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Journal of Chromatography A, 800 (1998) 267–273

JOURNAL OF CHROMATOGRAPHY A

Trace analysis of tributyltin and triphenyltin compounds in sea water by gas chromatography–negative ion chemical ionization mass spectrometry

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Received 26 August 1997; received in revised form 12 November 1997; accepted 14 November 1997

Abstract

An analytical gas chromatography–mass spectrometry (GC–MS) method using negative ion chemical ionization (NICI) has been investigated for the determination of trace tributyltin (TBT) and triphenyltin (TPhT) compounds in sea water. TBT and TPhT were extracted from samples as chloride under the acidic condition of HCl. Doping of the GC system with a dilute HBr–methanolic solution resulted in direct detection of the chlorides of TBT, TPhT and tripentyltin (TPenT, internal standard). As the result of HBr doping, a sharp peak of the respective organotin bromides appeared: during GC analysis, halogen exchange from the chloride to the bromide occurred. NICI-MS was highly selective and sensitive for the detection of TBT, TPhT and TPenT bromides. In the selected ion monitoring mode of NICI-MS, the minimum detectable amounts defined as the signal equal to three times the standard deviation (3σ) of the baseline noise were 20 and 25 pg ml^{-1} for TBT and TPhT, respectively. These amounts are approximately 250–400 times better than those in electron impact mode. The combination of GC using an apolar capillary column doped with a dilute HBr–methanolic solution and NICI-MS made it possible to determine TBT and TPhT at less than the ng l^{-1} level in sea water. © 1998 Elsevier Science B.V.

Keywords: Water analysis; Organotin compounds

1. Introduction

Tributyltin (TBT) and triphenyltin (TPhT) have been extensively used in marine fish net and boat paints as antifoulants for over 20 years. Concern about the accumulation on fish and shellfish and the adverse effect on marine life of these organotins caused legislation severely limiting their use in Japan. As a result, TBT and TPhT levels in some highly affected areas in Japan have been reported to exhibit a slow but steady decline [1,2]. However, it is now recognized that low level concentration of, especially, TBT in sea water can exert lethal and sublethal effects on a wide variety of marine organisms particularly in the case of sensitive juvenile life forms [3,4]. Current concern about the adverse effects of TBT on some shellfish began with the identification of TBT leachate from antifouling paints as being responsible for the imposex syndrome, the development of a penis and vas deferens in the female in several species of marine gastropods around UK and other jurisdictions [5–

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PII S0021-9673(97)01153-9

11]. Imposex has occurred in as low as 1 ng l^{-1} concentration levels of TBT in water [12]. Several gas chromatography (GC) analytical techniques for the determination of TBT and TPhT have been reported. Many of them are derivatization methods: alkylation by Grignard reagents and hydrogenation by sodium borohydride [13–21]. These methods are generally rather complicated and require many handling steps: these take analytical time and may affect the determination results such as in respect of accuracy and precision. Also, derivatization methods usually utilize flame photometric detection (FPD) or electron impact (EI) mass spectrometry (MS) for detection of organotin compounds with which it is difficult to detect trace levels of TBT and TPhT in sea water. To monitor TBT and TPhT concentrations at less than ng l^{-1} level in the environment, highly sensitive analytical techniques are essential. We have developed and reported the direct GC analysis of chlorides of TBT and TPhT using an electron-capture detection and a packed column which was treated with HBr or HCl [22,23], and the direct capillary GC analysis using FPD and HBr doping [24].

In this paper, capillary GC–negative ion chemical ionization (NICI) MS has been investigated for the direct determination of trace TBT and TPhT in sea water. TBT and TPhT extracted from sea water as the chlorides could be gas chromatographed directly by the capillary doped with a dilute HBr–methanolic solution. During GC separation, halogen exchange occurred from the chloride to the bromide. NICI-MS was used as a highly selective and sensitive method for the detection of organotin halides. GC–NICI-MS showed superior sensitivity for TBT and TPhT chlorides which were extracted from sea water under the acidic condition of HCl. Both detection limits in the selected ion monitoring (SIM) NICI mode were 250–400 times more sensitive than those in the SIM-EI mode. The combination of GC–MS using an apolar capillary column doped with HBr and SIM in the NICI mode made it possible to determine TBT and TPhT at lower than the ng l^{-1} level in sea water. The proposed method presents a highly suitable analytical technique for the ultra trace determination of TBT and TPhT in aqueous samples.

