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DETERMINATION OF 3,3',4,4'-TETRACHLOROAZOBENZENE IN WATER BY ISOTOPE DILUTION GAS CHROMATOGRAPHY/HIGH RESOLUTION MASS SPECTROMETRY

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ABSTRACT

A methodology, based on gas chromatography/high resolution mass spectrometry selected ion monitoring (GC/HRMS SIM) has been developed for the determination of 3,3',4,4'-tetrachloro-azobenzene (TCAB), a potential carcinogen, in water. As internal standard we prepared [$^{13}C_{12}$] labelled TCAB, from [$^{13}C_6$] labelled benzene. TCAB was extracted from water by hexane followed by a cleanup column chromatography over basic alumina. Recoveries of TCAB range from 80.0 - 88.3 %. The detection limit of TCAB is about 50 fg of TCAB or 5 ppq in 1,000 mL of water.

INTRODUCTION

3,3',4,4'-tetrachloroazobenzene (TCAB) is known to be a contaminant of 3,4-dichloroanilline (3,4-DCA) and its derived pesticides[1]. It is formed as an unwanted by-product during their production and the concentrations vary between 0.2 and 13 μg/g (ppm)[2-5]. The biological effects of TCAB are believed to be similar to those of the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Binding to the Ah receptor and induction of the aryl hydrocarbon hydroxylase (AHH) have been reported[1,6]. Environmental occurrence of TCAB may be due to application of contaminated pesticides or to their biotransformation. TCAB and it's oxidation product 3,3',4,4'-tetrachloro-azoxybenzene (TCAOB) are bio-synthesized by oxidative coupling of *N*-hydroxy-3,4-dichloroaniline, with 1,2-dichloro-4-nitrosobenzene, with itself or by a free radical process[7-9].
TCAB in pesticides are generally analyzed by GC/ECD[10,11], by HPLC/UV[12], by visible spectrometry[5] or by GC/MS[4]. The mass spectra of azobenzenes and azoxybenzenes have been investigated under EI and CI conditions by Waddell *et al.*[13] and Hsia and Burant[14]. Analogues of TCAB have been studied under EI conditions by Bowie *et al.*[15] and Efremov and Fedyaino[16]. High resolution mass spectrometry of TCAB was carried out by Lay *et al.*[17]. Capillary GC combined with HRMS SIM is a method of choice for the detection and quantification of environmental contaminants [18,19]. Because of its high diagnostic capability and sensibility SIM monitoring gives the best possible certainty of identification. In this paper we describe the GC/HRMS SIM analysis of TCAB in water. [$^{13}C_{12}$] labelled TCAB was prepared from [$^{13}C_6$] labelled benzene and used as internal standard.

MATERIALS AND METHODS

Preparation of ^{13}C labelled TCAB.

[$^{13}C_6$] labelled benzene (isotope purity of more than 99 %) was purchased from Isotec Inc. (U.S.A.). Urea nitrate and *N*-chlorosuccinimide were purchased from Knto Chemicals (Japan). Stannous chloride, palladium black and manganese dioxide were purchased from Wako Pure Chemical Industries (Japan). Preparative TLC plates were purchased from E. Merck (Silica Gel 60, F_{254} Art. 5717). Solvents and anhydrous potassium carbonate, purchased from Wako Pure Chemical Industries, were of analytical grade.

