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Trace analysis of trichlorobenzenes in fish by microwave-assisted extraction and gas chromatography–electron-capture detection

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Abstract

An analytical method consisting of extraction, clean-up, and analysis by gas chromatography–electron-capture detection (GC–ECD) was developed for the determination of trichlorobenzenes (TCBs) in fish samples. Two extraction methods, saponification and liquid–liquid extraction (S-LLE), and microwave-assisted extraction (MAE), were evaluated. In both cases, *n*-pentane was used as the extraction solvent. For S-LLE, the recoveries ranged from 66.6±9.1% for 1-bromo-4-chlorobenzene (4-BCB) to 93.5±4.9% for 1,2,4-trichlorobenzene (1,2,4-TCB). The recoveries were significantly lower, between 31.0±3.9% for 1,2,3-trichlorobenzene (1,2,3-TCB) and 52.3±3.0% for 1,3,5-trichlorobenzene (1,3,5-TCB), in the absence of fish. Proteins and glycerides of the fish tissue seemed to compete with TCBs for the base, and hence decreased their decomposition rate. In the case of MAE, the recoveries were highly dependent on the pressure applied during extraction. At 5 bar, much higher recoveries were obtained, from 66.7±15.6% for 4-BCB to 79.9±13.6% for 1,2,4-TCB, than at 1 bar. Sulfur formation was, however, observed at 5 bar, and interfered with the GC–ECD analysis of TCBs. Sulfur was adequately removed by copper powder treatment, which was shown not to affect the recovery of analytes. The recoveries of target analytes by S-LLE and MAE did not differ statistically (*t*-test, $\alpha=0.01$). Both methods were appropriate for the detection of TCBs at concentration levels typically observed in marine biota, i.e. ~1 ng/g. S-LLE was, however, more time consuming, and required larger volumes of high-purity organic solvents than MAE.
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1. Introduction

Trichlorobenzenes (TCBs) are used as solvents in industry and are intermediates in the production of several chemicals [1,2]. TCBs are also components of the dielectric fluids used in transformers and capacitors. They are toxic and have been detected in various environmental compartments, e.g. in sediment and living organisms [3]. Because of their widespread occurrence and environmental concern,

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regulations are in force in a number of countries. In California (USA), for example, the drinking water standard for 1,2,4-TCB was set at 5 μg/l in 1999 [4].

Based on their bioconcentration factors (ranging from 182 to 3200), TCBs are expected to accumulate in aquatic organisms [5,6]. Because of the potential threat to the marine environment, they were classified by OSPARCOM (Oslo and Paris Commission) as chemicals for priority action [7], and were proposed as chemical parameters in the Water Framework Directive by the European Commission [8]. While TCBs have been detected at ng/l concentrations in water samples of the North Sea, little is known regarding the concentrations and distribution in marine organisms.

Soxhlet extraction and analysis by gas chromatography–electron-capture detection (GC–ECD) was formerly used by Oliver and co-workers [5,6] to determine TCB isomers in fish and sediment samples. Roose and Brinkman [9] used purge-and-trap to enrich various volatile organic compounds (VOCs), including TCBs, from homogenized fish samples suspended in water. Elder et al. [10] analysed TCBs in water and sediment by solvent extraction, followed by gas chromatography–mass spectrometry (GC–MS) or gas chromatography–flame ionization detection (GC–FID). Borrelli et al. [11] determined 1,2,4-TCB in water by purge-and-trap and subsequent analysis by GC with FID, ECD or selected ion monitoring MS. Solid-phase microextraction was employed by He et al. [12] for the analysis of chlorinated benzenes in water. Recently, microwave-assisted extraction (MAE) has been introduced to isolate semi-volatile organic compounds from sediment samples. Lopez-Avila et al. [13] investigated the extraction efficiency of 1,2,4-TCB from soil samples by MAE. The recovery of 1,2,4-TCB with a *n*-hexane–acetone (1:1) mixture at 115 °C for 10 min was 59.2%. The chemical stability of 1,2,4-TCB was investigated by the same authors under MAE conditions in *n*-hexane–acetone (1:1), dichloromethane–acetone (1:1) and methyl *tert*.-butyl ether at various temperatures and extraction times. No chemical change was observed [14].