2. Experimental

2.1. Chemicals and reagents

Standard toluene solutions (1 mg l^{-1}) of TBT chloride [$(n\text{-}C_4H_9)_3SnCl$], TPhT chloride [$(C_6H_5)_3SnCl$] and tripentyltin (TPenT) chloride [$(n\text{-}C_5H_{11})_3SnCl$, internal standard (I.S.)] were obtained from Kanto (Tokyo, Japan). Working standard solutions were prepared by mixing the standard toluene solutions and diluting with hexane in the range from 0.1 to 2 ng ml^{-1}. TBT bromide and TPhT bromide were obtained from Aldrich (Milwaukee, WI, USA). TPenT bromide was prepared by treating the TPenT chloride toluene solution with a 10% HBr aqueous solution. HBr (25%)–acetic acid solution for doping was purchased from Wako (Osaka, Japan). HBr–methanolic solutions at the concentration of 1 m*M* and 0.5 m*M* were prepared by diluting the 25% HBr–acetic acid solution (3.3 *M*) with methanol. All solvents used were analytical grade. The standard solutions, the working standard solutions and HBr–methanolic solutions were stored at 4°C.

2.2. Apparatus

GC–MS was performed on a VG TRIO-1 quadrupole mass spectrometer (Thermo Quest, USA) interfaced by a direct transfer line to a Hewlett–Packard 5890A II gas chromatograph. The GC column used here was a fused-silica capillary column with a thin, immobilized stationary phase of DB-1 (15 m×0.25 mm I.D., 0.1 μm film thickness, J&W, Folsom, CA, USA). Injections were made in the splitless injection mode (1 min split time) with an inlet pressure of 50 kPa using helium as the carrier gas. A temperature program was employed in which the column temperature was initially held at 40°C for 1 min, then increased at 15°C min^{-1} to a final temperature of 240°C, and held for 1 min. The injector temperature was held constant at 250°C. Organotin species were ionized by using the EI or NICI source of the mass spectrometer. Conditions for the mass spectrometer were as follows: GC–MS interface temperature of 200°C, electron energy of 70 eV and ionizer temperature of 200°C for both EI and

NICI modes. In the NICI mode, isobutane or methane was used as reagent gas. The reagent gas pressure was adjusted to yield the maximum production of the reference gas (heptacosatrifluorotributylamine) ion at *m/z* 159, *m/z* 283, *m/z* 452 and *m/z* 633 for both methane and isobutane. Selected ions monitored in NICI-MS for TBT, TPhT and TPenT are listed in Table 1.

2.3. Pretreatment and doping of column

The column was pretreated with a HBr–methanolic solution to prevent the adsorption of TBT and TPhT chlorides within the column. After setting the capillary column to the gas chromatograph and holding the temperature at 40°C, pretreatment of the capillary column was performed by injecting three times 1 μl portions of 1 m*M* HBr–methanolic solution at 1-min intervals and programming the column temperature according to the GC conditions described above. The column end was connected to the mass spectrometer after the pretreatment with 1 m*M* HBr. One μl of 0.5 m*M* HBr–methanolic solution was doped at 40°C about 1 min prior to injection of a sample or standard solution in order to keep peaks sharp and sensitivity high.

