

## ANALYTICAL METHOD COMPARISONS FOR THE ACCURATE DETERMINATION OF PCBS IN SEDIMENTS

Masahiko Numata<sup>1</sup>, Takashi Yarita<sup>1</sup>, Yoshie Aoyagi<sup>1</sup>, Misako Yamazaki<sup>1</sup>, Akiko Takatsu<sup>1</sup>

<sup>1</sup>National Metrology Institute of Japan, AIST, Tsukuba

### Introduction

National Metrology Institute of Japan in National Institute of Advanced Industrial Science and Technology (NMIJ/AIST) has been developing several matrix reference materials, for example, sediments, water and biological tissues, for the determinations of heavy metals and organometallic compounds<sup>1</sup>. The matrix compositions of those certified reference materials (CRMs) are similar to compositions of actual samples, and those are useful for validating analytical procedures. "Primary methods of measurements" are essential to obtain accurate and SI-traceable certified values in the reference materials, because the methods have the highest quality of measurement<sup>2</sup>. However, inappropriate analytical operations, such as incomplete extraction of analytes or cross-contamination during analytical procedures, will cause error of analytical results, even if one of the primary methods, isotope-dilution, is utilized. To avoid possible procedural bias for the certification of reference materials, we employ more than two analytical methods which have been optimized beforehand.

Because the accurate determination of trace POPs in the environment is important to evaluate their risk, reliable CRMs are required by environmental chemists. Therefore, we have also been preparing matrix CRMs for the determination of POPs. To establish accurate analytical procedures for the certification of POPs, extraction is one of the critical steps as described above. In general, conventional extraction techniques for the determination of POPs, such as Soxhlet extraction (SOX) and saponification (SAP), have been characterized well, and introduced as official methods for environmental analysis. On the other hand, emerging techniques, such as microwave-assisted extraction (MAE), pressurized fluid extraction (PFE) and supercritical fluid extraction (SFE), give higher recovery yields of analytes with relatively short extraction time and small amount of solvent, by reasons of the high operating temperature and/or the low viscosity of extraction media<sup>3</sup>.

To evaluate these extraction techniques as analytical methods for the certification, the effects of the extraction conditions on the determination of some chlorinated biphenyl (CB) congeners in sediment samples have been investigated in this study. The analytical results obtained by the optimized extraction techniques have been used to determine certified values of the CB congeners in reference materials that we have planned to develop.

## Methods and Materials

**Sediment samples:** A marine sediment sample was used as a candidate of a reference material. The sediment was collected from a bay of Kyusyu island. The sediment was air-dried, pulverized, sieved (<106 $\mu$ m), homogenized and subsampled into 60-g portions by Environmental Technology Service (Japan). The bottled samples were sterilized by  $\gamma$ -ray irradiation of  $^{60}\text{Co}$  and stored at 4°C until use. Sediment CRMs, NIST SRM1944 (National Institute of Standards and Technology, USA) and BCR CRM536 (Institute for Reference Materials and Measurements, Belgium), were also used to validate the analytical results in some cases.

**SAP:** The analytical procedures were basically followed as a Japanese official method<sup>4</sup>. After the addition of a surrogate solution ( $^{13}\text{C}_{12}$ -CB congeners in 2,2,4-trimethylpentane), the sediment sample (2.0 g) was treated with 50 mL of ethanolic potassium hydroxide (1 M) solution with or without addition of water (10 mL) at 80°C with reflux, or at room temperature with shaking for 1 hour. The CB congeners in obtained supernatant were extracted with hexane. After shaking with sulfuric acid (98%), the hexane layer was passed through a glass column (10 mm i.d.) containing 5 g of silica-gel (moisture content: 5%) to remove polar constituents. The column was washed with 40 ml of hexane to recover the CB congeners.

**SOX:** The mixture of the sediment sample (2.0 g) and anhydrous sodium sulfate (10 g) was placed into a filter paper thimble, and the surrogate solution was added. The sample was loaded on an automated Soxhlet extraction system B-811 (BÜCHI, Switzerland) and then extracted for 6-48 hours. After extraction, activated copper powder (ca.10g) was added to the obtained extract to remove elemental sulfur. Then, the solution was cleaned up with a solid phase extraction cartridge (500 mg silica, International Solvent Technology, UK) and a normal phase liquid chromatograph equipped with YMC-Pack NH2 column (10 mm i.d. x 150 mm, YMC, Japan) by the method described elsewhere<sup>5</sup>.

**MAE:** After addition of the surrogate solution the sediment sample (2.0 g) was extracted with an MAE system (MARSX, CEM, USA) with 20 mL of solvent for 5-30 min at 100-145°C<sup>6</sup>. The extract obtained by MAE was centrifuged at 3,000rpm for 3min to remove residues. The extract was cleaned up by copper treatment, SPE, and nHPLC as described above.

