

# Analytical Procedure for the Determination of Chlorobenzenes in Sediments

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## Abstract

**This study presents the procedure for the determination of chlorobenzenes in sediment. It consists of solvent extraction (shaking overnight), extract clean-up with the use of a homemade glass column packed with activated silica gel and freshly activated copper, and slow solvent evaporation to a volume of 0.3 mL.**

**Two-microliter extract portions are analyzed by means of gas chromatography with an Rtx-624 capillary column (60 m × 0.32 mm,  $d_f = 1.8 \mu\text{m}$ ) coupled with mass spectrometry (in selected ion-monitoring mode). Deuterated 1,2-dibromobenzene is used as the recovery standard. The recovery of this method for all chlorobenzenes is high (ranging from 78% to 107%) with the exception of monochlorobenzene, which is 58%. The method is also characterized by good precision, which is commonly accepted in the analysis of trace organic pollution.**

## Introduction

Because of the substitution in the benzene ring by chlorine atoms it is possible to obtain 12 compounds, monochlorobenzene (MCB), three isomeric forms of dichlorobenzene (diCB), three isomers of trichlorobenzene (triCB), three isomers of tetrachlorobenzene (tetraCB), pentachlorobenzene (pentaCB), and hexachlorobenzene (hexaCB).

Chlorobenzenes are used mainly as: (a) intermediates in the synthesis of pesticides and other chemicals, (b) space deodorants and as a moth repellent (1,4-diCB), (c) components of dielectric fluids [the higher chlorinated benzenes (triCBs) and 1,2,3,4-tetraCB], and (d) a functional fluid in external combustion Rankine engines (1) and a component in heat transfer fluids in solar energy collectors (MCB) (2).

Chlorobenzenes are very persistent under the anaerobic conditions usually found in sediment and ground water, but many microorganisms from sediments and sewage sludge have been shown to degrade chlorobenzenes (higher chlorinated compounds are less readily degraded and such degradation occurs

only under aerobic conditions).

The toxicity of chlorobenzenes increases with the degree of chlorination of the benzene ring.

The following clinical symptoms and signs of excessive exposure in the case of chlorobenzenes have been observed (3): (a) central nervous system effects, (b) irritation of the eyes, (c) irritation of the upper respiratory tract (MCB), (d) hematological disorders (1,2-diCB), (e) hardening of the skin, and (f) hematological disorders including anemia (1,4-diCB).

A reduction of the widespread use and disposal of chlorobenzenes should be considered because: (a) they may act as precursors for the formation of polychlorinated dibenzodioxins/polychlorinated dibenzofurans (e.g., in incineration processes); (b) they can lead to taste and odor problems in drinking water and fish; and (c) residues persist in organically rich anaerobic sediments and soils and ground water (3).

The extraction of chlorobenzenes from the original matrix (i.e., sediment) is particularly difficult because of a large difference in the volatility between the volatile chlorobenzene and the non-volatile hexaCB. Therefore, the analysis of chlorobenzenes in sediments should not include a sample drying step using drying at a raised temperature ( $\sim 100^\circ\text{C}$ ), lyophilization (freeze-drying), or drying with a stream of inert gas at room temperature. The recovery of MCB was not quantitative under these conditions (4).

There are different extraction methods for removing chlorobenzenes from a sample that depend mainly on the matrix (i.e., air, water, and sediment). The extraction of chlorobenzenes from aquatic sediments or soil can be achieved by a solvent assisted by ultrasonic bath or shaking or Soxhlet extraction (5–10). The common solvents used are acetone, hexane, or both. The extract is generally dried using sodium sulfate, followed by clean-up on a Florisil column before gas–liquid chromatographic (GLC) analysis.

The analysis of chlorobenzenes in sediments at the concentration level of a few micrograms per kilogram requires the use of analytical tools characterized by high resolution and high capability for the identification of compounds (10,11).

The analytical technique of choice for the determination of chlorobenzenes in environmental samples is GLC.