The aim of this work was to develop and evaluate an analytical method for the determination of TCBs in fish samples, based on MAE and GC–ECD analysis. Saponification and liquid–liquid extraction (S-LLE) was used as a reference extraction method.

2. Experimental

2.1. Chemicals

n-Pentane, methanol and 2,2,4-trimethylpentane (for organic residue analysis) were obtained from J.T. Baker (Deventer, The Netherlands). *n*-Hexane (ECD tested, halocarbon-free grade) and silica gel (for column chromatography, 0.060–0.200 mm O.D., pore diameter ~4 nm) were obtained from Acros Organics (Geel, Belgium). Sodium sulphate, potassium hydroxide (ACS reagent), copper powder (99%, ~200 mesh), and activated and neutral alumina were purchased from Sigma–Aldrich (Bornem, Belgium). 1-Bromo-4-chlorobenzene (4-BCB), 1,4-dibromobenzene (4-DBB), 1,3,5-trichlorobenzene (1,3,5-TCB), 1,2,4-trichlorobenzene (1,2,4-TCB), and 1,2,3-trichlorobenzene (1,2,3-TCB) were supplied by Fluka (Sigma–Aldrich). 4-BCB was used as a recovery standard, while 4-DBB served as an internal standard.

Sodium sulphate was purified by Soxhlet extraction with *n*-pentane for 5 h, and dried at 260 °C for 4 h. Silica was activated at 160 °C for 24 h, and impregnated with concentrated sulphuric acid (30 g silica + 10 g sulfuric acid, 98%). Alumina was either activated at 300 °C for 24 h, or deactivated with 5% (w/w) water [15].

2.2. Instrumentation

A Varian CP-3800 GC system equipped with a CP-8410 auto-sampler and an ECD system was used for TCB analysis (Varian, Walnut Creek, CA, USA). Data processing was performed with a Star Chromatography Workstation, v. 4.51 (Varian).

Unknown chromatographic peaks were elucidated by GC–MS analysis. A Varian Model 3400 GC system with a 1075 split/splitless capillary injector was connected through a direct on-line inlet system to a Finnigan-MAT 355 ultratrace ion trap MS (Finnigan, San Jose, CA, USA). The MS was

operated at 70 eV in electron ionization mode from m/z 40 to 350.

A MarsX Microwave Accelerated Reaction System and GreenChem Plus PTFE vessels (CEM, Matthews, NC, USA) were used for MAE.

A Branson 2200 Ultrasonic cleaner (Bransonic Ultrasonic, Danbury, CT, USA) was used for ultrasonication.

2.3. Gas chromatography

The TCB isomers were separated on a CP-Sil 8 CB poly(5% diphenyl–95% dimethyl)siloxane Low Bleed/MS capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness; Varian). Nitrogen (Air Liquide, Liège, Belgium) was used as carrier gas at a flow rate of 5 ml/min. A 1-μl sample was injected into a CP 1177 injector in splitless mode. The injector temperature was held at 270 °C, and the purge valve was activated for 0.75 min after injection. The purge flow was 31.7 ml/min. The ECD temperature was held at 330 °C. The temperature of the GC oven was initially kept at 50 °C, and was then linearly increased from 50 to 130 °C at 5 °C/min, and from 130 to 180 °C at 10 °C/min. The oven temperature was held at 180 °C for 1 min.

Instrumental detection limits (IDLs) were calculated as the amount of analyte corresponding to a signal-to-noise ratio of 3. The retention time, linearity range and IDL of each TCB isomer, 4-BCB and 4-DBB are presented in Table 1.

Quantification was based on relative peak area values, normalized to the peak area of the internal standard.

2.4. Sample preparation

2.4.1. Standards and solutions

Standard stock solutions were prepared gravimetrically in methanol. Stock solutions were further diluted to obtain adequate concentrations for spiking and calibration purposes.

