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Comparison of Soxhlet, Shaking, and Microwave Assisted Extraction Techniques for Determination of PCB Congeners in a Marine Sediment

A. M. Cicero,[1] E. Pietrantonio,[1] G. Romanelli,[1] A. Di Muccio[2]

[1] ICRAM—Central Institute for Scientific and Technological Research Applied to the Sea, Via de Casalotti 300, Rome, Italy
[2] ISS—National Institute of Health, Viale Regina Elena 299, Rome, Italy

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Polychlorinated biphenyls (PCBs) are a mixture of aromatic chemicals that have been used commercially since 1930 as dielectric and heat-exchange fluids. They represent a general class of compounds that have become ubiquitous contaminants of global ecosystems during the twentieth century. Numerous chemicals are accumulated by sediments, that are a repository for many toxic organic chemicals released in aquatic systems, especially those which have a high lipophilicity and are slowly degradable by biotic or abiotic processes within the sediments. PCBs largely satisfy these criteria: they have a low water solubility; a high n-octanol/water partition coefficient; high persistence. In view of these characteristics, sediments are environmentally important for their accumulation in marine ecosystems (Mangani et al, 1991; Kelly et al.,1994).
Because the high affinity of PCBs to the organic content of the sediments, the extraction of these contaminants represents a critical aspect for determination of PCBs trace in marine sediments.

Many studies are reported about PCBs extraction procedures (Sherma, 1995); if the Soxhlet extraction represents the classical methodology for lipophilic compounds, recently some methodologies using microwave-assisted extraction were developed (Lopez-Avila et al., 1994; Lopez-Avila et al., 1995a and 1995b; Shu et al., 1997; Chiu et al., 1997; Pastor et al., 1997).
A procedure for extraction of PCBs in marine sediments by shaking with solvent was also used (Mecozzi et al, 1995; *Cicero et al.* in press).
In this paper a comparison of the performance of different extractive techniques of PCB congeners in marine sediment, like Soxhlet, shaking extraction or microwave extraction was conducted (Cicero et al,1999), using a sediment spiked with PCBs of environmental concern (IUPAC Nos. 28, 52, 101, 118, 137, 153, 180) (Duinker et al., 1988) and PCB 209.

MATERIALS AND METHODS

All solvents were for organic trace analysis, purchased from Merck, Darmstadt, Germany. Distilled water was extracted with dichloromethane (500 ml with 3 x 20 ml). Anhydrous sodium sulphate was heated at 600°C for 6 hrs. Analytical Reference Standards of PCBs were purchased from Ultra Scientific, and were

Correspondence to: A. M. Cicero

99% pure. Standard Reference Material HS1, a harbour marine sediment from Nova Scotia, was purchased from the National Research Council of Canada (N.R.C.), Halifax, N. S.

The extraction procedures were compared by mean of a home-made spiked sediment. The spiked sediment was prepared as follows: 80 g of inter-tidal sediment, sampled in a "blank area" during an oceanographic cruise and free from the analysed PCB congeners, was lyophilised and well homogenised to obtain an uniform size composition. The dried sediment was kept in contact with a spiking standard solution of PCBs congeners in iso-octane (IUPAC Nos. 28, 52, 101, 118, 137, 153, 180, 209). After 72 hours, the solvent was completely evaporated by rotary evaporator (bath temperature=40°C; reduced pressure); the obtained matrix was again homogenised in a mortar. The obtained concentration of PCBs in sediment is reported in table 1. At regular intervals, during 24 months from the treatment, different portions of the spiked sediment were analysed by GC/ECD to check the stability of the congeners.

The spiked sediment was extracted according to the following procedures.

The extraction by shaking with solvent was carried out on 1-3 g of dried spiked sediment, which was shaken for 2 minutes with 100 ml of an acetone/n-hexane (1:1 vol.) solution. 20 ml of water extracted with CH_2Cl_2 and 3 g of Na_2SO_4 were then added and the resulting mixture was shaken for 50 minutes. The organic phase was separated, removed and filtered through an anhydrous sodium sulphate column and collected into an Erlenmeyer flask; sediment was re-extracted with n-hexane (2 x 50 ml) and the organic phases collected. The combined fractions were carefully concentrated to dryness by rotary evaporator; the residue, dissolved in 10 ml of n-hexane, was purified according to the procedures described in the following paragraphs.

Microwave Assisted Extraction was carried out on 0,5 -1 g of dried spiked sediment placed in a quartz bottle. 20 ml of an acetone/n-hexane (1:1 vol.) solution were added and the bottle was placed in a PTFE vessel. 10 ml of n-hexane were placed in the hollow space to guarantee thermal continuity. The system, hermetically closed, was then exposed to microwaves (Milestone 1200 Mega High Performance Microwave Digestion Unit; maximum power: 1200 Watt) following the program: 10 min $\rightarrow$ 250 W; 10 min $\rightarrow$ 500 W; 10 min$\rightarrow$ 250 W; 10 min $\rightarrow$ 0 W. The organic extract and the n-hexane used to wash extracted sediment were dried on an anhydrous Na_2SO_4 column and carefully concentrated to dryness by rotary evaporator. The residue, dissolved in 10 ml of n-hexane, was purified according to the procedures described in the following paragraphs.

