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Measurements of polybrominated diphenyl ethers and polychlorinated biphenyls in a single drop of blood

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ABSTRACT

A quantitative method that requires only a small volume (50 μL) of blood has been developed for the determination of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs). Target analytes in both plasma sample (DBSV) and dried blood spot (DBS) were analyzed by a gas chromatography/high resolution mass spectrometer (GC/HRMS). Measurements of standard reference materials by the developed method were in agreement with those certified values. Linear correlation coefficients were found to be 0.9984 and 0.9965 for DBS and DBSV analysis, respectively. Other analytical criteria, such as limits of detection, recoveries, precision, accuracy and linearity of the proposed method are also reported. From recovery studies, the addition of formic acid to the extraction solvent was found to be effective in extracting PBDEs and PCBs from filter paper. The PBDE and PCB levels in spiked DBS were monitored at room temperature for up to 30 days and the variations of target analytes were found to be insignificant. Our results suggest that DBS sampling technique is feasible for PBDE and PCBs biomonitoring in human population.

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1. Introduction

Polybrominated diphenyl ethers (PBDEs), a group of brominated flame retardants (BFRs), have been used extensively over the past two decades in commercial and household products, including polyurethane foam, plastics, wire insulation, textiles and electronics. Polychlorinated biphenyls (PCBs) were historically used as fluid for heat-exchangers, transformers, and hydraulics, as well as an additive to paints, oils, joint caulking, and floor tiles.

Most of the PCB and PBDE congeners are chemically stable and therefore could undergo long range transport through aerosols and water system. Therefore, it is not surprising that PBDEs and PCBs have been found to be bioaccumulated in human, aquatic wildlife, and other ecosystems around the world [1–12]. Contamination in environment could include releases from manufacturing sites, poor management of hazardous waste sites that contain PCBs/PBDEs and disposal of PCB/PBDE products into non-hazardous waste. However, the exposure pathways through which PBDEs and PCBs get into human bodies remain unclear due to the limited information available from individuals, such as occupational exposure and house characteristics [13,14]. Even at low levels, PBDEs and PCBs can exert undesirable effects on wildlife and human beings. Emergent evidence from biomonitoring program suggested that PBDEs and PCBs, similar to other persistent organic pollutants (POPs), exert certain degree of neurological, endocrine-disrupting, immune-disrupting and genotoxic effects [15–18]. Therefore, these compounds have been included in biomonitoring programs as well as National Health and Nutrition Examination Survey (NHANES) held by Centers for Disease Control and Prevention (CDC) extensively.

Determination of PBDEs and PCBs in blood usually involves in laborious and time-consuming sample preparation (protein denaturation, liquid–liquid extraction and subsequent clean-up) and requires large volume of blood sample [19]. These factors limited population coverage in biomonitoring programs due to the costly operation of sampling, transportation and storage. Even though methods using solid phase extraction [20,31] and smaller sample volumes (>0.5 mL) for PBDE measurements have been recently developed [21], the use of syringes and large blood volume requirement (0.5–10 mL) hinder the expansion of current biomonitoring network. In order to extend the biomonitoring network to a wider area for the evaluation of human exposure to PBDEs and PCBs, there is a need to develop a new analytical approach, which requires a smaller amount of blood sample.

In addition to the requirement of large blood volume, human specimens collection and storage also present challenges to the current biomonitoring studies. To characterize variability in contaminant levels across diverse populations, large numbers of