Because of the volatility and easy degradation of MCB, it is very difficult to analyze all of the chlorobenzenes in a sediment sample

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using one sample preparation technique. The usually proposed extraction methods characterize the highest value of limit of detection (LOD) for the determination of MCB in comparison

with the others [i.e., 1500  $\mu\text{g}/\text{kg}$  for MCB, 5  $\mu\text{g}/\text{kg}$  for diCB, 0.4  $\mu\text{g}/\text{kg}$  for triCB, 0.2  $\mu\text{g}/\text{kg}$  for tetraCB, and 0.05  $\mu\text{g}/\text{kg}$  for pentaCB (12)].

There are also some problems with the separation of some of the chlorobenzenes isomers (i.e., 1,2,3,5-tetraCB and 1,2,4,5-tetraCB) when typical capillary columns are used (13).

Using our method it is possible to determine all of the congeners of chlorobenzenes in sediment samples at this same level of LOD and with satisfactory separation.

## Experimental

### Materials

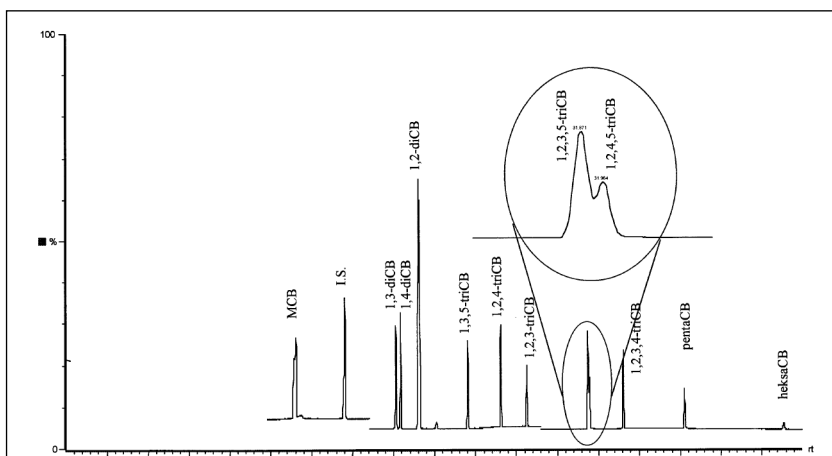
The following compounds were used in the analysis: monochlorobenzene; 1,2-diCB; 1,3-diCB; 1,4-diCB; 1,3,5-triCB; 1,2,4-triCB; 1,2,3-triCB; 1,2,3,4-tetraCB; 1,2,3,5-tetraCB; 1,2,4,5-tetraCB; pentaCB; and hexaCB (Supelco, Bellefonte, PA). All of these were chromatographic standards. Stock and working standard solutions were prepared in methanol (chromatographic grade) (Merck, Darmstadt, Germany). In addition, deuterated 1,2-dibromobenzene (Supelco) was employed as an internal standard to determine the recovery percentage of the analytes. 4-Bromo,1-fluorobenzene (Supelco) served as an internal standard for the gas chromatography (GC)–mass spectrometry (MS) quantitation. Glass solid-phase extraction columns (J.T. Baker, Griesheim, Germany) containing silica gel were used for fractionating the extract. Each column was conditioned and cleaned by using acetone and pentane (Merck). Wet sediment was dried with anhydrous sodium sulfate (POCH, Gliwice, Poland).

### Sample preparation

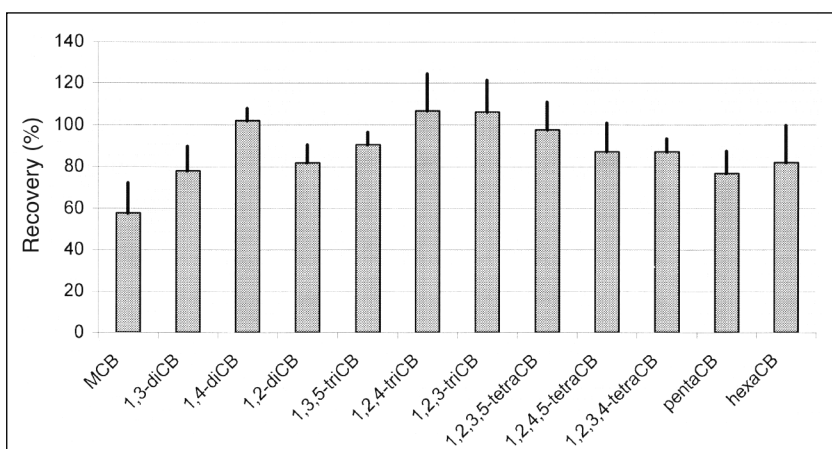
The samples were prepared and analyzed in the following way. The wet sediments were spiked with an internal standard (deuterated 1,2-diCB) and dried by adding anhydrous  $\text{Na}_2\text{SO}_4$ . They were extracted with 5 mL of a pentane and acetone mixture (4:1) on a shaker for 24 h at room temperature. The obtained extracts were decanted, and the extracts were cleaned using a short silica gel with activated copper powder at the upper layer of the column. The analytes were eluted with 10 mL of pentane; the eluate was evaporated under a stream of nitrogen to a volume of approximately 0.3 mL; 4-bromo,1-fluorobenzene was added as the internal standard; and an aliquot was analyzed by GC.