2.4. Sample preparation

Sample preparation for a sea water sample was carried out according to the flow chart of Fig. 1. TBT and TPhT were extracted from 200 ml of sea water three times as the corresponding chlorides each with 30 ml of hexane–diethyl ether (3:1, v/v) in the presence of HCl, methanol and ethyl acetate. The combined extract was concentrated to about 10 ml and then subjected to column chromatography using 3 g Florisil (lower) and 2 g anhydrous sodium sulfate (upper). Preparation of the column was as follows:

Table 1
Selected ions for NICI

Compound	Selected ion
TBT chloride,	*m/z* 311, 313, 315
TPhT chloride	*m/z* 349, 351, 352
TPenT chloride (I.S.)	*m/z* 339, 341, 343

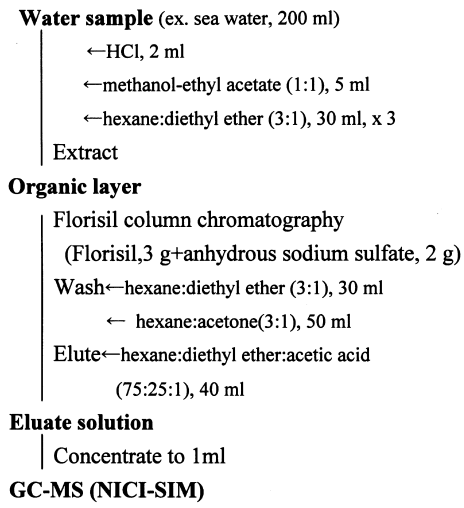

Fig. 1. Sample preparation procedure of TBT and TPhT compounds in a water sample.

Florisil or anhydrous sodium sulfate was suspended in hexane–diethyl ether (3:1, v/v) and packed into a glass column (30×1.0 cm I.D.). When the concentrate was poured into the column and the top of the solvent surface came to the anhydrous sodium sulfate layer, 30 ml hexane–diethyl ether (3:1, v/v), then 50 ml hexane–acetone (3:1, v/v) and 40 ml hexane–diethyl ether–acetic acid (75:25:1, v/v/v) were added. The final acid portion was collected and concentrated to 1 ml. TPenT chloride was added as an I.S. to the final sample solution at the 1 ng ml^{-1} level to control the precision of the manual injection. The solution obtained was subjected to GC–SIM-NICI-MS.

3. Results and discussion

3.1. Pretreatment of capillary column with HBr–methanolic solution

An apolar fused-silica capillary column with a thin, immobilized stationary phase of DB-1 (15 m× 0.25 mm I.D., 0.1 μm film thickness) was examined. When TBT and TPhT chlorides were injected into the gas chromatograph equipped with the capillary column not pretreated with HBr, the peaks of TBT

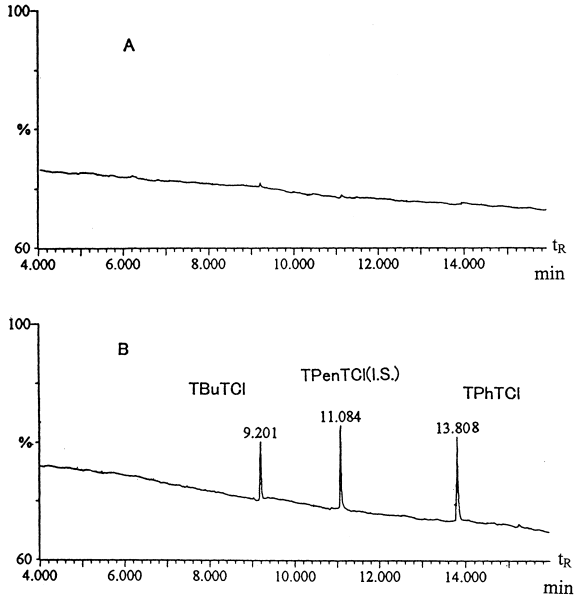

Fig. 2. MS total ion chromatograms of TBT chloride, TPhT chloride and TPenT chloride (I.S.) in NICI mode. (A) No doping, (B) doping with HBr.

and TPhT chlorides hardly appeared. Fig. 2A shows the GC–NICI-MS chromatogram when 0.1 ng each of TBT and TPhT chlorides including TPenT chloride (I.S.) was injected onto the not pretreated capillary column. No peak was observed.