2.4.2. Fish samples

Fish samples were prepared from cod (*Godus morhua*) obtained from the local market. The fish was filleted and homogenized with a commercial blender (Waring, Torrington, CT, USA), and small portions (~20 g) were stored in closed glass vessels at −10 °C. The water content, determined by gravimetry (the fish samples were dried at 105 °C until a constant mass was obtained), was 77.6±0.2% ($n=3$). The total lipid content was 3.4±0.1% ($n=3$), as determined by the Micro Folch method [16].

The samples were spiked with 10 ng (MAE) or 20 ng (S-LLE) of 1,3,5-, 1,2,4- and 1,2,3-TCB, and 20 ng of internal standard by injecting 10 and 20 μl, respectively, of a methanolic stock solution into the fish tissue. The tissue was mixed, and equilibrated overnight in a closed vessel at room temperature.

2.5. Extraction and clean-up procedure

2.5.1. Saponification and liquid–liquid extraction

A 20-g amount of spiked fish sample was refluxed with 100 ml 20% (w/w) potassium hydroxide in methanol for 2 h. The solution was then filtered and extracted with 2×50 ml of *n*-pentane. The combined *n*-pentane extracts were dried over anhydrous sodium sulphate, and 0.50 ml of 2,2,4-trimethylpentane

Table 1
Retention time, linearity range, and instrumental detection limit (IDL) of 1-bromo-4-chlorobenzene (4-BCB), 1,4-dibromobenzene (4-DBB) and trichlorobenzenes (TCBs)

Compound	Retention time (min)	Linearity range (μg/l)	Sensitivity (a)[a], $y = ax$	r^2	IDL (pg)
4-BCB	9.79	10–50	11 966	0.9425	1.07
4-DBB	12.42	10–50	31 918	0.9651	0.25
1,3,5-TCB	10.62	1–70	14 582	0.9928	0.47
1,2,4-TCB	11.93	1–70	9285	0.9911	0.53
1,2,3-TCB	12.87	1–70	20 334	0.9873	0.37

[a] x, concentration (μg/l); y, peak area (dimensionless); injected volume: 1 μl.

was added to avoid evaporation losses. The solution was concentrated to ~2 ml with a rotary evaporator (Buchi, Flawil, Switzerland). The concentrated extract was then cleaned by column chromatography. A Pasteur pipette was plugged with glass wool and filled, from bottom to top, with 500 mg of 5% deactivated alumina, 500 mg of silica impregnated with sulphuric acid and 100 mg of sodium sulphate [15].

The extract was eluted from the column with 2×5 ml of *n*-pentane. The eluent was concentrated to ~1 ml, and 20 μl of the internal standard (a 1-mg/l solution in *n*-hexane) was added. The extract was transferred to an autosampler vial.

2.5.2. Microwave-assisted extraction

A 10-g amount of fish spiked with TCBs was extracted with 20 ml of *n*-pentane in a microwave vessel. During extraction, the pressure was increased to 5 bar and held at that value for 15 min. The vessels were then cooled down, and the *n*-pentane extract was dried over sodium sulphate. After the addition of 0.5 ml of 2,2,4-trimethylpentane, the extract was concentrated to ~2 ml and cleaned up as above. After clean-up, the extract was concentrated to ~1 ml, treated with 1 g of copper powder, and sonicated for 15 min [17]. The solution was then filtered over glass wool, the internal standard was added, and the solution was transferred to an autosampler vial.

