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The effect of adding a standard on the result of determination of polychlorinated biphenyls in bottom sediment samples

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ABSTRACT

Bottom sediments are a very important component of aquatic ecosystems. The sediment matrix is highly diverse and heterogeneous; in consequence, compounds entering the aquatic environment from different sources are considerably enriched at its surface. Bottom sediments are regarded as natural sorbents, since they accumulate many harmful substances, such as heavy metals and stable organic contaminants.

Extraction is a key stage in every analytical procedure. It is during this stage that standards are added to samples. Standards are necessary not only for estimating analyte yields but also for validating the whole procedure. The question of the addition of standard substances to sediment samples has not been widely addressed in the subject literature, and yet it is of fundamental importance as regards obtaining reliable results of determinations.

This paper describes the results of a study on the effect of standard addition techniques on the results of determination of polychlorinated biphenyls in sediment samples (certified reference material: METRANAL™2—river sediment).

1. Introduction

Bottom sediments are a very important component of aquatic ecosystems [1]. Highly diverse and heterogeneous, the sediment matrix consists of two main parts [2–6]: an inorganic part made up of clays, silts, muds and sand, and a part of organic origin. The organic part can be further subdivided into:

- an amorphous phase (soft, plastic), consisting primarily of organic matter of animal and vegetable origin in various stages of decomposition;
- a condensed phase (hard, glassy), dominated by carbon geosorbents, e.g. various forms of carbon, including coal and kerogen [7–9].

These phases contain polar (–OH, –COOH, $–NH_2$, $–OCH_3$, =NH), non-polar and spatial (aromatic rings) fragments [10]. This diversity of composition means that compounds entering the aquatic environment from various sources undergo considerable enrichment at the sediment surface. Because they accumulate numerous harmful substances, like heavy metals and stable organic contaminants, bottom sediments are regarded as natural sorbents [5]. Among the stable organic contaminants that have received a lot of attention in recent years are polychlorinated biphenyls (PCBs) [11].

PCBs are entirely anthropogenic and are carried into the environment primarily with wastewaters. In sediments they are determined at considerably lower concentration levels—in only a few cases do their concentrations exceed 100 μg/kg [12,13]. The determination of both PCBs in sediments is required by international, national and local regulations.

Extraction plays an important part in isolating analytes from the sample matrix [14]. In view of the broad diversity of available techniques, recommending one that is optimal for isolating PCBs from sediments is not easy. The recovery of these compounds from sediments is not very efficient (17–30%) [15]: such a low yield and the diversity of the sediment matrix means that only the internal standard technique can be recommended for determining PCBs. An important stage in this procedure is therefore the addition of the internal standard to the sediment matrix. Once added to the sediment, the internal standard should be bound to it in much the same way as analytes are bound to it. The problem of adding standards to sediment samples has not been widely addressed in the subject literature, and yet it is of fundamental significance as regards achieving reliable results.

A perusal of the subject literature reveals that the yield estimated by some authors is very high, of the order of 81–119% [16] and 71–114% [17] for PCBs. However, either these authors do not

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describe precisely the technique they used to add the standard to the sediment samples, or they added the standard in an inappropriate way—to the solvent after extraction [17,18] or directly to the dry sediment [19–22]. The fault with adding standards to the final extract prior to chromatographic analysis lies in the fact that this approach takes no account of the yield of analytes from the sediment or of their loss during the successive sample preparation stages. Again, adding standards directly to the dry sediment does not ensure their proper dispersal within the sample. Standards are added only at certain points in the sediment, which does not reproduce the natural character of the analyte's bonds with the sediment [23].

That is why it is so important to choose the right techniques of adding standards to a sample—one that ensures their proper dispersal and also the reproduction of the natural character of the bonding between analyte and sediment.

This paper describes the results of a study on the effect of standard addition techniques on the results of determination of PCBs in sediment samples (certified reference material: METRANAL^{TM}2—river sediment).

2. Experimental

2.1. Reagents and standards

The solvents used during the study were dichloromethane (99.9%), methanol (99.8%) and acetone (99.9%) from Merck (Germany) and pentane (99.8%) from POCH (Poland). Individual solutions of seven selected PCB congeners (IUPAC Nos. 28, 52, 101, 118, 153, 138 and 180) [24–28] were obtained from Restek Corporation (Bellefonte, USA) as 10 μg/ml solutions. The stock solution of PCBs was prepared by mixing solutions (100 μl each) of these compounds. Certified PCB 209 (200 μg/ml in isooctane) standards were obtained from Dr Ehrenstorfer GmbH (Germany). River sediment certified reference material (METRANAL^{TM}2, Analytica Ltd.) was purchased from LGC Standards Sp. z o.o. (Poland), with certified concentrations of 15 PCB congeners. Copper powder and silica gel were from J.T. Baker.