The different synthetic steps for the preparation of the isotopically labelled form of the analyte are described in the literature. The [$^{13}C_6$] labelled benzene was nitrated by a mixture of nitric acid (60 %) and sulfuric acid (96 %) to give [$^{13}C_6$] nitrobenzene (20). [$^{13}C_6$] nitrobenzene was reduced to aniline by hydrogen in the presence of palladium black in ethyl acetate. Nitration with urea nitrate in concentrated sulfuric acid[21] gave 4-nitroaniline and a small quantity of 3-nitroaniline. The mixture was purified by preparative TLC. About 80 mg of the mixture was applied to one plate which was allowed to migrate in toluene. Elution of 4-nitroaniline (Rf 0.85) was achieved by methanol. 4-Nitroaniline was chlorinated by *N*-chlorosuccinimide in acetonitrile to [$^{13}C_6$] 2-chloro-4-nitroaniline [22]. Substitution of the amino group by a chlorine group by a Sandmeyer reaction gave [$^{13}C_6$] 3,4-dichloronitrobenzene. Reduction of the 3,4-dichloronitrobenzene by stannous chloride in ethanol 95 % and concentrated hydrochloric acid gave [$^{13}C_6$] 3,4-dichloroaniline. Oxidation of 3,4-DCA or [$^{13}C_6$] 3,4-DCA by manganese dioxide in dichloromethane at reflux yielded TCAB and [$^{13}C_{12}$] TCAB respectively which were recrystallized from dichloromethane at cold.

Stock solutions (100 µg/mL) were prepared by dissolving 2.5 mg of TCAB in 25 mL of HPLC grade toluene. Working solutions for the GC/HRMS calibration contained 1,000, 100, 10, 1 and 0.1 ng/mL native TCAB and 100 ng/mL of labelled TCAB.

Extraction and cleanup of TCAB from water

Extraction and work-up of TCAB was performed as described earlier[23]. The solution was extracted 4 times with hexane, using 50mL each time. The organic layer was dried over anhydrous sodium sulfate then reduced to 1 - 2 mL on a rotary evaporator under reduced pressure. The concentrated extract was added to a cleanup column containing 15 g of basic alumina (Aluminium Oxide 90 activity I, E. Merck). The column was filled with hexane and TCAB was eluted with a mixture of 200 mL of dichloromethane/hexane (60/40 v/v). The solution was concentrated on a rotary evaporator to about 1 mL, transferred to a centrifuge tube with hexane and the solvent was purged with a nitrogen beam. For recovery determination, the concentrate was taken up in 100 µL of toluene containing 100 ppb of [$^{13}C_{12}$] TCAB.

GC/HRMS analysis.

The GC/HRMS analysis were performed on a JEOL JMS SX-102 A high performance double focusing mass spectrometer. An aliquot (1 µL) of a sample was injected into a Hewlett Packard 5890 series II gas chromatograph equipped with a Spelco PTE-5 column (30 m × 0.32 mm i.d., film thickness 0.25 µm). The GC carrier gas was helium at a flow rate of 1.2 mL/sec. The GC column was maintained at 150 °C for 1 minute then the temperature was increased linearly to 260 °C at a rate of 20 °C/min. The final temperature was maintained for 5 minutes. The GC/MS interface temperature was 280 °C. The retention time of TCAB on the column was 7.76

min. The mass spectrometer was operated in the electron impact ionization (EI) mode at 42 V, source temperature 250 °C. Data acquisition and processing of the mass spectrometer were controlled by Hewlett Packard 98785 workstation. The EI mass spectra were recorded at an ionization potential of 70 V.
m/z = 144.9606 and 146.9584 for native TCAB and *m/z* = 150.9803 and 152.9773 for the internal standard, [$^{13}C_{12}$] TCAB. The mass profiles of the selected ions were obtained during GC elution. Identification was based on examination of isotopic ratio of *m/z* = 144.9606 and 146.9584 and retention time of the GC separation. The area of the mass profile peaks of the quantification ions was used for the quantitative analysis of TCAB.

Recovery studies of TCAB from water.
For recovery studies two 500 mL solutions, A1 and A2, containing TCAB at a concentrations of 10 ppt and two 500 mL solutions, B1 and B2, containing TCAB at a concentrations of 1 ppt were prepared. The calculated amount (1 mL) of a toluene solution containing TCAB at a concentration of 5 or 0.5 ppb respectively was evaporated by an air flash and the residue was dissolved in 500 mL of water.

Precision studies of TCAB from water.
For precision studies, five 500 mL solutions, C1 - C5, containing TCAB at a concentration of 1 ppt were spiked with [$^{13}C_{12}$] TCAB. The concentration of [$^{13}C_{12}$] TCAB was 20 ppt. Extraction and cleanup were as described above. The final residue was taken up in 100 µL of toluene.