3. Results and discussion

3.1. Saponification and liquid–liquid extraction

3.1.1. Recovery after evaporation

The analytical procedure based on S-LLE includes two evaporation steps. Considering the volatility of the target compounds, the recovery of analytes after evaporation was determined. TCB mixtures were added to 20-ml portions of *n*-pentane or *n*-hexane. The solution was then concentrated to ~1 ml, the internal standard was added and a sample aliquot was analysed by GC–ECD. The recoveries were highly dependent on the solvent used. Higher recoveries were obtained with *n*-pentane, and ranged from 92.7 to 97.4% for 1,3,5-TCB and 4-BCB, respectively ($n=5$), than with *n*-hexane, which ranged from 62.4 (4-BCB) to 75.5% (1,3,5-TCB) ($n=5$). This can be explained by the difference in boiling points of *n*-pentane (35–36 °C) and *n*-hexane (67–69 °C). Higher temperatures have to be applied to concentrate the *n*-hexane extract, and hence evaporation losses of target analytes are more considerable. The relative standard deviations ranged from 1.4% (1,2,4-TCB) to 4.8% (4-BCB) in the case of *n*-pentane, and from 9.8% (1,2,3-TCB) to 15.5% (1,2,4-TCB) for *n*-hexane. Consequently, *n*-pentane was used in all further experiments.

3.1.2. Recovery after clean-up

To determine possible losses due to sample clean-up, a standard solution spiked with TCBs and the recovery standard was chromatographed on the clean-up column. The column was eluted with 5-ml portions of *n*-pentane, with 0.5 ml of 2,2,4-trimethylpentane added to each portion after elution. Each fraction was then evaporated to a final volume of ~1 ml and an aliquot was analysed by GC. Between 78.7±3.5% (4-BCB) and 89.3±10.4% (1,2,4-TCB) ($n=5$) of the spiked amount was recovered in the first fraction, while another 1.6±0.8 and 2.9±0.5% ($n=5$) was found after the second elution for 1,3,5-TCB and 1,2,3-TCB, respectively. Accordingly, 2×5 ml of *n*-pentane seemed sufficient to recover most of the analytes from clean-up.

3.1.3. Total recovery of the method

Two experiments were performed to determine the total recovery of the analytical method. First, spiked standard solutions in methanol, without fish, were analysed. The analysis was then repeated with spiked fish samples. The results are given in Fig. 1. The recoveries obtained from the analyses of spiked fish samples were significantly higher (*t*-test, $\alpha=0.05$) than the results obtained from standard solutions in methanol. One possible explanation is that TCBs are partially decomposed during saponification. The fish tissue, with its proteins, glycerides and other constituents, competes for the base, thereby lowering the TCB decomposition rate.

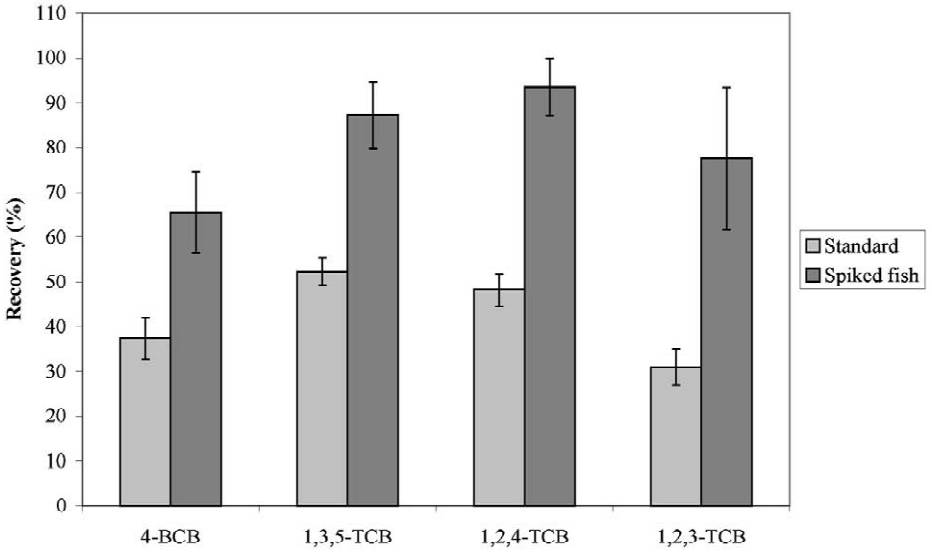

Fig. 1. Recovery of trichlorobenzenes (TCBs) and 1-bromo-4-chlorobenzene (4-BCB) by S-LLE and GC–ECD analysis of spiked fish samples, and standard solutions in methanol ($n=5$).