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samples must be collected to constitute a statistically representative study. More recently, the development of dried blood spot (DBS) sampling technique is growing rapidly. This sampling technique offers a significant cost reduction in sample transportation, storage and collection. In the United States, dried blood sample (DBS) is collected routinely from newborns for screening different genetic disorder or disease, including carnitine uptake defect, 5-oxoprolinuria, homocystinuria and argininemia, etc. After spotting a small drop of blood onto a specialized paper card, the card is then air dried and mailed to the analytical laboratory for analysis. Ease of use and small sample volume are also the advantages of DBS technology. Thus, the application of DBS has been spreading in pharmacokinetics studies such as screening for drug metabolites and disease markers [22–24]. Now, rapid advances in analytical technology, such as high resolution and tandem mass spectrometric instrumentation, enable environmental scientists to explore the practicality of archived DBS samples, in place of plasma samples, for the sensitive analysis of environmental chemicals. Picogram per microliters level of dichlorodiphenyldichloroethylene (*p,p′*-DDE) was detected in the DBS of infant [14]. Furthermore, analysis of perchlorate and polyfluoroalkyl chemicals in DBS has also been reported [25,26].

This study investigates the feasibility of an analytical method that requires only small volumes of blood (50 μL) for PBDE and PCB congener measurements in the forms of plasma blood sample (DBSV) and dried blood spot (DBS). The reduced blood sample size and the potential use of DBS sampling technique would not only reduce the operation cost of sample handling, but also allow us to increase the analytical capacity as well as to extend the current biomonitoring network.

2. Experimental

2.1. Samples

Human DBS were obtained from the laboratory's archives. National Institute of Standards and Technology (NIST) human serum reference material (SRM 1957, NIST) was used for method proficiency testing and was validated by spiking analytes in defibrinated sheep blood (Hemostat Laboratories, Dixon, CA, USA), either in the form of DBSV or in DBS as described elsewhere.

2.2. Chemicals

Pesticide grade hexane and acetone were supplied by Fluka (Steinheim, Germany), and formic acid was acquired from EMD (Darmstaldt, Germany). All native PCBs (100 μg mL^{-1} each in isooctane), including 5 dioxin-like PCBs (dl-PCBs, PCB77, PCB118, PCB105, PCB126 and PCB169), 6 marker-PCBs (PCB28, PCB52, PCB101, PCB153, PCB138 and PCB180) and 3 other PCBs (PCB194, PCB206 and PCB209), and native PBDEs (50 μg mL^{-1} each in nonane), including PBDE47, PBDE99, PBDE100, PBDE153 and PBDE183, and the corresponding 19 $^{13}C_{12}$-labeled PCBs and PBDEs solutions, were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). The concentrations of the $^{13}C_{12}$-labeled dl-PCB and PBDE congeners were 40 and 50 μg mL^{-1} (in nonane), respectively. The $^{13}C_{12}$-labeled marker-PCB, EC-4058's concentration was 5 μg mL^{-1} in nonane, and $^{13}C_{12}$-PCB 128 (40 μg mL^{-1} in nonane) was used as injection internal standard.

2.3. Preparation of standard solutions and DBS samples

Stock mixture solutions (1 μg mL^{-1}) of dl-PCBs, marker-PCBs and PBDEs were prepared from commercially available standard solutions and were diluted in nonane. A work mixture solution (0.1 μg mL^{-1}) was prepared by diluting the appropriate volume of each of these stock mixture solutions in nonane. The $^{13}C_{12}$-labeled stock mixture solutions (1 μg mL^{-1}) and $^{13}C_{12}$-labeled work mixture solutions (IS-0.1 μg mL^{-1}) were prepared by using the same procedure. $^{13}C_{12}$-PCB128 was diluted in nonane for the injection internal standard solution (0.01 μg mL^{-1}). A set of calibration standard solutions with eight levels (0.05–10 ng mL^{-1}) of native analytes, and constant levels of the $^{13}C_{12}$-labeled internal standards and the injection internal standard (5 ng mL^{-1} each), was prepared using the work mixture standard solutions. For preparing DBS quality control solution, sheep blood was aliquoted into three 10 mL subpools. One was used as a blank sample, and the other two were spiked with targeted PBDEs and PCBs to make a low-concentration (0.05 ng mL^{-1}) pool and a high-concentration (0.16 ng mL^{-1}) pool. After spiking, the pools were mixed for 10 min and equilibrated overnight prior to spotting onto filter paper cards. The cards were dried overnight in desiccates, and then stacked and placed in a Zip Closure bag, along with desiccant packs and a humidity indicator card. The DBS were stored at −20 °C and below 30% humidity. A 3 mm diameter disk was punched from the center of the DBS into a clean tube, followed by extraction procedures and GC/HRMS analysis.