The Soxhlet Extraction was carried out on 3-5 g of dried spiked sediment contained in a cleaned glass-fiber thimble that was extracted with n-hexane (300 ml) for 24 hours. The thimbles were cleaned by extracting them prior to the use in Soxhlet with n-hexane/acetone (1:1 vol.), and then with n-hexane. Then hexane

was renewed until the new addition was free from interfering peaks. The extract was carefully concentrated to dryness by rotary evaporator; the residue, dissolved in 10 ml of n-hexane, was purified according to the procedures described in the following paragraphs.

In order to remove sulphur, present in extracts of anaerobic sediments and having electrocaptive properties, 10 ml solution deriving from extraction was added with 5 ml of isopropyl alcohol and with 10 ml of a tetrabutylammonium hydrogen sulphate solution (TBA), obtained mixing 3.39 g of TBA with 100 ml of extracted water, and saturated with 25 g of sodium sulphite (Na_2SO_3). Solution was shaken for 1 minute and then added with 50 ml of extracted water and shaken for 3 minutes. The organic phase was collected and concentrated by rotary evaporator. To evaluate if the quantitation of the analytes is affected by the clean up treatment, two different procedures were followed: (a) sample was cleaned up according to the procedure described in the following paragraph and then analysed by GC/ECD or (b) sample was analysed by GC/ECD after the only TBA treatment for the removal of sulphur. Amounts of sulphur in the order of 22 mg/kg were determined, before the removal, by GC/ECD with reference to an external standard.

After the removal of sulphur, the raw extracts were cleaned up as follows. The residues deriving from sulphur removal treatment were dissolved in 1 x 3 ml of n-hexane and transferred onto the top of a column (1 cm id) prepared by packing 2.5 g of Florisil (60-100 mesh) activated at 130°C overnight, covered by 1 cm layer of sodium sulphate. The column was washed with 15 ml of n-hexane, that was discarded. Then the column was eluted with 60 ml of n-hexane. The fraction recovered was carefully concentrated to dryness in a rotary evaporator (bath temperature=40°C, reduced pressure), dissolved with 1 ml of isooctane and then analysed by GC/ECD.

The GC/ECD analysis were performed on a HP 5890 GC/ECD, using an HP Ultra 2 column (5% Diphenyl, 95% dimetilsiloxan, 25 m length, 0.32 mm i.d.).
Samples were injected by an automatic sampler HP 7673A.
GC operating conditions were: Helium (carrier gas), flow rate: 1.5 ml/min. Oven temperature: 80°C for 2 min, to 100°C at 10°C/min; then to 280°C at 3°C/min. Final temperature: 280°C held for 15 min. Injector: split/splitless, in splitless mode; purge-off time 1 min; temperature: 230°C. Detector temperature: 300°C.

The PCB congeners were identified by comparison of absolute and relative retention times to Aldrin.
Quantitation was performed with the external standard technique by comparison of peak areas in the sample with those obtained by injecting a standard mixture whose areas are close to the areas in the sample within $\pm$ 20%.

In order to apply a Quality Control measure procedural blanks were run to verify the absence of interfering peaks.

Furthermore, microwave, shaking and Soxhlet extraction procedures were tested with the reference material HS 1 (NRC, Certified Material Program) certified with Soxhlet extraction. The results, expressed as μg/kg d.w., are shown in the table 1, where recoveries of the single PCB congeners (101, 153, 180, 209) certified in HS1 reference material are reported.

TABLE 1. Results of recoveries of HS1 with the extraction procedures

Congeners N°	HS1 x_m (S.D.)	Shaking Extraction x_m (S.D.) (n=4)	Microwave Extraction x_m (S.D.) (n=4)	Soxhlet Extraction x_m (Δ/2) (n=2)
101	1.62 (0.21)	1.28 (0.21)	1.11 (0.23)	1.5 (0.21)
153	2.27 (0.28)	3.34 (0.46)	2.85 (0.61)	2.03 (0.25)
180	1.17 (0.15)	1.81 (0.42)	1.59 (0.37)	0.97 (0.23)
209	0.33 (0.10)	0.54 (0.21)	0.27 (0.02)	0.54 (0.10)

RESULTS AND DISCUSSION

Results obtained, by analysing the spiked sediment at 24 months after preparation with the three procedures, are reported in table 2.

TABLE 2. Results of the determination of eight PCB congeners spiked in a marine sediment analysed after 24 months from the preparation.