Fig. 2B shows the GC–NICI-MS chromatogram of TBT, TPhT and TPenT chlorides injected into the gas chromatograph equipped with the capillary column pretreated by injecting three times 1 μl of 1 m*M* HBr solution. The pretreated column gave sufficiently sharp peaks of TBT, TPhT and TPenT chlorides. Furthermore, 1 μl doping of 0.5 m*M* HBr solution prior to the injection of sample solution was successful in keeping the peaks sharp and sensitivity high. Doping of a 0.5 m*M* HBr solution had a persistent effect of sharpening peaks of TBT, TPhT and TpenT chlorides [24].

3.2. NICI-MS analysis: high-selectivity and sensitivity experiments

As a highly selective and sensitive analytical method for the detection of electronegative compounds such as TBT and TPhT chlorides, NICI-MS was very effective. Methane or isobutane was used

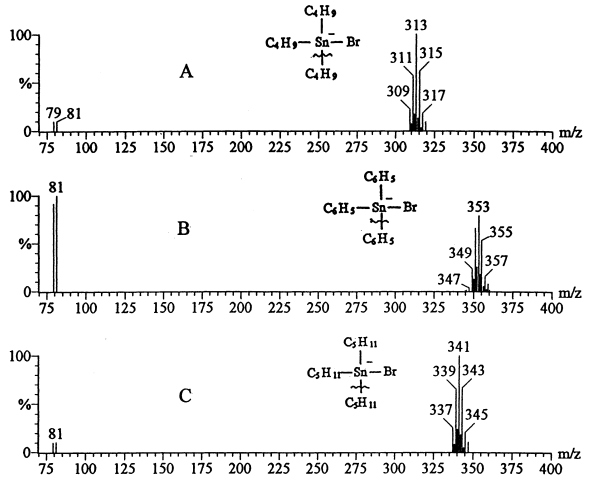

Fig. 3. GC–NICI mass spectra of TBT chloride (A), TPhT chloride (B) and TPenT chloride (C) separated by DB-1 capillary column under HBr doping.

as a reagent gas. Compared to the spectra of TBT, TPhT and TPenT in EI-MS, those in NICI-MS were relatively simple as shown in Fig. 3 (NICI) and Fig. 4 (EI). All spectra of TBT, TPhT and TPenT injected into the gas chromatograph as chlorides showed few molecular ions but fragment ions of $[M-R]^-$, where R represents the $-C_4H_9$, $-C_6H_5$ or $-C_5H_{11}$ group. Moreover, it was found that these fragments were assigned to the respective bromides: the cluster peaks from *m*/*z* 309 to 319 due to

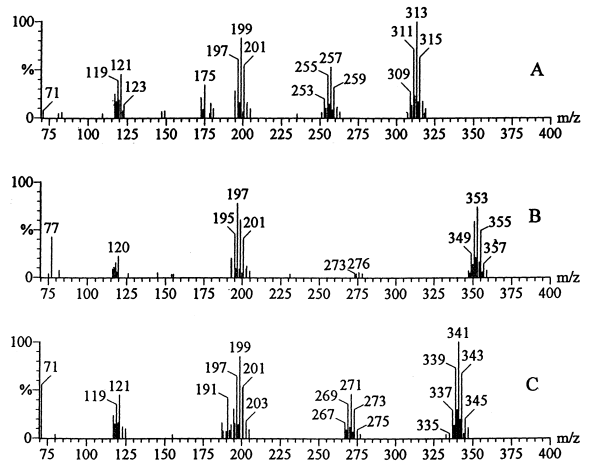

Fig. 4. GC–EI mass spectra of TBT chloride (A), TPhT chloride (B) and TPenT ckloride (C) separated by DB-1 capillary column under HBr doping.

$[M(TBT\ bromide)-C_4H_9]^-$, m/z from 349 to 359 to $[M(TPhT\ bromide)-C_6H_5]^-$ and m/z from 337 to 347 to $[M(TPenT\ bromide)-C_5H_{11}]^-$. Bromides showed the same MS spectra. Apparently, halogen exchange from chloride to bromide occurred during GC analysis [24].

