

Full Length Research Paper

Determination of indicator polychlorinated biphenyls (pcbs) by gas chromatography-electron capture detector

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An effective method for determination of indicator polychlorinated biphenyls (PCBs) has been validated using gas chromatography (GC) equipped with electron capture detector (ECD). The GC – ECD method was validated by determining the linear range (working range), minimum detectable quantities (MDQ), precision and accuracy of the method for analysis of the compounds. MDQ obtained for the compounds ranges from 0.0005 – 0.002 ng. Indeed the GC method was found to be more detectable as the number of chlorine atoms attached to the biphenyl increases. The precision and accuracy of the GC method validated ranges from 2.4 -14.5 % and -7.0 – 14.6 % respectively. Coefficient of variation associated with the repeatability of the retention times and corresponding peak areas was found to be 0.0001- 0.0007 for the retention times and 0.0014 – 0.059 for the peak areas. Thus, the method is more repeatable for determination of retention times than for peak areas. Percentage recoveries for the compounds were in the range of 95.7 – 101.0 %. The validated method was then applied to determine levels of indicator PCBs in sediments sampled from eleven sampling points along the Bosuntwi Lake in Ghana and the highest PCB load of 19.17 ng/g was recorded at Pipie no.2. PCB 52 and PCB 101 were found to be the most ubiquitous indicator PCBs in the study area, both with 90.91 percentage occurrence.

Keywords: Polychlorinated biphenyls, Gas chromatography, Electron capture detector, Validation, Detectable, Precision, Accuracy

INTRODUCTION

A polychlorinated biphenyl (PCB) is an organochlorine with two to ten chlorine atoms attached to biphenyl (UNEP, 1999; Mahan, 1998). The Stockholm convention on Persistent Organic Pollutant also defines PCBs as a group of aromatic compounds formed in a manner that the hydrogen atoms on the biphenyl molecule (two benzene rings bonded together by a single carbon–carbon bond) may be replaced by up to 10 chlorine atoms (UNEP, 2001). PCBs are amongst the group of

chemicals that are classified as persistent organic pollutants (POPs) because they are known to persist in the environment and bioaccumulate in living systems. Indicator PCBs are PCBs that are known to be persistent in the environment and bioaccumulate in the food chain. They are therefore assumed to be a suitable representative for all PCBs. They are the predominant congeners in biotic and abiotic matrices. Mixtures of PCBs are generally assessed on the basis of a chemical analysis of the sum of the seven indicator PCBs. The seven indicator PCBs are PCB #28, 52, 101, 118, 138, 153, and 180. Polychlorinated biphenyls have been produced commercially for some five decades starting about 1920, by direct chlorination of biphenyl (Bakker et al., 2003). This chlorination occurs with one to ten

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chlorine atoms, resulting in 209 possible PCB congeners.

PCB congeners are odourless, clear to pale-yellow, viscous liquid. PCBs are practically insoluble in water but are soluble in most organic solvents, hydrophobic media such as fatty or oily substances. They have high dielectric constants, high thermal conductivity and are chemically inert. Indeed, they are extremely resistant to oxidation, reduction, addition, elimination and electrophilic substitution reactions (Boate et al., 2004). Commercially, they are attractive because they are chemically inert liquids and are difficult to burn. Because of these properties, they were used extensively as dielectric and coolant fluids in power transformers, capacitors and electric motors. Indeed in Ghana PCBs had been used as dielectric fluid in transformers and capacitors by Electricity Company of Ghana and Volta River Authority (Buah-kwofie et al, 2011). Many congeners of PCBs are formed and released to the environment during various anthropogenic processes such as incineration, combustion, smelting and metal reclamation (Falandysz, 1998; Bullscmhiter, 1987).

Due to PCBs' toxicity and classification as a persistent organic pollutant, PCBs production has been banned by the Stockholm Convention on Persistent Organic Pollutants in 2001 (UNEP,1999). According to the U.S. Environmental Protection Agency (EPA), PCBs have shown to cause cancer in animals, and there is also evidence that they can cause cancer in humans (US EPA, 1998). A number of peer-reviewed health studies have also shown a causal link between exposure to PCBs and non-Hodgkin Lymphoma, a frequently fatal form of cancer (Epidemiology, 2010). The most commonly health effect observed in people exposed to extremely high levels of PCBs are skin conditions, such as chloracne and rashes but these were known to be symptoms of acute systemic poisoning. Studies in workers exposed to PCBs have shown changes in blood and urine that may indicate damage. In Japan in 1968, 280 kg of PCB-contaminated rice bran oil was used as chicken feed, resulting in a mass poisoning, known as Yushō disease, in over 14,000 peoples (Bakker et. al, 2003). Studies have also shown that transformer oils containing PCBs are known to be associated with a number of health related problems such as cancer (Giwercan and Skakkebaek, 1992; Davis et al., 1993; Barron et al., 1994; Hoyer et al., 1998; Laden et al., 2001; Cocco, 2002; Charlier et al., 2003); neurological disorder (National academies press, 1999), immune system failure (US EPA, 2008), reproductive failure (Oliva et al., 2001; Andric et al., 2005; US EPA, 2008) and the endocrine system as endocrine disruptors (US EPA, 2008).