**PFE:** The sediment sample (2.0 g) was weighted in a stainless steel cell. After, anhydrous sodium sulfate and the internal standard solution were added into the cell. The contents was extracted at 40-150°C, 9-18 MPa using a PFE system (ASE200, Dionex, USA)<sup>7</sup>. The obtained extract was cleaned up as described above.

**SFE:** The sediment sample (1.2 g) was weighed in a stainless steel cell. After, anhydrous sodium sulfate, activated copper powder, and the surrogate solution were added into the cell, the contents was extracted with supercritical carbon dioxide at 40-140°C, 10-30 MPa using an SFE system (SFE400, SUPELCO, USA). The extracted components were trapped onto an ODS-silica gel column (4.6 mm i.d. x 50 mm), and eluted with 10 ml of acetone. After concentration with a

nitrogen gas stream, the eluent was directly injected to GC/HRMS system to determine CB congeners.

**Quantification:** Analysis of PCBs in the extracts was performed using a GC/HRMS system (AutoSpec, Micromass, UK)<sup>5</sup>. The PCB congeners were separated using a gas chromatograph equipped with HT-8 capillary column (0.22 mm i.d. x 50 m, film thickness 0.25  $\mu\text{m}$ , SGE, Australia). Abundant congeners (CB#3, 15, 28, 31, 70, 101, 105, 138, 153, 170, 180, 194, 206, and 209) in commercial PCB products were determined by isotope-dilution method, and concentrations of other congeners were also evaluated by using the above <sup>13</sup>C-labeled congeners as internal standards.

## Results and Discussion

**Optimization of the extraction conditions:** The effects of the extraction conditions, such as temperature, pressure (in the cases of PFE and SFE), time, and solvents (hexane, dichloromethane, acetone, hexane/acetone mixture, and toluene) on the determination of the CB congeners have been investigated to optimize them.

**1) SAP:** Because saponification is efficient procedure to eliminate concomitants, such as lipid, elemental sulfur, it has been introduced as some official methods for the PCB determination in sediments, wastes, wastewaters and biological tissues. However, dechlorination of highly chlorinated biphenyls (octa- to deca-CBs; especially 2,3,4,5,6- substituted congeners) was observed in the case of high-temperature saponification (80°C). On the other hand, the obtained concentration values (i.e., extraction efficiencies) of mono- and di-CB congeners were low when room-temperature treatment was applied. The low degradation of highly chlorinated biphenyls and the high recovery of less-chlorinated biphenyls were compatible, when the residue of the room-temperature saponification was re-extracted at 80°C, and both obtained extracts were combined.

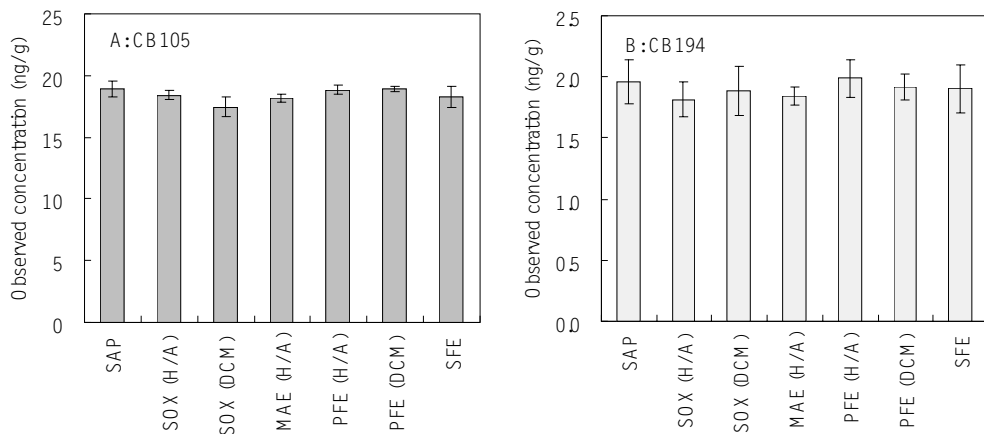
**2) SOX:** The SOX technique has been introduced as official method for environmental analysis, and as the technique for the certification of reference materials in many cases. For the tested sample, the optimal extraction conditions were 24 hours (or more) with hexane/acetone (1:1) or dichloromethane.

**3) MAE:** In addition to the high recovery yields of analytes, high sample throughput is realized by a simultaneous sample treatment by MAE. However, application of the MAE technique to the certification of reference materials has not been reported yet. Although optimization of MAE procedures and comparison with other extraction techniques were reported in many articles, a combination of the MAE technique and the isotope-dilution method has not been employed, except a few cases. The optimal extraction conditions were 145°C, 10 min with hexane/acetone (1:1) in tested ranges.

