DETERMINATION OF BUTYLTIN COMPOUNDS IN SEDIMENTS BY GC/MS AFTER THEIR CONVERSION TO METHYL DERIVATIVES

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A selective and rapid procedure was developed for the determination of trace amounts of butyltin compounds in sediments by capillary gas chromatography with mass spectrometric detection. Analytes were isolated from the sediment samples by extraction with a methanolic solution of HCl (0.5 mol l^{-1}) followed by reextraction into a hexane solution of tropolone (0.1%). The organotin compounds were converted to the nonpolar methyl derivatives by methylation with a Grignard reagent. The recovery was determined for each step of the procedure. This efficient separation method combined with the ion trap based mass detection ensures a high selectivity even for concentrations approaching the detection limit (0.15 pg for Bu_3SnMe) in real sediments. Chemical ionization using acetonitrile as the reaction gas, exhibiting a similar sensitivity of determination and reducing the background level, can serve as a convenient alternative of detection for sediments heavily contaminated by organics. The method was applied to a set of real samples and validated by analysis of a certified reference material.

Key words: Tin; Sediments; GC/MS.

Environmental determination of various species (speciation) of metals, which differ dramatically in their toxicity, has been attracting interest. Among such metal species are butyltin compounds, which are biocides and are used as components of coatings against mould and ship coatings and as pesticides. Till 1995, organotin compounds were also produced in the Czech Republic in quantities about 500 tons annually, contributing thus roughly one per cent to the world production. The determination of trace amounts of such compounds has not been receiving due attention in the Czech Republic despite the fact that, *e.g.*, tributyltin is capable of penetrating through cell membranes and accumulating in living organisms and sediments. A tentative analysis of a few

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Czech samples was only performed within the German study[1] of heavy metals and their compounds in the Labe (Elbe) river.

A survey of analytical methods employed in the determination of organotin compounds can be found in a review by Szpunar-Lobinska and coworkers[2]. Such methods usually involve analyte extraction from sample, treatment and conversion of the organotin compounds to suitable derivatives, chromatographic separation, identification, and determination. Gas chromatography is the most popular method, although the use of high performance liquid chromatography, atomic absorption spectrometry, voltammetric techniques[3], and capillary electrophoresis[4] has also been reported.

In our previous work[5] we concentrated on the optimization of conditions for the determination of trace amounts of organotin compounds in waters by gas chromatography/mass spectrometry; we found that the application of chemical ionization during the use of the mass detector working on the ion trap principle is convenient for the detection of organotin compounds and that extremely low detection limits can be routinely achieved while maintaining the identification potential of this type mass detector.

Existing analytical procedures for the quantitation of traces of butyltin compounds in sediments include time consuming analyte isolation from sample and suffer from improperly low detection selectivity and sensitivity. The present paper describes optimization of the sediment treatment process for subsequent quantitation of the butyltin compounds by gas chromatography/ion trap mass detection. Assets of chemical ionization in the mass detection are identified. Particular attention is paid to the validation of results, including analysis of a certified reference material.

EXPERIMENTAL

Apparatus

A Magnum bench top GC/MS system manufactured by Finnigan MAT (U.S.A.) was used; this system works on the ion trap principle and allows operation in the electron ionization (EI) as well as chemical ionization (CI) mode. The system included a Varian 3400 gas chromatograph and a Varian SPI injector. The instrument was computer controlled and the results were processed by means of the MAGNUM 2.4 software. Acquisition proceeded in the Automatic Gain Control mode by setting standard parameters obtained by automatic tuning. Chemical ionization was carried out at a filament current of 10 μA, the preset target value was 60% of the determined value for the electron impact ionization mode. The CI mode parameters for the reaction gases employed have been described previously[5].

A DB 5 ms chromatographic capillary column with a chemically bonded methylphenylsiloxane type phase (5% phenyl groups) was employed. The column was 30 m long, 0.25 mm i.d., film thickness 0.25 μm. Helium served as the carrier gas at an input pressure of 76 kPa, average linear velocity of 33.1 cm s^{-1}, flow-rate 0.97 ml min^{-1}, hold-up time 90.6 s (all at 60 °C). The temperature programme was as follows: 60 °C for 1 min, temperature gradient 60–100 °C at a rate of 5 °C/min, 100–250 °C at 8 °C/min, with a final hold at 250 °C for 5 min. The injectior was heated from the initial 125 to 250 °C at 200 °C/min, kept at 250 °C for 2 min, and allowed to cool to the initial temperature.

The injection volume was 1 μl. The acquisition parameters and injection procedure have been described in detail in ref.[5].