Determination of leachate water sample from DCPA contaminated soil.
Dichloropropanil (DCPA) contaminated soil was packed in soil column (250 mm height × 200 mm i.d.) and 500 mL of water was sprayed at the top of the column every day. Leachate water (4 L) was collected and analyzed. Leachate water sample (2 L) was spiked with 10 ng of internal standard, [$^{13}C_{12}$] TCAB, and was extracted with a portion of 200 mL of hexane twice. The extract was removed solvent by using evaporator to about 3 mL and subjected to alumina column chromatography. TCAB was recovered in dichloromethane/hexane (60/40 v/v) fraction and the fraction was concentrated by using evaporator and nitrogen gas purging to almost dryness. It was dissolved in 100 µL of decane and analyzed by GC/HRMS.

RESULTS AND DISCUSSION

Identification of mass numbers and quantitative analysis.
The mass spectra of native TCAB (a) and [$^{13}C_{12}$] TCAB (b) are represented in Figure 1. The elemental composition and the relative intensities of the main peaks are given in Table 1.
The ions used for identification and quantification of TCAB were chosen on the basis of maximum selectivity (presence of chlorine atoms) and maximum abundance (base peak and second most intensive peak). The mass spectrum of [$^{13}C_{12}$] TCAB has no significant ions at *m/z* = 144 and 146, thus the internal standard does not interfere with the analysis of TCAB. The criteria used for determination and quantification of TCAB were the retention time (7.76 ± 0.05 min) and the isotope abundance ratio of *m/z* = 144.9606 to 146.9584 (0.64 ± 0.13).

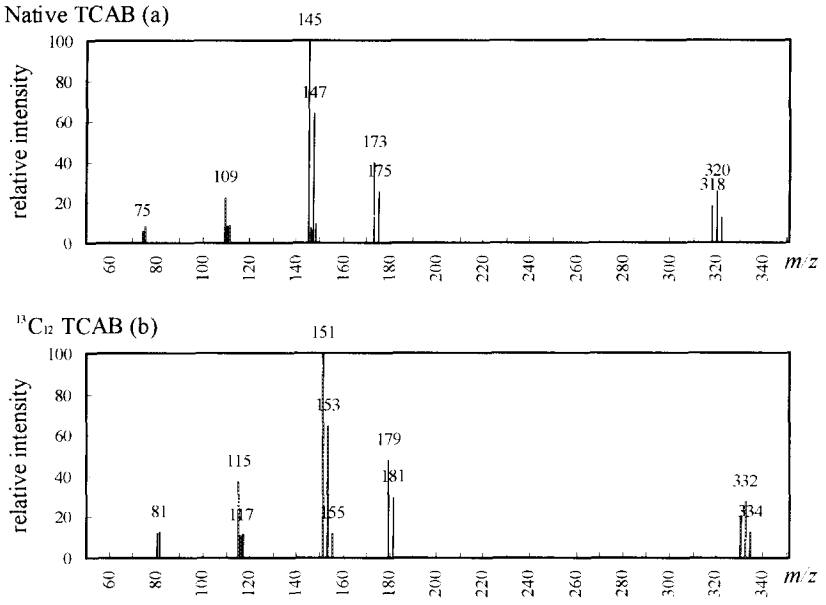

Figure 1. Mass spectra of native TCAB (a) and $^{13}C_{12}$ TCAB (b).

m/z	Relative intensity	Composition
321.9194	9.4	$C_{12}H_6N_2{}^{37}Cl_2{}^{35}Cl_2$
319.3219	23.9	$C_{12}H_6N_2{}^{37}Cl^{35}Cl_3$
317.9254	17.9	$C_{12}H_6N_2{}^{35}Cl_4$
172.9676	39.8	$C_6H_3N_2{}^{35}Cl_2$
146.9584	63.5	$C_6H_3{}^{37}Cl^{35}Cl$
144.9606	100	$C_6H_3{}^{35}Cl_2$

Table 1. Relative intensities and elemental compositon of the main fragment of native TCAB.