2.2. Gas chromatographic analysis

All experiments were performed using a gas chromatograph (TRACE GC), a mass spectrometric detector (TRACE MS) and an on-column injector maintained at 280 °C. The capillary column was a ZB-5MS unit (30 m; 0.25 mm i.d.; 0.25 μm film thickness; 5% phenyl + 95% dimethylpolysiloxane). The carrier gas (helium) was maintained at a constant pressure of 70 kPa. The GC oven temperature was programmed as follows: from 40 to 120 °C at a rate of 40 °C min^{-1}; then at a rate of 5 °C min^{-1} up to 280 °C, where it was held for 5 min. The MS was operated in electron ionization (EI) mode with the ion source temperature at 220 °C. The mass spectrometer was operated in selected ion monitoring mode; the following ions were monitored: (m/z) 256, 258, 290, 292, 324, 326, 358, 360, 392, 394, 496, and 494. An injection volume of 2 μl was selected for all analyses. The interface temperature was maintained at 280 °C.

2.3. Procedure for PCB determination in sediment samples

A ca. 1-g sample of sediment was extracted with 5 ml dichloromethane in shaker for 24 h. The extract obtained was decanted and then evaporated to a volume of 1 ml under a gentle nitrogen stream. Then extract was transferred to SPE columns filled with SiO_2 and activated copper (added to bind sulfur containing compounds). Prior to use freshly activated copper (in 5 ml HCl–water 1:1, v:v) was placed at the column front After column loading the analytes were eluted with dichloromethane (1 ml/min) and 8-ml fractions were collected. The next stage consisted of the following operations:

- evaporation of a specified extract volume to dryness; extraction with pentane (3× 100 ml) of the dry residue in an ultrasonic bath;
- fractionation of the pentane extract in glass columns filled with freshly conditioned silica gel (8 h at 140 °C);
- collection of the fraction containing PCBs (8 ml) and evaporating it to dryness under a gentle stream of nitrogen; and
- dissolution of the dry residue in 30 ml of hexane.

Then, 2-μl aliquots of the hexane extract were injected into the chromatographic column, separated and analysed by means of GC–MS. The change in the solvent from dichloromethane to pentane allowed for a preliminary purification of the extract through the primarily separation of polar impurities.

The scheme of the procedure for determining PCBs in sediment samples is given in detail in Wolska [1].

2.4. The effect of standard addition technique on the result of PCB determinations

The aim of the study was to assess the effect of adding a solution containing an internal standard to sediment samples on the results of analyte determinations. Samples of standard were added to 1 g of sediment using three different techniques:

(1) adding a solution containing the standard to a sediment moistened with acetone and leaving the sample for 24 h to allow the solvent to evaporate;
(2) adding the standard directly to the extraction solvent;
(3) adding a solution of the standard directly to the dry sediment,

and then adding 2 μl (variant A) or 20 μl (variant B) of standard solution PCB 209.

2.5. Calculation mode of the analytes amount introduced to the chromatographic column

In order to perform the calculations of the analytes quantity in the sample introduced to the chromatographic column, samples were dosed to the system in the following order:

- first—the standard solution, where the content of analytes and internal standard is known (standard solution contained analytes from PCB group at concentration of 100 ng/ml PCB 209 and 100 ng/ml of PCB mixture);
- second—the investigated sample containing internal standards (internal standard contained of 30 ng/ml of PCB 209).

The quantity of investigated analytes was calculated on the basis of formula presented below [23]:

$$\frac{pY^p/mY^p}{pC^p/mC^p} = \frac{pY^{st}/mY^{st}}{pC^{st}/mC^{st}} \quad (1)$$

where pY^p is the peak area of a determined substance Y on a chromatogram obtained after injecting extract of a sediment sample into the chromatographic system; mY^p the mass of a determined substance Y on a chromatogram obtained by dosing extract of a sediment sample into the chromatographic system; mC^p the mass of a standard C on a chromatogram obtained by dosing extract of a sediment sample into the chromatographic system; pC^p the peak area of an internal standard C on a chromatogram obtained by dosing extract of a sediment sample into the chromatographic system; pY^{st} the peak area of a determined substance Y on a chromatogram

Table 1
Determined values of LOD and LOQ of applied GC-MS system for particular chemicals of PCB group.