Chemicals

Tributyltin chloride (95% chromatographic purity) was obtained as a gift from the University of Pardubice (Czech Republic). Tributylmethyltin was prepared from tributyltin chloride by methylation[5]. Tropolone (2-hydroxy-2,4,6-cycloheptatrienone, 98%) was purchased from Aldrich. The Grignard reagent – methylmagnesium iodide – was prepared as a solution in diethyl ether (2 mol l^{-1}) by the standard procedure under dry nitrogen and kept at 4 °C for a period not exceeding 2 weeks. The following chemicals were also used: methanol p.a., hydrochloric acid p.a. (both Lachema, Brno, Czech Republic), cyclododecane puriss. (Koch Light Laboratories, U.K.), hexane (Litvinov Chemical Works, Czech Republic, redistilled from an all-glass still), and CRM 462 certified reference material of the EC Reference Bureau. The other chemicals used were of the highest purity commercially available.

Treatment of Sediments

Sediment samples were kept in polyethylene bottles in a refrigerator at a temperature about 4 °C. Prior to use, the sediments were freed from tiny stones, pieces of leaves, roots, and grass by screening through a sieve about 1 mm mesh size. Before analysis, the polyethylene bottle contents were agitated for homogenization. A fraction of the sediment (about 1.5 g) was weighed into a glass vessel approximately 60 ml volume, 25 ml of HCl (0.5 mol l^{-1}) in methanol were added, and the whole was treated in an ultrasonic bath for 2 h. The solid phase was separated in a Hermle Z 510 centrifuge, operated at 2 000 rpm for 20 min. A volume of 20 ml of the liquid phase was taken for further processing.

Spiked samples were prepared by adding methanolic solution of Bu_3SnCl to the weighed-in sediment in the glass bottle to be inserted into the ultrasonic bath.

The dry matter fraction of the sediment samples was determined separately by drying a weighed portion of the sediment at 105 °C to constant weight (approximately 3 h) to eliminate the hazard of loss of the organotin compounds during analysis.

Extraction of Butyltin Compounds

Four extraction procedures for transferring the butyltin compounds into ether were examined:

Extraction procedure I. The 20 ml volume of the liquid phase was extracted with 2 × 5 ml of hexane solution of tropolone (0.05%). Each extraction took 3 min and the phases were allowed to separate during 7 min. To the combined extracts Na_2SO_4 was added, and after drying, 1 ml of the hexane solution was transferred into a test tube a subjected to derivatization with the Grignard reagent.

Extraction procedure II. The 20 ml volume of the liquid phase was diluted with distilled water to 200 ml and extracted with 2 × 10 ml of hexane solution of tropolone (0.1%). The extraction procedure was as above, and 2 ml of the hexane solution was taken for derivatization.

Extraction procedure III. The methanolic solution of HCl was concentrated to roughly 5 ml using a vacuum rotary evaporator. To this concentrated solution 200 ml of distilled water was added, and the extraction was carried out as above. Two ml of the hexane solution were taken for derivatization.

Extraction procedure IV. The hexane solution for derivatization obtained by extraction procedure *III* was concentrated to a volume about 2 ml in a rotary vacuum evaporator and subjected to derivatiza-

tion. The concentration step required precise and careful experimental work, and the procedure was not used on a routine scale within this study.

Analysis of Certified Reference Material

Before opening the bottle with the CRM, the contents were homogenized by shaking as recommended by the supplier. Two parallel analyses of approximately 1 g of the material were carried out. Extraction procedure *IV* was applied.

Derivatization

To the extract emerging from the extraction procedure applied was added 1 ml of the derivatization reagent, and derivatization was performed as described elsewhere[5]. Subsequently, 1 ml of the internal standard solution (cyclododecane in hexane, 1 μg ml^{-1}) was added to 1 ml of the hexane solution, and the chromatographic analysis was run.

Preparation of Bu_3SnMe Calibration Solutions

Calibration solutions at concentrations of 0.001–10 μg ml^{-1} were prepared by consecutive dilution of the stock solutions (10 ng ml^{-1}, 100 ng ml^{-1}, 1 μg ml^{-1}). Each calibration solution contained the internal standard at 0.5 μg ml^{-1}.