As the result of PCBs toxicity, there is an international intervention under the Stockholm Convention aimed at total elimination of PCBs and other compounds by 2025 (Buah-Kwofie et al., 2011). State parties to the convention therefore have to develop a strategy of

identifying, determining and eliminating persistent organochlorine compounds including PCBs from their environment. It is against this background that GC - ECD methodology has been validated for determination of indicator PCBs as it is generally known that electron capture detectors (ECDs) have a limited linear calibration range (Booij et al., 1998) for .determination of target compounds. The validated method has been applied for determination of indicator PCBs in sediment sampled from the Bosumtwi Lake in Ghana. The method was applied to measure indicator PCBs in Bosumtwi Lake because of the Lake critical national and international importance.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used for the investigation were of high purity and they were analytical grade. Hexane (96+%), acetone (99 %), ethyl acetate (99.8%), anhydrous sulphate were purchased from Sigma-Aldrich, Germany. Florisil adsorbent was purchased from Hopkin and William Limited, England. The PCBs standards were supplied by United Nation Environmental Program (UNEP)

Gas Chromatograph

The gas chromatograph was Varian GC model CP-3800 equipped with ⁶³Ni Electron Capture Detector of activity 15 mCi with an auto sampler. Chromatographic conditions for GC resolution are described as follows

Oven program:

70°C (2min) 25°C/min to 180°C (1min) to 300°C 5°C/min.

Injector settings:

Mode: Pulsed splitless

Temperature: 270°C

Purge flow: 55.4 ml/min

Purge time: 1.4 min

Column:

Capillary column VF-5ms, 30 m x 0.25 mm x 0.25 µm film thickness

Detector setting:

EDC detector at 300°C of activity 15 mCi

Carrier gas (mobile phase): Nitrogen at 1.0ml/min, Make up gas: Nitrogen at 29ml/min.

Validation of GC-ECD method

Linear range determination

Linear range for the determination of the indicator PCBs

were obtained by analysis various standards solution of the PCBs in the concentration range of 0.01 – 0.30 µg/ml. PCB 18 was the internal standard and calibration curves for PCB made by plotting the peak area ratio (peak area of a particular PCB / peak area of PCB 18) against the corresponding concentration of the particular PCB

Minimum detectable quantity (MDQ)

Minimum detectable quantity considered as the smallest quantity of the standard material resulting in definite peak three times the baseline peak was obtained by analyzing various amount of PCB standard in a range of 0.1 – 0.001 ng. The amount of PCB whose chromatogram was equal to three times the baseline peak was considered as the MDQ.

Precision and accuracy of the GC methodology

The precision and accuracy of the GC method was determined by analyzing 5 g soil sample spiked with 1 ml of 50ng/ml and 150 ng/ml of PCBs standard solution to generate PCB-sediment concentration of 10.0 and 30.0 ng/g respectively. Five independent determinations were made for each spiking level.

Application of the validated GC method

The GC- ECD method validated was tested by analysis sediment samples for levels of the indicator PCBs collected from the Bosumtwi Lake in Ghana.

Sampling and sample preparation

Sediments samples were collected from eleven communities dotted around the lake. The sampling points stretches from Ankaase to Essase. At each point three sediment samples were taken. Samples were collected at various points using Eckman grab. Sediments were scoop to about a depth of 20 cm. The samples were then wrapped in aluminium foil and bagged in polyethylene bags. All samples were stored in an ice-chest container and transported to the laboratory. In the laboratory the samples were dried at room temperature and they were then milled with pestle and mortar and sieved with 500 µm mesh size sieve to remove stones and other debris. The sieved samples were then wrapped in aluminium foil and kept at room temperature in a clean cupboard.