PCB	LOD [ng/g]	LOQ [ng/g]
28	0.35	1.06
52	0.24	0.72
101	0.28	0.84
118	0.052	0.16
153	0.045	0.13
138	0.045	0.13
180	0.14	0.42

obtained by dosing standard solution into the chromatographic system; mY^{st} the mass of a determined substance Y on a chromatogram obtained by dosing standard solution into the chromatographic system; pC^{st} the peak area of an internal standard C on a chromatogram obtained by dosing standard solution into the chromatographic system; mC^{st} is the mass of an internal standard C on a chromatogram obtained by dosing standard solution into the chromatographic system.

2.6. Statistical analysis

To compare the importance of differences between mean determined value and true one the Student t-test ($f = n - 1$, $\alpha = 0.05$, $t_{crit.} = 3.182$) has been applied. This test was employed to examine if calculated average concentration are not statistically different.

LOD has been calculated based on SD value of set of signals and slope angle of the calibration curve. LOD has been determined by applying the following relationship:

$$\mathrm{LOD} = \frac{3.3 \cdot s}{b} \tag{2}$$

where s is the SD of free term of the calibration curve used, b the slope of the calibration curve, and LOQ has been determined by applying the following relationship:

$$\mathrm{LOQ} = 3 \cdot \mathrm{LOD} \tag{3}$$

Determined values of LOD and LOQ are shown in Table 1, with LODs ranging from 0.0045 to 0.35 ng/g and LOQs ranging from 0.13 to 1.06 ng/g, observing that the higher LODs and LOQs were obtained for PCBs with greater number of chlorine atoms in the molecule—PCB 118, PCB 153, PCB 138 and PCB 180.

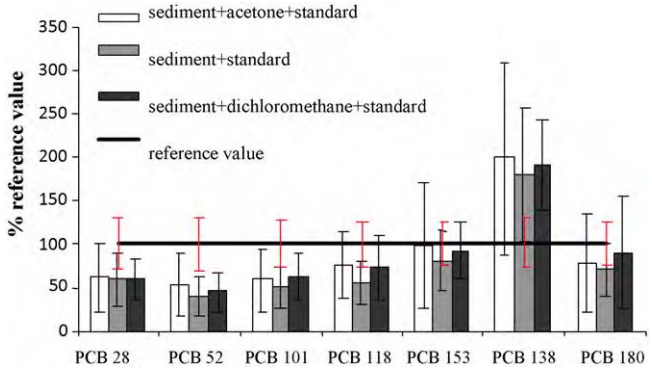

Fig. 1. Comparison of the results of determination of PCB analytes in sediment samples with reference value, obtained using three different standard addition techniques: (a) sediment + acetone + standard; (b) sediment + standard; (c) standard; variant A—addition of 2 μl of internal standard to samples.

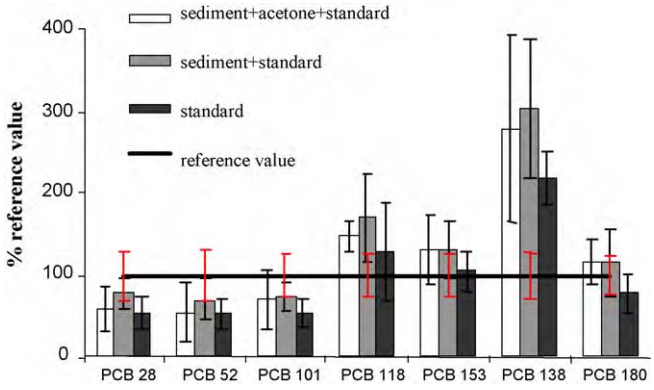

Fig. 2. Comparison of the results of determination of PCB analytes in sediment samples with reference value, obtained using three different standard addition techniques: (a) sediment + acetone + standard; (b) sediment + standard; (c) standard; variant B—addition of 20 μl of internal standard to samples.

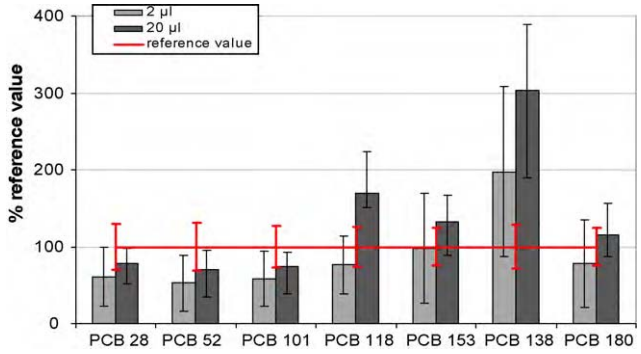

Fig. 3. Comparison of the results of determination of PCB analytes in sediment samples with reference value, obtained using two different volumes—2 and 20 μl of added standard (standard addition techniques: sediment + acetone + standard).