Determination of TCAB from water and detection limit.

The recovery of TCAB from 10 ppt (A1 and A2) and 1 ppt (B1 and B2) water solutions after hexane extraction and cleanup on basic alumina column is shown in Table 2.

The recovery yields vary between 80.0 and 88.3 %. A blank (not listed in the table) showed no contamination of the water sample or GC/MS system. The standard deviation and the relative standard deviation are larger for the 1 ppt solutions than for the 10 ppt solutions. This situation is generally observed when quantities approaching

Analysis	Recovery (%)			
	A1 (10 ppt)	A2 (10 ppt)	B1 (1 ppt)	B2 (1 ppt)
1	79.2	88.9	88.6	106.2
2	74.1	71.0	98.3	92.6
3	81.2	84.4	86.1	77.9
4	94.1	81.6	68.2	76.9
5	84.3	75.1	81.4	87.7
AVE[a] ± SD[b]	82.6 ± 7.4	80.0 ± 7.2	84.5 ± 11.1	88.3 ± 12.0
RSD[c] (%) n[d]=5	9.0	9.0	13.1	13.6

Table 2. Recovery of native TCAB from water after extraction and column cleanup.
a = average, b = standard deviation, c = relative standard deviation, d = number of determinations

Solution	Native TCAB (ppt)	$^{13}C_{12}$ TCAB (ppt)	Recovery of native TCAB (%)
C1	1	20	93.0
C2	1	20	117.1
C3	1	20	77.8
C4	1	20	107.8
C5	1	20	81.6
AVE[a] ± SD[b]			95.5 ± 17.1
RE[c] (%)			-4.5
RSD[d] (%) n[e]=5			17.9

Table 3. Accuracy and precision data for TCAB determination in water.
a = average, b = standard deviation, c = relative error,
d = relativestandard deviation, e = number of determination

the detection limit are analyzed.

The accuracy and precision data for TCAB determination in water by liquid phase extraction, column cleanup and GC. HRMS analysis are given in Table 3. The mean value for the determination of TCAB come very close to the actual "contamination" level. The relative error (RE) is 4.5 %.

The detection limit of TCAB at a peak to noise ratio of 3 are about 20, 50,100 and 100 fg respectively for the ions of *m/z* = 144.9606, 146.9584, 317.9285 and 319.9256. This corresponds to theoretical detection of 2 - 10 ppq of TCAB in a 1,000 mL water sample, considering that the final extract is concentrated to 100 µL and that 1 µL of this extract is injected into GC/MS system. The detection limit may be lowered if larger water samples are analyzed or if the final extract is further concentrated. The chromatograms for the selected ions (*m/z* = 144.9606

and 146.9584) of a 0.05 ppb spiked water sample are represented in Figure 2. The limit may be higher in natural samples because of interferences with other compounds, because of lower recovery yields and a higher noise level.

Determination of leachate water sample from DCPA contaminated soil.

The method is applied to leachate water sample from DCPA contaminated soil. In these samples, the use of higher numbers (m/z = 317.9285 and 319.9256) as selected monitoring ions are preferred because of the lesser extent of chromatographic interference. Figure 3 shows the chromatogram of a leachate water sample. TCAB concentration in the sample was calculated as 0.22 ppt. TCAB may be found as an impurity of DCPA herbicides. In fact two commercially available DCPA formulations we analyzed contained 0.3 and 0.65 ppm TCAB[23]. TCAB may also be present in the soil as a metabolite of the herbicide.

CONCLUSION

We developed a method for specific determination of TCAB in water samples at very low concentration levels. Extraction with hexane and cleanup of the samples over a alumina column are easy and give good recovery yields. The GC/HRMS SIM analysis is the method of choice for determination of low contamination levels of TCAB. This method will allow to monitor the transportation and biotransformation of TCAB in the environment.

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