Extraction of indicator PCBs from sediment

Two gram of the sample were accurately weighed and

sonicated in an ultrasonic bath (Branson 220, Branson Ultrasonic Cleaner, USA) for 3 hours at 40 °C with 50 ml of hexane/acetone mixture (3:1). PCB 18 was added as internal standard. The sonicated method was earlier optimized by varying the time of sonication. After sonication the extract was concentrated to about 3 ml and subjected to clean up using 2.0 g florisil adsorbent with 1.5 g anhydrous sodium sulphate packed on top of the florisil (Nyarko et al, 2011). PCBs were eluted with 8 ml of hexane. The eluted extract was evaporated to almost dryness on rotary evaporator and residue picked up in 1.5 ml ethyl acetate for GC analysis.

Method evaluation

Method was evaluated by determining the repeatability of retention times and the corresponding peak areas of the compounds as well as recovery of the compounds from fortified sediment samples.

Repeatability of retention time and peak area

Repeatability of retention times and corresponding peak areas for GC – ECD determination of the compounds was determined by analyzing a mixed standard of indicator PCBs at concentration level of 0.01µg/ml. PCB 18 was used as internal standard. Five replicate determinations were made and 1µl volume of the standard was injected for each replicate analysis

Recovery studies

Five gram of untreated sediment samples were fortified with 1 ml of 50ng/ml of mixed standard of the PCBs to generate concentration of 10 ng/g. After fortification samples were kept for 24 hours and subjected to extraction as described per section 2.4.2. Recovery was found to be between 94 – 102 %

RESULTS AND DISCUSSION

Table 1 shows the data obtained for the linear range determination of the indicator PCBs. The data shows the linear range concentration, the regression equation and the coefficient of correlation (R^2) between the concentrations and the peak areas. The coefficient of correlation ranged from 0.981 – 0.999, indicating good correlation between concentrations and peak areas in the linear or working range. In all, the linear range concentrations were between 0.01 – 0.25µg/ml. The differences in the linear range for the indicator PCBs

Table 1. Data on linear range determination of the indicator PCBs.

Compound	Linear range concentration($\mu\text{g/ml}$)	Regression equation	Coefficient of correlation (R^2)
PCB 18	0.01 - 0.15	$Y = 73936x + 17444$	0.999
PCB 28	0.01 - 0.25	$Y = 10.87X + 1.136$	0.999
PCB 52	0.01 - 0.10	$Y = 5.150X + 1.041$	0.998
PCB 101	0.01 - 0.10	$Y = 9.505X + 1.114$	0.981
PCB 153	0.01 - 0.20	$Y = 19.99X + 2.310$	0.997
PCB 138	0.01 - 0.20	$Y = 13.19X + 2.320$	0.985
PCB 180	0.01 - 0.15	$Y = 36.42X + 2.429$	0.998

Table 2. Minimum detectable quantities for the indicator PCBs measured with Varian GC-ECD on capillary column VF-5ms, 30 m x 0.25 mm x 0.25 μm film thickness; injector, 270 $^{\circ}\text{C}$; detector, 300 $^{\circ}\text{C}$.

Compounds	Amount(ng)
PCB 18	0.002
PCB28	0.002
PCB 52	0.002
PCB 101	0.001
PCB 153	0.001
PCB 138	0.001
PCB 180	0.0005

Table 3. Analysis of sediment spiked with mixed standard of PCBs.

Compound	Level of spiking (ng/g)	Mean (ng/g)	CV	Precision (%)	Accuracy (%)
PCB 18	10.0	10.6 \pm 1.33	0.25	11.3	-6.0
	30.0	28.4 \pm 2.86	0.14	9.4	5.3
PCB 28	10.0	10.5 \pm 0.80	0.10	8.0	-5.0
	30.0	25.6 \pm 3.45	0.17	13.9	14.6
PCB 52	10.0	9.6 \pm 0.42	0.07	4.3	4.0
	30.0	29.3 \pm 4.17	0.17	14.5	2.3
PCB 101	10.0	9.8 \pm 0.49	0.07	5.1	2.0
	30.0	30.8 \pm 1.84	0.07	5.3	-2.6
PCB 138	10.0	10.8 \pm 1.02	0.11	7.8	-2.0
	30.0	27.6 \pm 1.59	0.08	5.9	8.0
PCB 153	10.0	10.4 \pm 0.84	0.12	10.0	-6.0
	30.0	29.6 \pm 0.50	0.06	2.4	1.3
PCB 180	10.0	10.3 \pm 0.81	0.09	7.7	-7.0
	30.0	28.6 \pm 2.52	0.10	8.7	4.7

shows the differences in the ECD detector response factors to the individual PCBs.