Quantitative Evaluation

The quantitative evaluation was based on the chromatographic peak areas. The MAGNUM 2.4 software was employed to set up a procedure for routine processing of the chromatograms, where the analyte peak was identified within the preset retention time window, the average mass spectrum of the peak with background subtraction was created, this spectrum was compared with the reference spectrum, and if the spectra matched each other and the signal-to-noise ratio was adequate, the peak area integration process was started. In the internal standard method, the peak area parameters were related to those of the internal standard and the analyte content was calculated by using a calibration set. The ADSTAT statistical software (Trilobyte, Pardubice, Czech Republic) was applied to the comparative calculation of the calibration curve parameters and linearity testing (*F*-test). The detection limit was calculated as the minimum detectable amount of analyte, *i.e.* amount producing a chromatographic signal equal to the triple noise, using a chromatographic data where the signal-to-noise ratio (*S/N*) was lower than 10. Similarly, the limit of determination was calculated for $S/N = 10$ from an experiment where the signal-to-noise ratio was not higher than 20.

RESULTS AND DISCUSSION

The retention data of the substances examined are given in Table I. The chromatographic peaks were symmetrical and exhibited baseline separation.

The compounds give characteristic and well-reproducible mass spectra. Tin has 10 stable isotopes, and therefore the occurrence of clusters is a typical feature of the spectra of tin compounds[5,6]. In the electron ionization mode, the substances studied did not exhibit the molecular ion, only fragment ions due to the elimination of the alkyl groups were observed in accordance with published data[5,6]. To identify organotin compounds isolated from matrices as complex as sediments, it is possible to match the spectra

against those included in the NIST Library (National Institute of Standards and Technology Mass Spectrometry Library, 1990 edition). The match was very good at concentrations corresponding to the injected amount not lower than 5 pg Bu_3SnMe.

Determination of Butyltin Compounds

Preliminary analyses gave evidence that sediments near the manufacturing plant contained only tributyltin (TBT) compounds in detectable quantities. Therefore, the quantitative determination method was aimed at this type of substance. The chromatograms were evaluated making use of our earlier findings[5]. The optimum signal-to-noise values and linear range of the method were obtained for selectively chosen ions with m/z = 245 + 247 + 249 for Bu_3SnMe and 83 + 111 for cyclododecane as the internal standard. The calibration straight lines met the linearity requirement. With the acquisition parameters so chosen, the linear range of the detector spanned roughly over 4 orders of magnitude. Figure 1 shows the chromatogram obtained on injection of sample containing 0.4 pg Bu_3SnMe, where the S/N ratio was 7.8, giving a detection limit of 0.15 pg Bu_3SnMe in the volume injected for S/N = 3. The limit of determination, calculated for S/N = 10, was 0.5 pg Bu_3SnMe, or 0.2 pg Sn, in the injection.

The recovery from the derivatization reaction was examined within the range of 0.01 to 13 μg ml^{-1} (5 measurements), and the value of 98.2 ± 6.9% (confidence interval) was obtained, in a very good agreement with published data[5,7].

Recovery of the Butyltin Compounds in the Extraction from Sediments

The extraction procedures investigated differed in the methanol concentration during extraction, and the recovery values were different as well. In the extraction procedure *I*, the recovery was 29 to 42% for the spiked solutions. This increased to 74–79% in extraction procedure *II* owing to the lower concentration of methanol. The low recovery from procedure *I* can be explained in terms of the partial miscibility of methanol and hexane. The recoveries were highest for extraction procedures *III* and *IV*, *viz*. 82 to 95%. These

TABLE I
Retention data of the compounds studied

Compound	Relation time t_{Ri}, s	Relative retention[a] r_G
Bu_2SnMe_2	525	1.47
Bu_3SnMe	697	1.96
Bu_4Sn	819	2.30

[a] The analyte-to-decane retention time ratio t_{Ri}/t_{Rs} with t_{Rs} = 356 s.

procedures resemble the procedure applied by Quevauviller and coworkers[8] in the determination of organotin compounds in biological materials for the preparation of certified reference material, where the recovery of TBT was 91 ± 9%. The recovery from the extraction procedures *III* and *IV* is consistent with published data[9] and is considered sufficiently high. The repeatability of the extraction recovery for the addition of 100 μg of Bu_3SnMe to the sediment prior to processing was 83.0 ± 1.6%. Extraction procedure *IV*, involving an additional preconcentration step, is designed for the quantitation of extremely low TBT contents. Too time consuming and placing high demands on experimental work, this procedure was not applied routinely, but it demonstrates the feasibility of extending further the concentration range of the method. The optimum procedure chosen can be summarized as follows:

1. Add 25 ml of a 0.5 M solution of HCl in methanol to approximately 1.5 g of sediment, and expose the suspension to sonication for 2 h.

2. Separate the liquid phase by centrifugation (2 000 rpm, 20 min), and concentrate 20 ml of this phase roughly to 5 ml.

3. Dilute to 200 ml with water and extract with 2 × 10 ml of a 0.1% solution of tropolone in hexane, combine the extracts and dry them with $MgSO_4$.