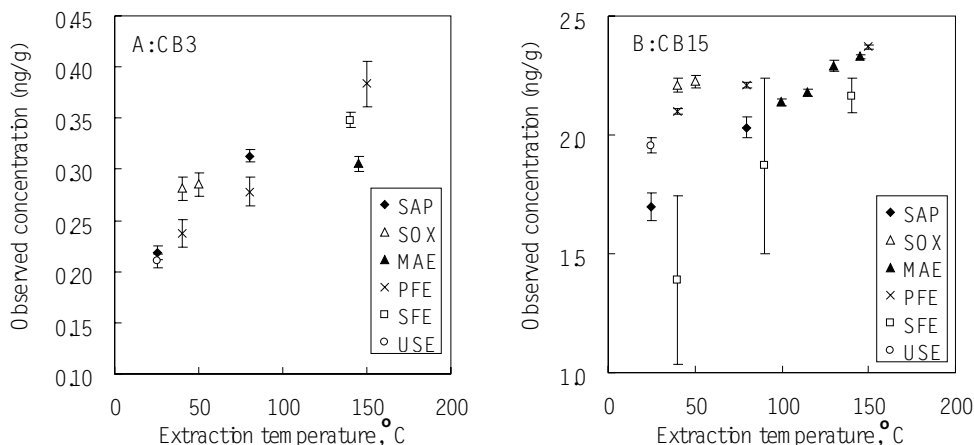
**4) PFE:** The PFE technique is known as a highly efficient extraction method and has come into wide use in the field of environmental analysis. It has also been used for the determination of certified values of PCBs and other organic contaminants concentrations in some reference materials. On investigation, the optimal extraction conditions were 150°C, 15 MPa, and 30 min x 2 cycles using hexane/acetone (1:1) or dichloromethane as extraction solvent. We found loss of 4,4'-DDT (unpublished data) and increase of the CB2 concentration during the high-temperature PFE treatment (150°C). The CB2 would be produced from the higher chlorinated biphenyls, because the phenomenon occurred when diluted Kanechlor was treated under same conditions without the sediment sample. The MAE treatment under high temperature in the Teflon vessels did not cause such effect. Consequently, the stainless steel extraction cell might work as a catalyst of the dechlorination.

**5) SFE:** Compared with other emerging techniques, SFE is known as a highly specific method. Relatively less-polar components, CB congeners, were recovered effectively, and any interference on the chromatograms of the CBs was not found. The optimal extraction conditions were 140°C, 30 MPa in tested ranges. Combination of 15 min of static extraction mode and 30 min of dynamic mode gave high recovery yields. Addition of modifiers (methanol or acetone) did not affect analytical results significantly.

**Method comparisons:** The analytical results of CB congeners obtained under optimum conditions of each extraction techniques were compared. For most CB congeners, no significant differences were observed (Fig. 1). However, the obtained concentration values (i.e., extraction efficiencies) of mono- and di-CB congeners mainly depended on the extraction temperature (Fig. 2; including the optimized conditions and other tested conditions). In particular, uncertainty of CB3 concentration associated with the differences between these optimized methods was much larger than uncertainties associated with other factors, such as homogeneity of the sample, concentration of the GC/MS calibration solution, and so on. Because aged pollutants are tightly adsorbed on the surface of the soil particles or trapped in the three-dimensional structure of clay minerals or humic substances, the dependence of analytical values on the temperature would be explained by slow exchange with isotope-labeled surrogates and the low extraction speed of such aged pollutants<sup>8</sup>.



**Figure 1:** Examples of analytical results of CB congeners in the sediment sample, obtained by the optimized analytical procedures (see text). (A: CB105; B: CB194), H/A: hexane/acetone (1:1); DCM: dichloromethane, Error bars: SD (n=5).



**Figure 2:** Observed concentration values of CB3 (A) and CB15 (B) in the sediment sample as a function of the extraction temperature (USE: ultrasonic extraction at room temperature with acetone). Error bars: SD (n=3-5).

Because the analytical results of most congeners obtained by different extraction techniques were consistent enough, combinations of these optimized methods and gas chromatography/isotope-dilution mass spectrometry have been applied for certification of a marine

sediment reference material. However, the certified value of a mono-CB congener (CB3) has relatively large uncertainties mainly caused by the temperature dependence of extraction. The reference material, NMIJ CRM 7304-a (Polychlorinated Biphenyls and Organochlorine Pesticides in Marine Sediment – High Pollutant Concentrations) having SI-traceable certified values of 14 CB congeners and 4 organochlorine pesticides, is distributed from NMIJ/AIST with its certificate, and another CRM (lower pollutant level) is in preparation<sup>9</sup>.

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