### Apparatus

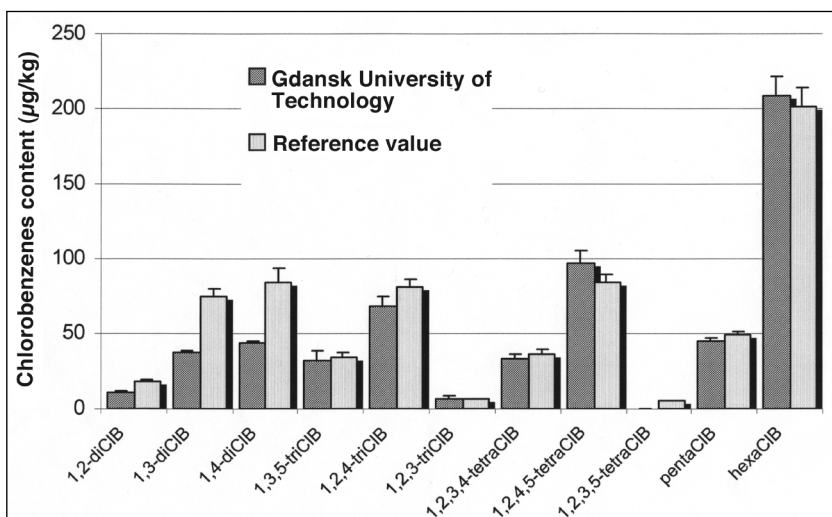
The extracts were analyzed by GC–MS using a



**Figure 1.** Chromatogram of a standard mixture of chlorobenzenes obtained under the proposed chromatographic conditions.



**Figure 2.** Recovery (with standard deviation) of chlorobenzenes from sediment spiked with a standard solution.



**Figure 3.** Recovery (with standard deviation) of chlorobenzenes from reference materials.

GC 8000 gas chromatograph equipped with an MD-800 quadrupole mass spectrometer, which were both from Fisons Instruments (Milan, Italy).

Analytes were quantitated in the selected ion-monitoring (SIM) mode. The carrier gas was helium at a flow rate of 2 cm<sup>3</sup>/min (100 kPa). The oven temperature started at 60°C, then was programmed at 5°C/min to 200°C and held isothermally at 200°C for 3 min, and then was programmed at 10°C/min to 250°C and held at 250°C for 9 min. The injection volume of the extract was 2 µL using oncolumn injection.

**Table I. Description of the Analyzed Compounds**

Compound	Boiling temperature (°C)	Retention time (min)	Ion detected (m/z)
MCB	130–133	13.2	[77],[112]
1,3-diCB	172.5	19.3	[146],[148]
1,4-diCB	174	19.5	[146],[148]
1,2-diCB	180–183	20.7	[146],[148]
1,3,5-triCB	208	23.6	[180],[182]
1,2,4-triCB	213–214	25.6	[180],[182]
1,2,3-triCB	218–219	27.2	[180],[182]
1,2,3,5-tetraCB	246	30.7	[214],[216]
1,2,4,5-tetraCB	240–246	30.8	[214],[216]
1,2,3,4-tetraCB	254	33.0	[214],[216]
pentaCB	277	36.8	[248],[250]
hexaCB	332	42.5	[284],[286]