4. Take 2 ml of the hexane extract to derivatization by addition of 1 ml of a solution of MeMgI (2 mol l^{-1}).

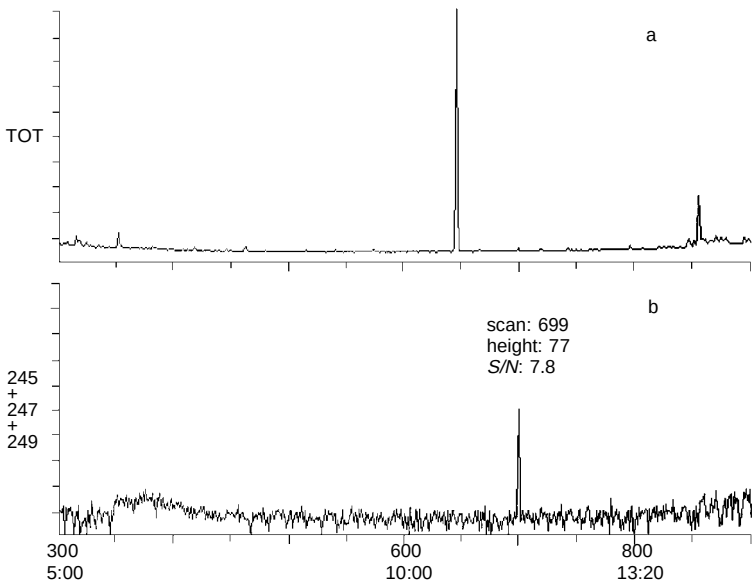

FIG. 1

a Chromatogram obtained by recording the total ion current; b reconstructed chromatogram for ions *m/z* = 245, 247 and 249 in dependence on the number of scans (s^{-1}) and time (min:s)

5. Add 1 ml of internal standard solution to 1 ml of the solution so obtained, and inject 1 μl of this mixture into the gas chromatograph.

Method Validation by Using Certified Reference Material

Certified reference material CRM 462 was employed to test whether the method is free from bias. This material is a sample of a river sediment with a very low butyltin compound content (TBT^+ = 70 ± 14 ng per g of dry sediment, DBT^{2+} = 128 ± 16 ng per g of dry sediment) and matches well the matrix of the samples studied. Another commercial certified reference material for organotin compounds in sediments, PACS-1 (National Research Center Council of Canada), is not suitable for validation of the present method because of its too high organotin content (1 270 ± 220 ng Sn per g) and a different nature of matrix. In the CRM 462 sample, 76 ng TBT^+ per g of dry matter was determined. This value lies within the confidence interval for the CRM, which is 70 ± 14 ng TBT^+ per g of dry sediment. This gives evidence that the method developed is free from bias.

Uncertainty Estimation

The uncertainty of measurement characterizes an estimate of an interval within which the true value lies[10,11]. For the method under study, the uncertainty was estimated by using results of analysis of the certified reference material. In this case, the total expanded uncertainty can be calculated according to Marchandise[12] by the equation

$$U = \sqrt{U_R^2 + U_C^2 + \Delta^2}\,, \qquad (1)$$

where U is the total expanded uncertainty, U_R is the uncertainty arising from random effects during analysis, U_C is the uncertainty of the CRM value (14 ng TBT/g), and Δ is the difference between the experimental result (X_L = 76 ng TBT/g) and the certified value (C = 70 ng TBT/g; Δ = 70 – 76 = –6 ng TBT/g).

For values up to tenfold the limit of determination, the U_R value was estimated to 35 ng TBT per g of dry sediment, based on the repeatability of the accomplished determinations of real sediment samples. Inserting this value, along with the above U_C and Δ values, in Eq. (*1*), the total expanded uncertainty is 34.2 ng TBT/g dry sediment; this is roughly 50% of the determined TBT concentration in the CRM. The uncertainty is most contributed to by the U_R value. This contribution is lower for higher TBT concentrations. Therefore, for concentrations higher than tenfold the limit of determination the uncertainty estimate will be one-half lower. The uncertainty contribution of steps that are included in the real sample analysis procedure but not in the CRM analysis proce-

dure is insignificant as compared to the total uncertainty of determination and can be neglected.

Application to Real Samples

Five sediment samples were analyzed. The samples were taken from 2 sites: from a site near the town of Bohumín, where chemical agents containing butyltin compounds used to be manufactured, and from the Vltava (Moldau) river for a comparison. Since the sediments differed in their water contents, conversion to TBT per gram of dry matter was performed. Depending on the dry matter content of the sediment, the lower concentration limit of the method is 20 ng Sn/g dry matter for a 25% dry matter content of the sediment. This value can be tenfold reduced by applying extraction procedure *IV*. Data of the samples, dry matter contents, and tributyltin compound concentrations in them are given in Table II.