3.3. SIM determination

Three ions were selected for each organotin compound for SIM-NICI, as follows: m/z 313, 311 and 315 for TBT, m/z 353, 351 and 355 for TPhT, m/z 341, 339, and 343 for TPenT. Fig. 5 shows the GC–SIM-NICI-MS chromatogram each of 1 pg for three organotin chlorides. This result showed superior sensitivity compared with that obtained by the SIM-EI mode. Table 2 shows detection limits of SIM in EI and NICI mode. Detection limits in the SIM-NICI mode were about 250–400 times more sensitive than those in the SIM-EI mode.

3.4. Calibration curves and detection limits

Calibration curves for TBT and TPhT chlorides were prepared by plotting the relative peak area to TPenT chloride as I.S. vs. the concentration. The SIM-NICI-MS showed excellent sensitivity and linearity of response from 0.1 to 2.0 ng ml^{-1} for both compounds.

The detection limits were defined as the signal equal to three times the standard deviation (3σ) of the baseline noise. For 1 μl of standard solution, the detection limits were 20 pg ml^{-1} for TBT and 25 pg ml^{-1} for TPhT, respectively (Table 2). If the analysis started from 200 ml of sea water and the final volume of sample solution was 1 ml, the levels of limit detection for sea water were 0.10 and 0.13 ng l^{-1}, respectively. It is emphasized that the SIM-NICI-MS achieves low detection limits for the determination of TBT and TPhT in sea water.

3.5. Reproducibility and recovery test

The repeatability for TBT and TPhT was determined by five consecutive manual injections of 1 μl of standard solution containing 1 pg of TBT chloride and TPhT chloride. The peak area measurement gave relative standard deviations (R.S.D.s) of 0.43% and 0.50%, respectively.

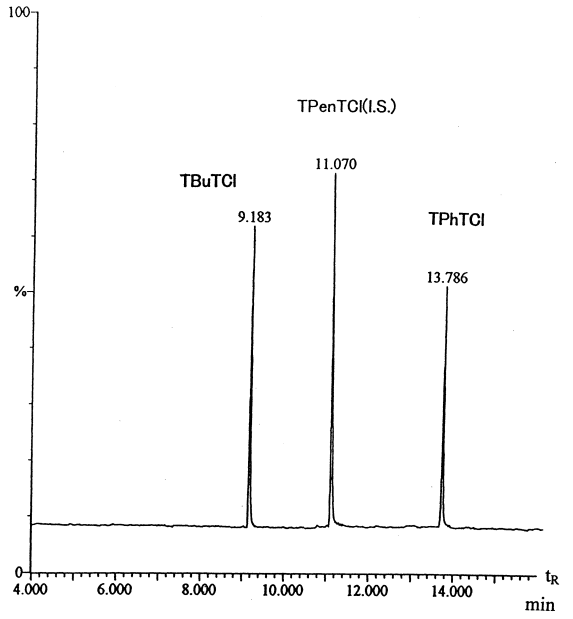

Fig. 5. GC–NICI-MS-SIM chromatogram of TBT chloride, TPhT chloride and TPenT chloride (I.S.). Experimental conditions; DB-1 capillary column, 0.5 m*M* HBr doping, TPenT (I.S.), 1 ng ml^{-1}, and injection volume, 1 μl.

A less polluted sea water sample (levels<0.1 ng l^{-1}) was used for the spiked experiment. TBT and TPhT standard solutions were added to sea water to produce final concentrations of 100 and 10 ng l^{-1}, and a recovery test was performed. The results are shown in Table 3. The mean recoveries and R.S.D.s for each compound ($n=5$) ranged from 90.3 to 98.5% and from 4.5 to 6.8%, respectively. Quantitative recoveries were obtained with the method developed.