		MICROWAVE EXTRACTION			
PCB	Spiked Conc. (ug/kg)	TBA Treatment* x (S.D.) n=6		Complete clean up* x (S.D.) n=6	
		μg/Kg	%	μg/Kg	%
28	12.0	n.d.**	n.d.**	n.d.**	n.d.**
52	9.0	n.d.**	n.d.**	8.7 (2.8)	94.4 (28.1)
101	8.0	7.4 (0.3)	93.0 (3.2)	6.5 (0.6)	81.3 (7.6)
118	9.0	9.8 (0.5)	108.5 (5.5)	7.3 (0.8)	81.3 (8.8)
153	15.0	14.3 (0.5)	95.1 (3.5)	12.3 (0.9)	81.9 (6.3)
137	11.0	12.2 (0.5)	111.1 (4.1)	9.0 (0.6)	81.4 (5.9)
180	13.0	12.9 (0.7)	99.4 (5.2)	10.4 (1.0)	80.1 (7.4)
209	9.0	10.2 (1.3)	113.4(14.7)	8.0 (1.4)	88.7 (15.8)

		SOXHLET EXTRACTION		**SHAKING EXTRACTION**			
PCB	Spiked Conc.	Complete clean up X (Δ/2) n=2		TBA Treatment* X (S.D.) n=6		Complete clean up* X (S.D.) n=6	
	(ug/kg)	μg/Kg	%	μg/Kg	%	μg/Kg	%
28	12.0	13.1	109.5	n.d.**	n.d.**	12.2(1.0)	101.5(8.3)
52	9.0	7.4	82.3	8.2(1.0)	91.3(10.7)	6.6(0.6)	73.1(6.9)
101	8.0	6.2 (0.7)	77.8 (8.6)	7.7(1.0)	96.3(12.4)	6.7(0.7)	84.3(8.1)
118	9.0	7.7 (0.8)	85.4 (8.6)	10.7(1.7)	118.3(18.5)	8.5(1.2)	93.9(13.8)
153	15.0	12.4 (1.5)	82.9 (9.6)	14.8(1.8)	98.0(11.6)	13.4(1.3)	89.3(8.5)
137	11.0	9.8 (1.2)	88.6 (10.5)	12.3(1.1)	111.9(10.1)	9.2(1.4)	83.4(12.7)
180	13.0	11.3 (1.5)	86.4 (11.3)	13.2(1.9)	109.1(15.2)	11.4(2.1)	87.8(15.9)
209	9.0	9.0 (0.8)	89.0 (7.4)	11.0(2.7)	110.7(18.8)	9.2(0.7)	100.8(13.5)

*Values as average of 6 measures.
**n.d.= not detectable
$\Delta/2 = (X_{max} - X_{min})/2$

Efficiency of different extractive techniques was calculated considering results derived either after sulphur removal (referred to «TBA Treatment») or after complete clean up procedure (including TBA + Florisil).
Results of Soxhlet extraction without Florisil clean up cannot be calculated because of coextractive interference.

Microwave Extraction recoveries following TBA treatment were in the range 93-113% with S.D. in the range 3-15%, yet the high S.D. value of 14.7 is due only to CB209. After the Florisil clean up procedure the recoveries of analytes were somewhat lower (10-30 %); the higher S.D. values were due to CB52 (S.D.=28) and CB209 (S.D.=15.8). Under the analytical conditions adopted CB28 cannot be calculated because of the presence of a large band due to co-extractives while the CB52 is detectable only after complete clean up. Consequently, although results after Florisil clean-up are somewhat lower, it is considered an important step for the identification of PCBs congeners.

In the analytical conditions adopted for Shaking Extraction the recoveries after TBA treatment were in the range 91-118%; on the average, an higher standard deviation (about 10-18%) than microwaves was observed. Also in this case the results after Florisil treatment are somewhat lower (10-30%).
As observed in microwave extraction, CB28 cannot be calculated after the only TBA treatment, because of the presence of co-extractives; this congener can be measured only following TBA + Florisil clean up.

The Soxhlet extraction was used just to have results from a conventional method to which compare results obtained from the two other treatments. The Soxhlet procedure showed recovery values (78-109%) comparable to the results of the other two methods. Because of the limited number of trials conducted with Soxhlet, the accuracy for this procedure is reported in table 2 as the dispersion of average range.

In general, the recoveries obtained from the three procedures are similar; microwave extraction showed a better reproducibility of the measures compared to the other two methods.
Work is in progress to improve analytical conditions for detection of CB28 and CB52.
The advantages and the disadvantages of different procedures are summarised in table 3.

TABLE 3. Comparative valuation of tested extraction procedures.

Microwave Extraction

Advantages	*Disadvantages*
Good recoveries	Cost of the instrument
Small amount of solvent (~20 ml)	Need of expert operator
Small amount of sample (~0.5-1 g)	
Opportunity of extracting 6 sample simultaneously	
Reduced handling of the samples	
Shorter time of extraction (~40 min)	
Good accuracy	

Shaking Extraction

Advantages	*Disadvantages*
Good recoveries	Large amount of solvent (~200 ml)
Inexpensive system	More handling of the sample
Easy to use	Longer time of extraction (~2 hrs)

Soxhlet Extraction

Advantages	*Disadvantages*
Good recoveries	Large amount of solvent (~300 ml)
Traditional technique for certification of standard materials	Single extraction
Inexpensive system	Very long time of extraction (~24 hr)
Easy to handle	Conditioning of thimbles (~30 hrs)

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