For the Bohumín sediments, samples taken in 1994 and 1996 exhibited different TBT concentrations, apparently due to the fact that the manufacture of agents containing organotin compounds was discontinued there since September 1995. The concentration decrease is roughly 60%. Although a higher number of compounds pass into the hexane phase during extraction and consequently, the chromatogram displays more peaks, a sufficient peak resolution can be achieved by using a suitable data processing procedure based on selectively chosen ions (Fig. 2).

The Use of Chemical Ionization in Real Sample Analysis

The sediment samples were analyzed by using chemical ionization with the aim to ascertain whether the sensitivity with respect to organotin compounds is higher and/or the chromatograms from real sediments are simpler as compared to the electron ionization mode. This experimentally undemanding option is offered by the ion trap type

TABLE II
Data of samples analyzed

Sampling site and date	Dry matter content, %	Sn found ng/g dry sediment
Lidicky potok brook, 25 March 1996	22.16	272.4
Lidicky potok brook, March 1994	25.69	739.8
Bohuminska Struzka brook, 25 March 1996	18.40	149.2
Bohuminska Struzka brook, March 1994	36.42	337.4
Vltava river, Rez near Prague, 16 April 1996	13.46	<38.5[a]

[a] Below the limit of determination; tributyltin compounds detected only.

mass detection, where the ionization mode can be changed by software means in a very short time.

When using the conventional reaction gas, *viz*. methane, the background (chromatographic peaks) in the butyltin compound elution region decreased slightly; this advantage, however, was offset by the decrease in the detector sensitivity.

Some advantage can be gained by applying chemical ionization with acetonitrile as the reaction gas. The spectra of the butyltin compounds are characterized by peaks of adducts of the $(M_{\text{fragment}} + 42)^+$ type[5]. A dominant formation of the similar $(M + CH_2CN)^+$ stable adducts was also observed for higher alkanes, for which – in contrast to the organotin compounds – a peak corresponding to the molecular ion can also be found[13,14]. With respect to the detection of organotin compounds, the detector sensitivity in the chemical ionization mode using acetonitrile is comparable to the EI mode, and in some cases even slightly higher. The relative peak heights are also different. For a sample which was used in the two modes applying identical injection volumes, the *S/N* ratio was 12.9 in the EI mode and 20.2 in the CI mode with acetonitrile (response of selectively chosen ions for Bu_3SnMe = 288 + 290). The background level for real samples was also lower. Hence, chemical ionization with acetonitrile is very convenient

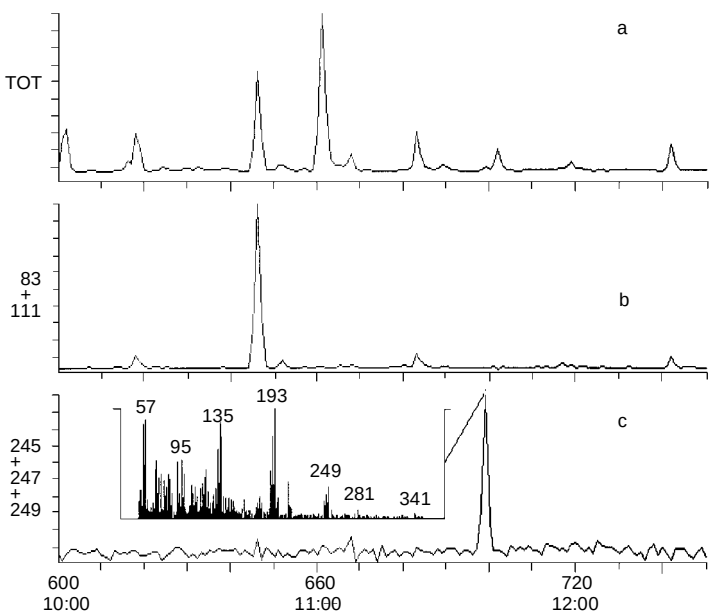

FIG. 2

A segment of the chromatogram of a sediment containing 149.2 ng Sn/g dry matter. a Total ion current; b reconstructed chromatogram for ions *m/z* = 83 and 111 (cyclododecane), c reconstructed chromatogram for ions *m/z* = 245, 247 and 249 (Bu_3SnMe) in dependence on the number of scans (s^{-1}) and time (min:s)

for suppression of background interference for sediments contaminated by higher hydrocarbons or, in general, substances exhibiting a low response in the chemical ionization mode.

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