**Table II. Statistical Evaluation of the Data**

Compounds	Ion	Intercept	Slope	R <sup>2</sup> *	LOD (ng/mL)
MCB	[77]	22463	6173	0.9983	0.002
	[112]	40802	19428	0.9949	
1,3-diCB	[146]	42288	5804	0.9977	0.002
	[148]	31091	148	0.9998	
1,4-diCB	[146]	47948	12372	0.9951	0.001
	[148]	36625	1774	0.9999	
1,2-diCB	[146]	21094	27555	0.9830	0.003
	[148]	17784	13742	0.9926	
1,3,5-triCB	[180]	27824	1878	0.9993	0.001
	[182]	26517	1891	0.9994	
1,2,4-triCB	[180]	21656	-1755	0.9998	0.002
	[182]	20956	1077	0.9996	
1,2,3-triCB	[180]	22933	1374	0.9993	0.002
	[182]	21603	1609	0.9992	
1,2,3,5-tetraCB	[214]	21129	-6254	0.9990	0.001
	[216]	23642	-1184	0.9996	
1,2,4,5-tetraCB	[214]	22238	760	1.0000	0.002
	[216]	28801	-77	0.9999	
1,2,3,4-tetraCB	[214]	21501	-5666	0.9993	0.002
	[216]	24304	-883	0.9998	
pentaCB	[248]	19121	-5962	0.9991	0.003
	[250]	21675	342	0.9997	
hexaCB	[284]	12088	-316	0.9997	0.004
	[286]	9491	97	0.9993	

\* R<sup>2</sup>, correlation coefficient.

## Results and Discussion

### GC-MS analysis

In our procedure, a 60-m capillary column coated with a 1.8-µm film of medium polarity Rtx-624 (6% cyanopropyl-phenyl-94% dimethyl polysiloxane) stationary phase was used. As shown in Figure 1, good separation of the analyzed chlorobenzenes was achieved. Under the conditions described, the quantitative analysis of 1,2,3,5-tetraCB and 1,2,4,5-tetraCB was possible. This previously could not be attained with a DB-5 (5% diphenyl-95% dimethyl polysiloxane) column (25 m × 0.32 mm, d<sub>f</sub> = 0.25 µm) in routine use.

A low-resolution mass spectrometer in SIM mode was used for detection, and each chlorobenzene was examined for the presence of two chosen ions (Table I).

### Calibration curve and limit of detection

Calibration curves were constructed for five different concentrations ranging from 0.006 to 40 ng/µL. The measurements for each data point were taken at least in triplicate.

Within the concentration range investigated the relationship between a detector signal (peak area) and the concentrations of the particular substances was linear and characterized by a high value of the correlation coefficient (Table II). The LOD was calculated as three times the standard deviation for the lowest concentration of each chlorobenzene (Table II).

### Sample preparation for the GC analysis

In the procedure presented, sample drying with a well-known drying agent (Na<sub>2</sub>SO<sub>4</sub>) was proposed. Before adding Na<sub>2</sub>SO<sub>4</sub> to the sample, the still-moist sediment was spiked with deuterated 1,2-diCB, which served as the recovery standard. Next, the sample was subjected to an overnight extraction in a 5-mL mixture (4:1) of pentane and acetone on a shaker. In general, the high concentrations of sulfur in the sediment samples were removed by using copper powder during the fractionation of the extract on activated silica-gel columns.

The cleaned-up extract was evaporated in a very slow stream of clean nitrogen gas until it reached a volume of 0.3 mL. Before injecting the extract onto a chromatographic column, a known volume of bromofluorobenzene was added for GC-MS quantitation.

### Recovery and precision

The recovery of the proposed procedure, expressed as a percentage of the true value for samples spiked with a known amount of a standard mixture, is shown in Figure 2. The lowest was for MCB reaching only 58%, and for the remaining compounds it was above 78%. This fact has been reflected in the formula for calculating the MCB content in a sediment by using a correction factor of 1.72. For concentrations ranging from 15 to 40 µg/kg, the precision of the proposed method (expressed as the relative standard deviation) did not exceed 20%, and it reached a value of only approximately 25% for MCB.