3.2. Microwave-assisted extraction

3.2.1. Effect of extraction pressure and temperature

The extraction procedure was first performed according to the following program: the pressure was increased to 1 bar within 5 min, and was held at that value for 15 min (65 °C). The recoveries were relatively low, ranging from 21.2±1.2 to 38.7±4.5% for 1,3,5-TCB and 1,2,3-TCB, respectively ($n=4$). When 5 bar was applied (100 °C), the recoveries increased to 66.7±15.6 and 89.9±13.6% for 4-BCB and 1,2,4-TCB, respectively. The results are illustrated in Fig. 2.

Polar solvents are generally used in MAE as they absorb microwaves. Unless the sample itself is able to absorb microwaves, apolar solvents can only be used in combination with polar solvents. Apolar solvents, however, are more selective and avoid co-extraction of polar substances. Consequently, the sample clean-up is faster and simpler. Because of its high water content (~80%) the fish tissue absorbs microwaves. As the pressure increases within the sample, the cellular structure is disrupted and target compounds are more easily released and extracted by the solvent. If the pressure is too low, the fish tissue remains intact, resulting in low extraction efficiencies. This might explain the difference in recoveries observed at 1 and 5 bar. Similar results have been observed for MAE based analyses of soil, sediment and plant samples [18–20].

3.2.2. Sulfur formation

At a pressure of 5 bar (100 °C), several interfering peaks were found in the chromatograms, as can be seen in Fig. 3. The largest peak was identified as elemental sulfur (S_8) by GC–MS analysis. It is well known from literature that sulfur is released from sediment samples during MAE [21]. However, its formation in fish samples has not previously been observed.

Sulfur was adequately removed by copper treatment and ultrasonication (Fig. 3) [17]. The copper powder had not been activated before. Control experiments demonstrated that the copper treatment did not affect the recovery of analytes.

3.2.3. Background contamination and carry-over

In trace and ultra-trace level analysis, background contamination and carry-over are limiting factors which affect the limit of detection (LOD) of the analytical method [22]. Düring and Gäth [23] used MAE for the extraction of polychlorinated biphenyls from soil and solid waste samples. Considerable

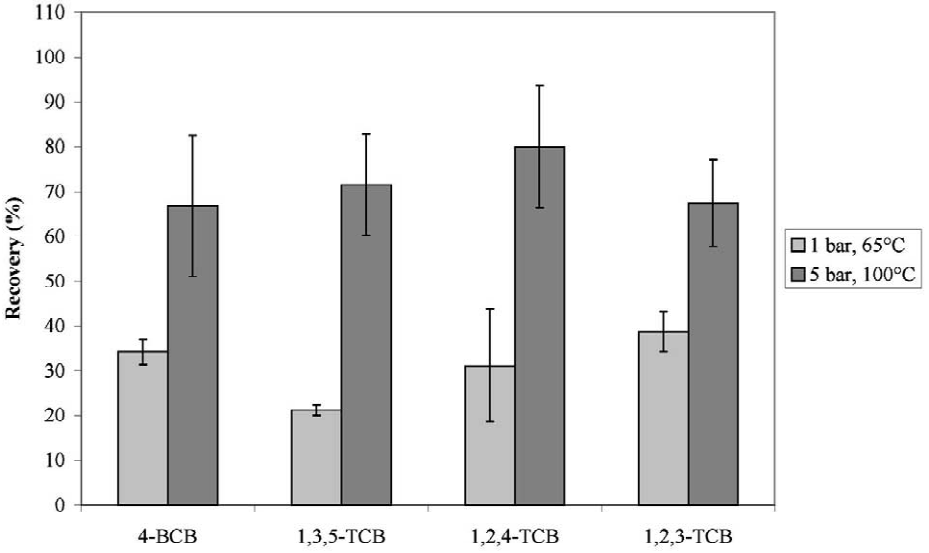

Fig. 2. Recovery of trichlorobenzenes (TCBs) and 1-bromo-4-chlorobenzene (4-BCB) with MAE and GC–ECD analysis at 1 bar (65 °C), and 5 bar (100 °C) ($n=5$).

cross-contamination was observed during microwave extraction. They applied a thorough cleaning procedure to diminish this memory effect.