3. Results and discussion

In Figs. 1–5 there are presented results of model studies of the PCB analytes extraction process based on the reference material METRANAL™2 with application of different standard substance added (Figs. 1 and 2) and different volumes of the standard added (Figs. 3–5).

Results obtained are presented in the form of analytes recovery under assumption that the reference recovery value reaches 100%. Results of PCB group analytes content determination in sediment samples obtained using different standard addition techniques are not statistically different among each other (Figs. 3–5). It indicates,

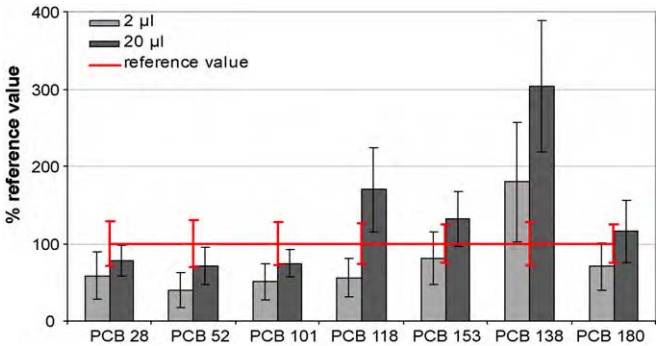

Fig. 4. Comparison of the results of determination of PCB analytes in sediment samples with reference value, obtained using two different volumes—2 and 20 μl of added standard (standard addition techniques: sediment + standard).

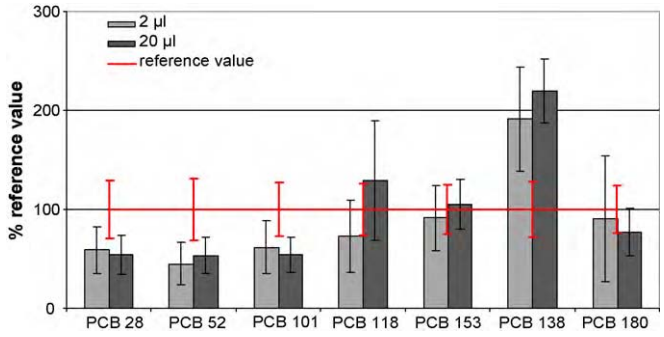

Fig. 5. Comparison of the results of determination of PCB analytes in sediment samples with reference value, obtained using two different volumes—2 and 20 µl of added standard (standard addition techniques: sediment + dichloromethane + standard).

that in every case the recovery of internal standard is similar, independently on the addition technique. However it could be observed that results closer to the certified value were obtained in variant B (addition of 20 µl of internal standard to the sample), while results obtained in variant A (addition of 2 µl of internal standard to the sample) are lower (Figs. 1 and 2). Additionally, in case of addition of the 20 µl internal standard (variant B), the closest to the certified value results were obtained in case of the I type of the standard addition technique (sediment + acetone + standard).

On the basis of the results obtained it can be stated that the amount of internal standard addition has influence on the extraction results obtained. The results closest to the reference values were obtained by adding the 20 µl of standard to the sediment moistened with a small quantity of acetone and leaving the sample for 24 h to allow the solvent to evaporate.

Therefore, this technique is believed to be the best one from all investigated (truly imitates association of analytes with sample matrix, which occurs in aqueous environment). It also gives an additional advantage—in case of adding internal standards to sediment sample wetted with acetone it is not necessary to previously dry or freeze-dry a sample.

4. Conclusions

A standard is usually added to a sediment sample in only a small quantity of organic solvent (0.1–1 µl). The results of this study demonstrate, however, that a standard should be added to the sediment in a large volume of solvent, or should be added to the solvent that was earlier used to moisten (drench) the sample. The standard should then be vigorously mixed with the sample, and the solvent ensures that the sample is properly moistened. The next step should be to allow the solvent to evaporate gradually; thereafter, once a suitable time interval has elapsed (reproduction of the ageing process), one can move on to the extraction step. Adding the standard to the acetone-moistened sediment has the further merit that the sediment samples do not require prior drying or lyophilization.

Internal standards should be added during the first stage of sample preparation for analysis. Only in this way they will follow exactly the same stages as an analyte occurring naturally in a sample.

However, the possibility of usage of the isotope labelled compounds, as recovery standards, in multistage procedure of PCBs isolation and determination substantially contributed to the improvement of determinations' accuracy and precision however unfortunately also increases the cost of analysis.

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