

# DETERMINATION OF POLYCHLORINATED BIPHENYLS USING ACCELERATED SOLVENT EXTRACTION

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## Introduction

Polychlorinated biphenyls (PCBs) have many characterizations which are a group of highly lipophilic, chemical and thermal stable, persistent contaminants and low vapor pressure at ambient temperature etc.<sup>1</sup> PCBs were used as industrial chemicals (e.g. dielectrics, flame retardants, ink solvents)<sup>2</sup> for more than half a century.<sup>3</sup> Because it was harmful to the health of animal and human, the use of PCBs has been banned.<sup>2</sup> In recent years, the number of studies focused on determining the levels of persistent organic pollutants (POPs) such as PCDD/Fs, PCBs in foodstuffs. The traditional extraction techniques such as Soxhlet are time-consuming and require large volumes of organic solvents.<sup>4,5</sup> New techniques have been developed in order to reduce extraction solvent volumes, extraction times, and sample preparation costs and to improve method precision and accuracy.<sup>6</sup> The Accelerated Solvent Extraction (ASE) is not only one of the most recent sample preparation techniques but also able to extract in a very short time and so simple to operate.<sup>1,2</sup> The purpose of this study was to develop optimized extraction conditions using ASE and to apply extraction of fat for PCBs analysis. In this study, Soxhlet and ASE were compared with the content of extracted fat using milk powder, which is commercially merchandise from domestic market. Also, the determination of PCBs was checked by analyzing CARP-2 which is certified reference materials (CRM) in various ASE conditions.

## Materials and methods

### (1) Chemicals

n-Hexane, dichloromethane (DCM) and nonane were purchased at ultra-residue grade for dioxin analysis (Wako, Japan) and other chemicals used analytical grade. Washed silica gel was obtained from Supelco (USA) and anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), sulfuric acid ( $\text{H}_2\text{SO}_4$ ) and sodium hydroxide (NaOH) were manufactured from same company with solvent. Native standard of PCBs (PCB IUPAC No. 28, 52, 101, 118, 138, 153 and 180), labelled-PCBs ( $^{13}\text{C}_{12}$ ) and recovery spike (RS) solution (PCB IUPAC No. 70L, 111L and 170L) were purchased from Wellington Laboratories Inc. in Canada. CARP-2 was obtained from National Research Council Canada.

### (2) Optimization of extraction for PCBs analysis

The milk powder samples for fat extraction prepared approximately 10g to compare Soxhlet with ASE 350 (Dionex Corporation, USA). Sample for Soxhlet was extracted for 24 h with 300 mL DCM/hexane (3:1 v/v). The extracts were transferred into a 200 mL tube and reduced to drought approximately in nitrogen concentrator. To analyze PCBs was carried out on ASE 200 (Dionex Corporation, USA). 1-2g CARP-2 were mixed with 35g anhydrous sodium sulfate powder and completely transferred to the extraction cells. The sample was spiked with an internal standard (isotopically labelled PCBs mixture). The conditions of ASE were as follows: pressure 1500 psi, oven heat-up time 5 min, static time 5 min, flush volume 60% of extraction cell volume, purge time 60 s, solvent DCM/hexane (1:1 v/v). Optimal conditions for ASE method were determined in the experiment and the different ASE parameters were oven temperature (90, 100 and 125°C) and extraction cycle (1-3 cycles). The extracts loaded onto the multi layer chromatography clean-up column.<sup>7</sup> After eluants were spiked with RS solution, the samples were evaporated to approximately 100 $\mu\text{L}$  by using nitrogen gas.

### (3) GC-MS analysis

GC-MS analysis was performed using an Agilent 6890N (Wilmington, USA) gas chromatography that was connected to a Agilent 5973 mass spectrometer (Wilmington, USA) in selected ion monitoring (SIM) mode using the molecular ions of individual compounds.<sup>8</sup> The chromatography analysis was achieved by splitless

injection of 1 $\mu$ L on two different capillary columns: DB-1 column (60m x 0.25 mm ID x 0.25 mm FT, J&WScientific, USA) and DB-5MS column (30m x 0.25 mm ID x 0.25 mm FT, J&WScientific, USA). The temperature conditions of chromatographic analysis using DB-1 column were described as follows: the initial column temperature was held at 100 °C, increased to 160 °C at a rate of 10°Cmin<sup>-1</sup> and maintained for 2min, increased to 200 °C at 5°C min<sup>-1</sup> and held for 2 min, increased to 210°C at 2°C min<sup>-1</sup> for 5 min, and then increased to 300°C at 10 °C min<sup>-1</sup> for 10 min, it was lastly maintained to 300°C for 5 min. Another column condition (DB-5MS): initial oven temperature 100°C, 15°C min<sup>-1</sup> to 200°C (7min), 2°C min<sup>-1</sup> to 220°C (5min), 6 °C min<sup>-1</sup> to 250°C, 300°C min for 5min (postrun), carried gas by helium.