3.6. Analysis of environmental samples

Analyses of real sea samples were carried out to

Table 2
Comparison of detection limits in SIM-NICI mode and SIM-EI mode

Organotin	SIM-NICI	SIM-EI
TBT chloride	20 pg ml^{-1}	5 ng ml^{-1}
TPhT chloride	25 pg ml^{-1}	10 ng ml^{-1}

Table 3
Recoveries of TBT and TPhT from spiked sea water

	100 ng l^{-1} Spiking level		10 ng l^{-1} Spiking level	
	Recovery (%)	% R.S.D. (n=5)	Recovery (%)	% R.S.D. (n=5)
TBT	98.5	4.5	94.9	5.3
TPhT	93.2	5.7	90.3	6.8

Table 4
Determination of TBT and TPhT in sea water[a]

Sample	TBT (ng l^{-1})	TPhT (ng l^{-1})
1	16.70±0.72	2.94±0.17
2	8.83±0.55	1.11±0.08

Results given are average of triplicate analyses.
[a] Collected from Tokyo Bay in 1997.

demonstrate the applicability of the proposed method. Each analysis was performed in triplicate. Table 4 gives the results for three water samples collected from Tokyo Bay in 1997. The analytical result by SIM-NICI-MS of a sea water sample (sample 1 in Table 4) is shown in Fig. 6. Detection limits for TBT and TPhT were significantly improved by the SIM-NICI-MS, relatively small amounts of sea sample (ex. 200 ml) could be used, subsequently the disadvantage by matrix interference of sample could be avoided.

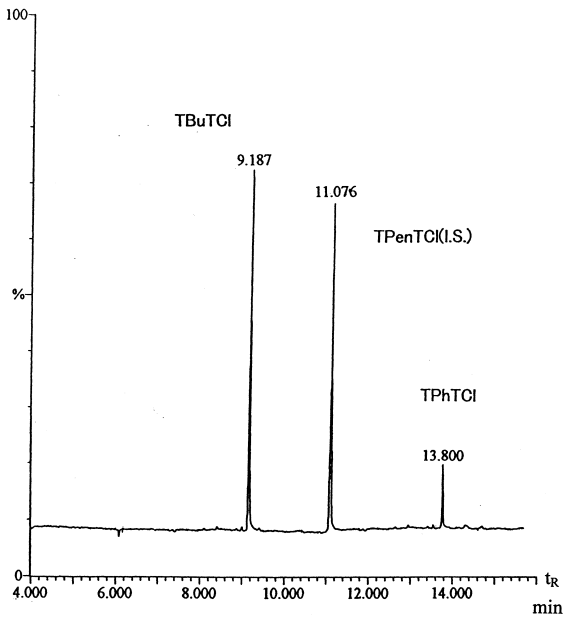

Fig. 6. GC–NICI-MS-SIM chromatogram of sea water sample. Experimental conditions as in Fig. 5.

4. Conclusion

The combination of GC using a pretreated column with HBr–methanolic solution to MS detection on SIM-NICI using isobutane or methane as a reagent gas gave enough sensitivity to determine trace levels of TBT and TPhT compounds in sea water for the investigation of the imposex phenomena. The pretreatment of column with HBr produced good results in that TBT and TPhT chlorides eluted smoothly without adsorption. Consequently, TBT and TPhT chlorides could be determined directly without derivatization. From the NICI-MS spectra, it was found that TBT and TPhT chlorides converted to and eluted as the respective bromides by the reaction with HBr remaining in the column. NICI-MS provides wide superior linear response, dynamic range, and detection limit for the analysis of TBT chloride and TPhT chloride. In SIM-NICI mode, minimum detectable amounts defined as the signal equal to three times the standard deviation (3σ) of the baseline noise were found to be about 20 pg ml^{-1} for TBT and 25 pg ml^{-1} for TPhT, respectively. These amounts were approximately 250–400 times better than those in the SIM-EI mode.

The results showed that the proposed method of pretreatment of a column with HBr–methanolic solution and SIM-NICI-MS measurement presents a highly suitable analytical technique for the trace determination at less than the ng l^{-1} level of TBT and TPhT in sea water. Hence the technique was significantly selective and sensitive, amounts of sample were comparatively small, subsequently the interference of sample matrix and the amounts of reagents used for extraction analysis could be minimized, and might be also applicable for trace level concentration of TBT and TPhT in small biological samples.

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