Owing to the sorptive nature of PTFE, TCBs were expected to absorb into the MAE vessels and slowly desorb during subsequent analyses. Considering the concentration levels targeted in this study, i.e. 10–20 ng/g, it was important to determine, and eventually reduce, any background contamination of the system.

The PTFE vessels were first ''contaminated'' with each TCB. For this, three extraction vessels were filled with 8 ml of water and 20 ml of *n*-pentane containing 10 ng of each TCB isomer. The MAE procedure was run for 15 min at 5 bar. The vessels were cleaned with chromic acid for 5 h at room temperature. They were then rinsed with water and acetone, dried and extracted with *n*-pentane–water (20 ml:8 ml) at 5 bar for 15 min. An aliquot of *n*-pentane was injected into the GC–ECD system to determine the amount of TCBs recovered after clean-up. The procedure described above was repeated with 10% nitric acid, chloroform and acetone for 15 min at 5 bar. The amount of TCBs recovered after each clean-up procedure is given in Table 2. Background levels were lowest after the acetone treatment. This cleaning procedure was therefore applied in all subsequent experiments.

The LOD was calculated for each TCB isomer using the blank levels obtained after the acetone treatment. The following equation was used [24]:

$$LOD = c_{bl} + 3s_{bl} \quad (1)$$

where c_{bl} is the average blank concentration and s_{bl} the standard deviation.

The calculated LOD values are listed in Table 3. Due to cross-contamination, the LOD increased by a factor of 3.4, 1.7 and 2.1 for 1,3,5-, 1,2,4- and 1,2,3-TCB, respectively, as compared with the IDL. However, the LODs are still sufficiently low to allow the determination of concentration levels of ~1 ng/g, i.e. those generally observed in marine biota [9].

3.3. Comparison of microwave-assisted extraction with saponification and liquid–liquid extraction

The main characteristics of the two extraction methods are summarized in Table 4 for each target analyte and the recovery standard.

The recoveries of 4-BCB and TCBs by S-LLE and MAE do not differ statistically (*t*-test, $\alpha=0.01$). S-LLE requires five times more solvent, and the extraction is six times longer than MAE. On the other hand, the LOD obtained by S-LLE is lower by