## Results and discussion

### (1) Extraction of fat using ASE methods

Soxhlet extraction for comparison with ASE result was performed in milk powder. The rate of fat extraction was 23.3% by traditional soxhlet method and ASE obtained 22.2% and 25.5%, relatively 1 cycle and 2 cycles. This result was shown that the ASE method is more reliable and fast than soxhlet extraction method. The result indicated Table 1. Furthermore the soxhlet extraction method requires use of large solvent volumes and long extraction times. While ASE method can decrease extraction time and solvent consumption, which should solve the crisis with increasing demands on analytical laboratories to perform fast and reliable PCB analysis of various foods.<sup>1</sup>

**Table 1. Comparison soxhlet with ASE on fat content in milk powder**

Extraction	Soxhlet (%)	ASE (%)	
		1 cycle	2 cycle
Average	23.3	22.2	25.5
SD*	1.13	1.46	0.62

\* Standard deviation

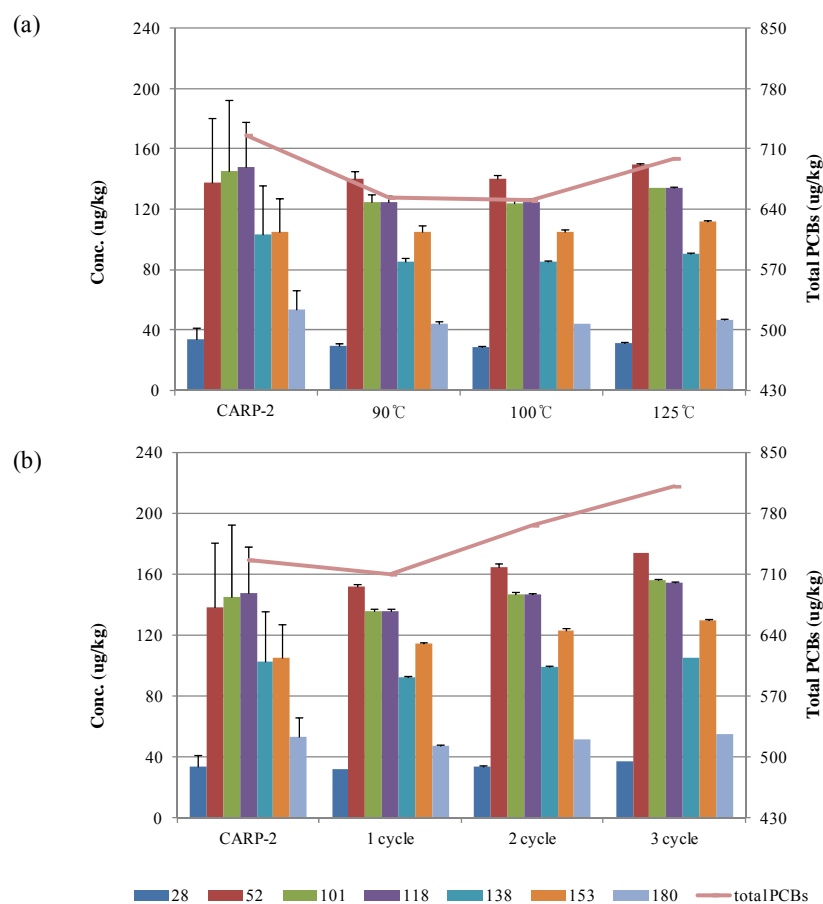
### (2) Optimization for PCBs analysis in CRM

To make a calibration curve for the quantitative determination of PCB congeners, all calculations were performed by using the ratio between isotopically labeled PCBs and the corresponding native ones. The linear regression of 7 congeners gives good correlation coefficients (> 0.999). The indicator congeners (PCB IUPAC No. 28, 52, 101, 118, 138, 153 and 180) play a major role in the estimation of total PCB in almost all the environmental samples. Therefore 7 indicator PCBs were used to compare the experimentally determined concentrations with the certified concentrations and to confirm the accuracy and recovery of the whole analytical procedure on established in the studies. The conditions of ASE carried out the experiments in the previously same procedures. Accordingly, this study focused mainly on the two most influential parameters temperature and cycle. As results, PCBs contents were detected within range of standard error regardless of temperature and cycles in CARP-2. The result is shown in Figure 1. The average recoveries of CARP-2 were measured to 82.0-101.8%, 81.9-101.4% and 87.0-108.6 at 90, 100 and 125°C respectively. Taken as a whole, 125°C was indicated the highest recovery in all congeners. Furthermore the recoveries measured 89.5-110.2%, 96.1-119.3% and 102.6-125.9% when ASE cycle increased 1, 2 and 3 times respectively (Table 2). The performance of the extraction techniques ASE and soxhlet for PCBs analysis is compared. In general, the two techniques showed equivalent results in other study.<sup>6</sup> Taking into account general parameters such as extraction time, solvent consumption, sample amount, and practical conditions, ASE is the preferable techniques. Additionally, two different capillary columns were used for comparison of resolution on relatively PCB congeners. DB-1 column has the outstanding recovery rate (100.32%) better than DB-5MS regarding 28 congener which was excessively increased recovery rate (198.14%) (Table 3). Two columns are a representative non polar column but DB-5MS was relatively polarity. Because DB-5MS have 95% methyl- and 5% phenyl-, DB-1 was well separated non polar substances.

**Table2. Average percentage recovery rates of experimentally determined CRM (CARP-2) at different extraction temperature and cycle**

Congeners	Temperature			Cycle		
	90°C	100°C	125°C	1 cycle	2 cycle	3 cycle
28	86.9	84.6	92.4	94.5	100.3	108.7
52	101.8	101.4	108.6	110.2	119.3	125.9
101	86.3	85.2	92.3	93.5	101.3	107.5
118	84.3	84.0	90.9	91.8	99.3	104.6
138	82.3	82.3	87.9	89.7	96.0	101.8
153	100.1	99.9	106.7	108.9	117.5	123.7
180	82.0	81.9	87.9	89.5	96.1	102.6

(Unit:%)



**Fig.1. The concentration of individual PCB congeners in CARP-2 for different ASE parameters (a) ASE conditions:oven temperature90, 100 and 125°C; (b) ASE conditions: static cycle 1-3 cycles**

**Table 3.Comparison between two different columns for PCBs analysis in CRM (CARP-2) (Unit: ug/kg)**

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