### The verification of the method by using reference material

The presented procedure of sediment sample preparation was

subjected to verification during the international interlaboratory exercise organized by the International Odra Project (Polish–Germany Project concerning transboundary Odra River pollution). Reference material SRM EC-2 from the National Institute of Standards and Technology was used in the trial.

Figure 3 shows a comparison between the reference values and the mean value obtained with the proposed method from at least triplicate measurements. The MCB content of the reference material was not given.

The spread of measurements (expressed as relative standard deviation) was dependent on the concentration level of the analytes. For a concentration level of a few micrograms per kilogram it reached 30%; for a concentration of several micrograms per kilogram it amounted to 15%, and for 20 µg/kg it was a few percent. Such values of measurement precision are generally accepted in the analysis of trace organic pollutants.

## Conclusion

The proposed method for the determination of chlorobenzenes in sediment allows the identification of all chlorobenzenes during one analytical cycle. The use of a 60-m capillary column coated with a 1.8-µm film of a medium-polar Rtx-624 stationary phase resulted in a good separation of the analyzed chlorobenzenes and, in particular, of the resolution of 1,2,3,5-tetraCB from 1,2,4,5-tetraCB. In the analysis, a sample of 1 g of sediment was used, which resulted in a much lower volume of solvents than in the case of other methods. The precision of the method, while dependent on analyte concentration, is comparable with the precision of methods generally used in trace organic analysis.

Verification of the method for the determination of chlorobenzenes in sediment with the use of reference material has proven the method to be accurate.

## References

1. H.M. Curran. Use of organic working fluids in Rankine engines. *J. Energy* **5**: 218–23 (1981).
2. J.L. Boy-Marcotte. 100–1000 kW (el) medium-power distributed-collector solar system. *Electr. Power Syst. Res.* **3**: 41–51 (1980).
3. B.G. Oliver and K.D. Bothen. Extraction and clean-up procedures for measuring chlorobenzenes in sediments and fish by capillary gas chromatography. *Int. J. Environ. Anal. Chem.* **12**: 131–39 (1982).
4. C.-L. Lee and M.-D. Fang. Sources and distribution of chlorobenzenes and hexachlorobutadiene in surficial sediments along the coast of southwestern Taiwan. *Chemosphere* **35**: 2039 (1997).
5. B.G. Oliver and K.D. Nicol. Chlorobenzenes in sediments, water, and selected fish from lakes Superior, Huron, Erie, and Ontario. *Environ. Sci. Technol.* **16**: 532–36 (1982).
6. V. Lopez-Avila, R. Northcutt, J. Onstot, M. Wickham, and S. Billets. Determination of 51 priority organic compounds after extraction from standard reference materials. *Anal. Chem.* **55**: 881–89 (1983).
7. F.I. Onuska and K.A. Terry. Determination of chlorinated benzenes in bottom sediment samples by WCOT column gas chromatography. *Anal. Chem.* **57**: 801–805 (1985).
8. J. Namieśnik, B. Zygmunt, M. Wiergowski, and L. Torres. Organic pollutants of soils and sediment. Methods of sample preparation and isolation–preconcentration prior to their final determination. *Pol. J. Environ. Stud.* **5**: 5–25 (1996).
9. J. Namieśnik. Trends in environmental analytics and monitoring. *Crit. Rev. Anal. Chem.* **30**: 221–69 (2000).
10. J. Namieśnik and T. Górecki. Preparation of environmental samples for the determination of trace constituents. *Pol. J. Environ. Stud.* **10**: 77–84 (2001).
11. J. Namieśnik and B. Zygmunt. Role of reference materials in analysis of environmental pollutants. *Sci. Total Environ.* **228**: 243–57 (1999).
12. C.-L. Lee, H.-J. Song, and M.-D. Fang. Concentration of chlorobenzenes, hexachlorobutadiene and heavy metals in surficial sediments of Kaohsiung coast, Taiwan. *Chemosphere* **41**: 889 (2000).
13. M.E. Meek and M.J. Giddings. "International Programme on Chemical Safety, Environmental Health criteria 128, Chlorobenzenes other than hexachlorobenzene". <http://www.ccohs.ca>.

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