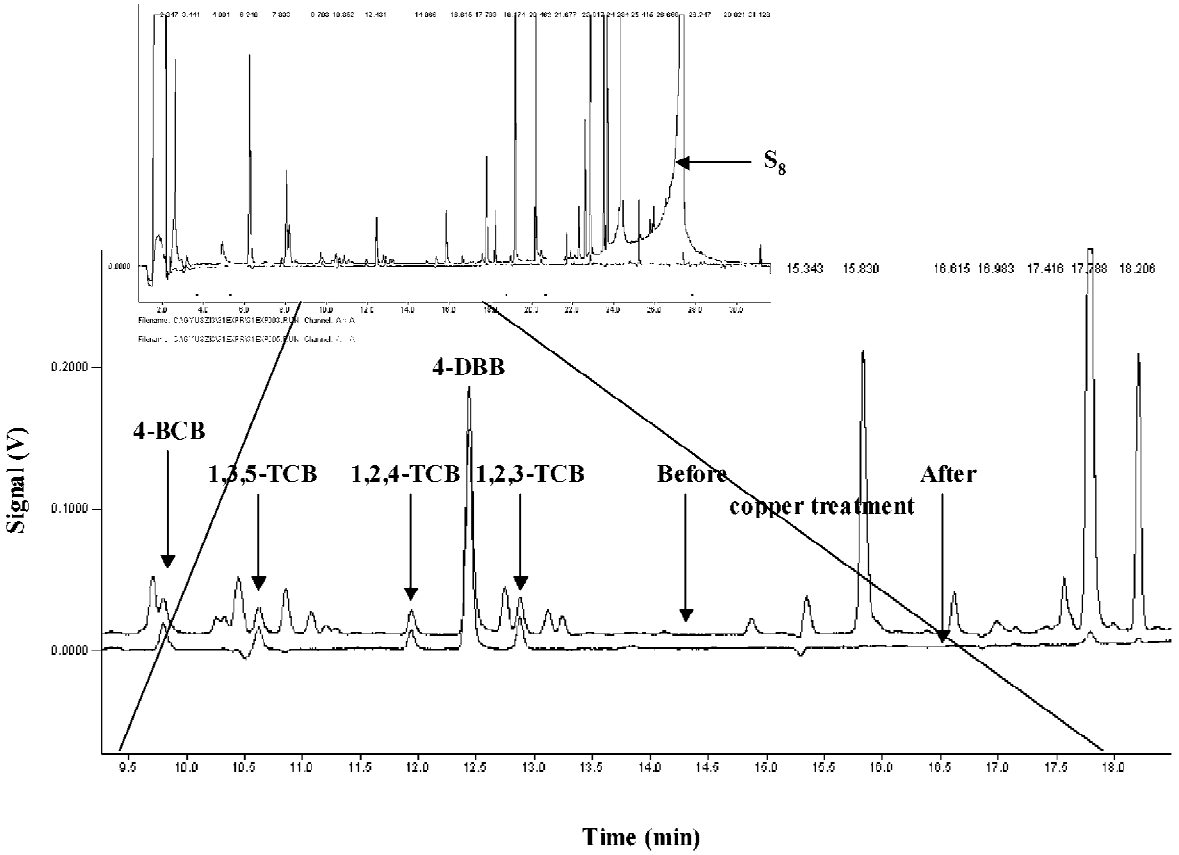

Fig. 3. Chromatogram resulting from MAE and GC–ECD analysis of a spiked fish sample, before (upper) and after (lower) copper treatment.

Table 2
Amount of trichlorobenzenes (TCBs) extracted after various cleaning procedures

Treatment	Extracted amount (ng)		
	1,3,5-TCB	1,2,4-TCB	1,2,3-TCB
Chromic acid[a]	1.16±0.41	0.30±0.25	0.80±0.22
10% Nitric acid[a]	1.41±0.38	1.41±0.44	1.29±0.32
$CHCl_3$[a]	0.87±0.42	0.37±0.32	0.69±0.41
Acetone[b]	0.50±0.36	0.23±0.22	0.19±0.18

[a] $n=3$ parallel measurements.
[b] $n=10$ parallel measurements.

Table 3
Instrumental detection limits (IDLs) and limits of detection (LODs) obtained by MAE and GC–ECD analysis of trichlorobenzenes (TCBs) in fish[a]

	1,3,5-TCB	1,2,4-TCB	1,2,3-TCB
IDL (pg/g)	65.7	66.4	54.9
LOD (pg/g)	221.8	110.8	113.7

[a] Calculated from the amount of fish sample used (10 g wet mass), the sample volume (1 ml), the injected volume (1 μl) and the recovery of each analyte.

a factor of 4.1, 1.6 and 2.4 for 1,3,5-, 1,2,4- and 1,2,3-TCB, respectively. The difference in LOD is mainly due to background contamination and carry-over of the MAE vessels.

4. Conclusions

An analytical method consisting of extraction, clean-up and GC–ECD analysis was developed for

Table 4
Main characteristics of saponification and liquid–liquid extraction (S-LLE), and microwave-assisted extraction (MAE) for the determination of trichlorobenzenes (TCBs) in fish

	Recovery (%)				Solvent (ml)	Extraction time (min)	LOD (pg/g)		
	4-BCB	1,3,5-TCB	1,2,4-TCB	1,2,3-TCB			1,3,5-TCB	1,2,4-TCB	1,2,3-TCB
S-LLE	66.6±9.1	87.8±7.3	93.5±4.9	78.9±15.8	100	125	53.8	69.4	47.8
MAE	66.7±15.6	71.5±11.1	79.9±13.6	67.4±9.7	20	20	221.8	110.8	113.7

the determination of TCBs in fish samples. Two extraction techniques, S-LLE and MAE, were evaluated. In each case, *n*-pentane was used as the extraction solvent.