The results of the minimum detectable quantity (MDQ) considered as the smallest quantity of the standard material resulting in a definite peak or chromatogram three times the baseline peak (Afful et al., 2010) are presented in Table 2. The results suggest that the

detector showed higher detectability as the number of chlorine atoms bonded to the biphenyl increases. For example the detector could response to smaller change in PCB 180 with seven chlorine atoms compared to PCB 153 and 138, both with 6 chlorine atoms.

Table 3 shows the results of precision and accuracy of the method for the determination of the PCBs. For five

Table 4. Levels (ng/g) of indicator PCBs in sediments from Lake Bosumtwi.

Compounds	Esase	Ayina.	Abaase	Aboro.	Obo	Nkawi	Pipie 2	Bode.	Obonu	Adwafo	Ankase
PCB 28	nd	nd	nd	0.55±0.22	nd	0.91±0.31	5.90±1.05	3.53±0.90	5.25±0.74	nd	nd
PCB 52	5.79±1.23	5.51±1.34	4.87±1.53	5.45±0.15	5.43±0.96	nd	4.85±1.20	4.26±1.12	5.24±1.92	5.74±1.81	3.88±0.60
PCB 101	0.78±0.61	1.14±0.55	1.060±0.09	1.37±0.04	0.70±0.08	1.10±0.22	0.39±0.07	0.16±0.02	0.33±0.08	nd	1.43±0.04
PCB 153	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PCB 138	nd	nd	nd	nd	nd	nd	0.95±0.10	2.30±0.10	3.90±0.63	nd	nd
PCB 180	nd	nd	nd	5.03±0.12	nd	2.07±0.43	7.08±1.23	7.55±1.33	nd	nd	5.65±1.06

nd = not detected, Ayina = Anyinatiase, Aboro = Aborodwom, Bode = Old Bodekwamo

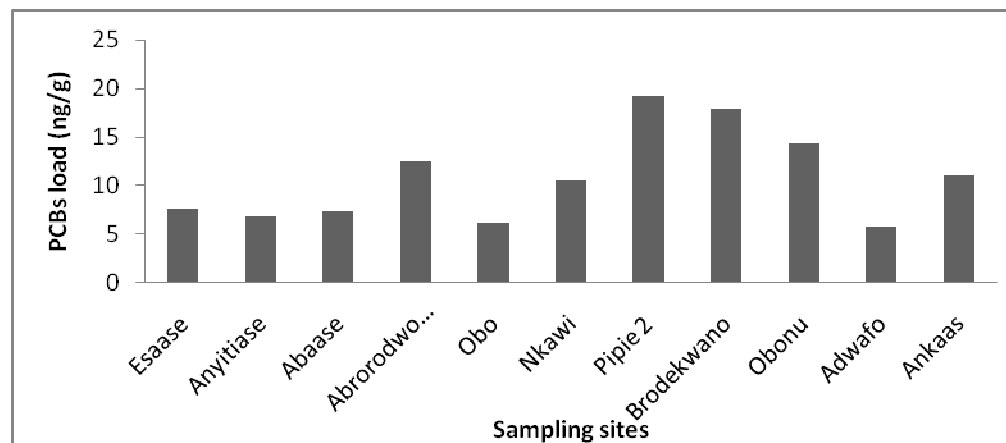


Figure 1. Total PCBs load (sum of all the PCBs measured) at the various sampling sites along Lake Bosumtwi.

independent determinations at spiking levels of 10.0 and 30.0 ng/g, coefficient of variation ranged from 0.07 – 0.25. Margin of errors associated with the mean of the measured PCBs are mean deviations based on the five replicate determination of each compound.

The levels of indicator PCBs recorded when

the validated method was applied to determine the compounds in the sediment samples from eleven sampling points along the Bosumtwi Lake are presented in Table 4, while Figure 1 also shows total PCB load (sum of the indicator PCBs) obtained at each sampling point. From the results PCB 52 and PCB 101 are the most ubiquitous

indicator PCBs in the study area both with more than 90 percent occurrence (Table 4). Indeed the two PCB congeners were detected in all the sampling locations except one. The highest PCB load (sum of the entire indicator PCBs) of 19.17 ng/g was recorded at Pipie no. 2 as shown in figure 1. The lowest PCB load of 6.13 ng/g was

