for tin (0.5 pg of tin absolute or less than 1 ng/ml) and selectivity over carbon of greater than 30 000. SPE provides a fast simple method for the extraction and concentration (concentration factors of up to 1000 times are possible) of tin species. With a concentration factor of 1000 and the ability to detect ng/ml solutions directly, environmental levels of 10 pg/ml can be detected with the MIP after extraction. On-line hydride conversion allows the GC of alkyltin species without time-consuming sample preparation or sample loss in the chromatographic system. This combination results in a method capable of detecting pg/ml levels of alkyltin species in marine water samples with minimal sample preparation. This technique is robust, utilizes commercially available instrumentation and is capable of automation.

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