When spiked fish samples were used, the recoveries by S-LLE ranged from 66.6±9.1 to 93.5±4.9%. The recoveries were significantly lower in the absence of fish. Proteins and glycerides of the fish tissue probably compete with TCBs for the base, and hence lower their decomposition rate.

For MAE, the results were highly dependent on the extraction parameters. The recoveries at 5 bar were significantly higher than those at 1 bar, and ranged from 66.7±15.6 to 79.9±13.6%. At 5 bar, however, sulfur formation was observed. Sulfur interfered with the GC–ECD analysis of TCBs, but was efficiently removed with copper powder. This was shown not to affect the recovery of the target compounds.

Both extraction methods are appropriate for the detection and quantification of TCBs at concentration levels typically observed in marine biota, i.e. ~1 ng/g. S-LLE is, however, more time consuming, and requires larger volumes of high-purity organic solvents than MAE.

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References

[1] R.J. Lewis, in: Hawley's Condensed Chemical Dictionary, 12th ed, Van Nostrand Reinhold, New York, 1993, p. 1169.
[2] US Environmental Protection Agency, Health Assessment Document for Chlorinated Benzenes. Part 1. USEPA-600/8-84-015A, Washington, DC, 1984, pp. 4–18.
[3] V.A. Elder, B.L. Proctor, R.A. Hites, Environ. Sci. Technol. 15 (1981) 1237.
[4] California Environmental Protection Agency, Public Health Goal for 1,2,4-Trichlorobenzene in Drinking Water, CA, USA, 1999.
[5] B.G. Oliver, K.D. Nicol, Environ. Sci. Technol. 16 (1982) 532.
[6] B.G. Oliver, M.N. Charlton, R.W. Durham, Environ. Sci. Technol. 23 (1989) 200.
[7] Annual Report 2000–2001, OSPAR Commission, London, 2001, p. 41.
[8] The European Parliament and the Council of the European Union, Directive 2000/60/EC of the European Parliament and the Council action in the field of water policy. Official Journal of European Communities, L331/2, 2001, p. 5.
[9] P. Roose, U.A.T. Brinkman, Analyst 123 (1998) 2167.
[10] V.E. Elder, B.L. Proctor, R.A. Hites, Environ. Sci. Technol. 15 (1981) 1237.
[11] R. Borrelli, T. Fiorani, P. Golfetto, J. High. Resolut. Chromatogr. 19 (1996) 457.
[12] Y. He, Y. Wang, H.K. Lee, J. Chromatogr. A 874 (2000) 149.
[13] V. Lopez-Avila, R. Young, W.F. Beckert, Anal. Chem. 66 (1994) 1907.
[14] V. Lopez-Avila, R. Young, W.F. Beckert, J. AOAC Int. 81 (1998) 462.
[15] M. Cleemann, B.P. Gudrun, E. Storr-Hansen, A. Fromberg, J. AOAC Int. 82 (1999) 1175.
[16] J. Folch, M. Leea, G.H.S. Stanley, J. Biol. Chem. 226 (1957) 497.
[17] M.W. Jacobs, J.J. Delfino, G. Bitton, Environ. Toxicol. Chem. 11 (1992) 1137.
[18] O.F.X. Donard, B. Labere, F. Martin, R. Lobinski, Anal. Chem. 67 (1995) 4250.
[19] J. Szpunar, V.O. Schmidt, O.F.X. Donard, R. Lobinski, Trends Anal. Chem. 15 (1996) 181.
[20] J.R.J. Paré, J.M.R. Bélanger, S.S. Stafford, Trends Anal. Chem. 13 (1994) 176.
[21] F. Smedes, J. de Boer, Trends Anal. Chem. 16 (1997) 503.
[22] J. Versieck, F. Barbier, R. Cornelis, J. Hoste, Talanta 29 (1982) 973.
[23] A. Düring, St. Gäth, Fresenius J. Anal. Chem. 368 (2000) 684.
[24] E. Prichard, G.M. MacKay, J. Points (Eds.), Trace Analysis: A Structured Approach Obtaining Reliable Results, Thomas Graham House, Cambridge, 1996, p. 33.