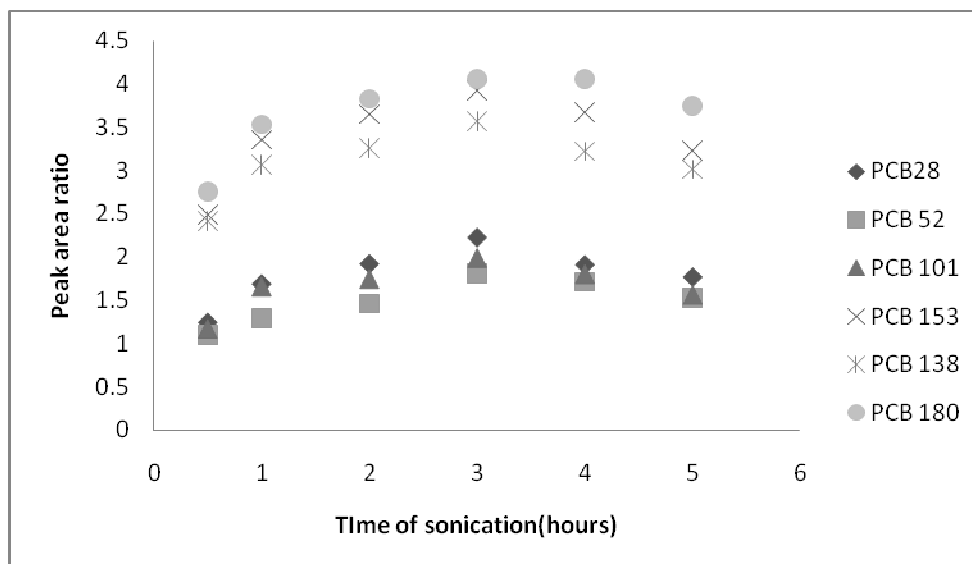


Figure 2. Sonication extraction yields of the PCBs with time in US Branson 220 ultrasonic bath at 40°C.

Table 5. Retention times, relative retention times based on PCB18 and peak areas for the PCBs determined with Varian GC – ECD, oven program, 70°C (2min) 25°C/min to 180°C (1min) to 300°C 5°C/min; column, capillary column VF-5ms, 30 m x 0.25 mm x 0.25 µm film thickness; injector, 270°C; detector, 300°C.

Compound	Average RT	RRT(PCB 18)	CV	Average PA	CV
PCB 18	20.804±0.002	1.000	0.0001	25444±1523	0.059
PCB28	21.810±0.015	1.050	0.0007	30796±444.7	0.014
PCB 52	22.448±0.011	1.079	0.0005	28318±1094	0.038
PCB 101	24.106±0.003	1.158	0.0001	30080±542.8	0.018
PCB 153	25.664±0.005	1.234	0.0002	62473±1923	0.031
PCB 138	26.173±0.005	1.258	0.0002	61174±1512	0.025
PCB 180	27.388±0.005	1.316	0.0002	68575±1237	0.018

RT= retention time, RRT = relative retention time, PA = peak area

was also recorded at Obo.

In the determination of the optimum sonication time, the sonication method applied for extraction of the PCBs was optimized by varying the period of sonication from 0.5 to 5 hours. Figure 2 shows the results of the optimization procedure. The results indicate that higher yield was obtained for the PCBs in 3 hours of sonication at 40°C. After 3 hours of sonication, yield begins to decline as shown in figure 2.

Results of repeatability of retention times and corresponding peak areas for GC analysis of the indicator PCBs are presented in Table 5. Margin of errors are standard deviation based on replicate measurement of the retention times and peak areas. Table 5 also shows the coefficient of variation (CV) associated with the measurement of retention times and the peak areas. Coefficient of variation for measurement of retention

times and peak areas ranges from 0.001 – 0.007 and 0.018 – 0.059 respectively. From the coefficient of variation it can be inferred that it is more repeatable in determining retention times compared to peak areas. The relative retention times indicated in Table 5 were calculated based on the internal standard (PCB 18) as the reference compound.

CONCLUSIONS

The gas chromatography – electron capture detector method validated for determination of indicator PCBs is simple, fast and effective and it is recommended for routine analysis of PCBs congeners and related compounds in sediments and similar environmental samples. Minimum detectable quantities for the com-

pounds determined with the method ranged from 0.0005 – 0.002 ng and that the method was found to be more detectable as the number of chlorine atoms attached to the biphenyl increases. PCB 52 and PCB 101 were found to be the most ubiquitous indicator PCBs in the study area, both with more than 90 percent occurrence. The highest PCB load (sum of the entire indicator PCBs) was 19.17 ng/g and was recorded at Pipie 2.

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