The stability of targeted PBDEs and PCBs in DBS form was also studied. To monitor the concentration of targeted analytes in both spiked (0.1–0.2 ng mL^{-1}) and unspiked DBS over a 30-day test period, the DBS filters were wrapped in aluminum foil at room temperature (as a surrogate for storage under non-optimal conditions).

Passive absorption of targeted analytes onto filter paper during transportation and storage was also studied. Blank filter papers were pre-washed with a mixture of hexane and dichloromethane. After washing, filter papers were wrapped with aluminum foil and exposed it to laboratory air. Blank of filter papers were then monitored during the 30-day test period.

2.4. Sample pretreatment for DBSV

With modifications, liquid–liquid extraction (LLE) was used as described previously [27]. Before extraction, 20 μL of the $^{13}C_{12}$-labeled internal standard (IS) spiking solution, which was prepared by diluting the $^{13}C_{12}$-labeled work mixture solution in acetone, was spiked into the specimen (50 μL) and equilibrated for 2 h. After adding 250 μL of formic acid–acetone (3:2, v/v) as a protein denaturation agent, the samples were extracted for 2 min, immediately followed by mixing with 1.0 mL of a solvent mixture of dichloromethane and hexane (1:4, v/v). Then, the samples were centrifuged for 2 min at 3500 rpm to obtain clear phase layers. The supernatant was transferred to a concentration vessel, and the extraction was repeated with an addition of 1 mL hexane. The combined extract was concentrated under a gentle stream of nitrogen to approximately 0.2 mL and transferred to a GC vial insert for further evaporation. After the extract was reduced to near dryness, 10 μL of injection internal standard ($^{13}C_{12}$-PCB128 at 5 ng mL^{-1} in nonane) was added before GC/HRMS analysis.

2.5. Sample pretreatment for DBS

One dried blood spot (corresponding to 50 μL blood) was cut into small pieces and placed into a 20 mL glass centrifuge tube (Fisher Scientific, Pittsburgh, PA). 20 μL $^{13}C_{12}$-labeled IS solution, 500 μL of formic acid–acetone mixture (2:3, v/v) and 1 mL of hexane–dichloromethane mixture (4:1, v/v) were added to each sample. The samples were extracted in an ultrasonic bath for 20 min, and then mixed well. Subsequent procedures of centrifugation, transfer, repeated extraction, concentration, addition of injection internal standard and reconstitution, were identical to the DBSV method. Other samples (such as spiked samples, blank

samples, blank filter paper, and stability experiment samples) were prepared and processed using this same procedure.

2.6. Instrumental analysis by GC/HRMS

Samples were analyzed by Trace Ultra GC coupled with a DFS High Resolution Gas Chromatography/Mass Spectrometer (DFS GC/HRMS) (Thermo Scientific, Waltham, MA, USA). The mass spectrometer was operated in EI mode, with 1 A of emission current, 45 eV of electron energy. Ion source temperature was set to 200 °C, and perfluorotributylamine (FC43) was used as the reference gas during analysis. Before injecting each batch of samples/standards, the resolution (R) of the DFS HRGC/MS was tuned to 10,000 ± 500, where the resolving power is defined as the mass number (m) of the observed mass divided by the difference between two masses (Δm) that can be separated:

$$R = \frac{m}{\Delta m}$$

Multiple ions detection (MID) mode was used to achieve the maximum sensitivity and selectivity. Two isotopic ions of known relative abundance, representing a group of isomers, were monitored for each molecular ion cluster and for each ^{13}C-labeled standard. The two most abundant molecular isotope masses of each PBDE and PCB were detected ($[M+2]^+$ and $[M+4]^+$ for tetra-BDE, $[M+4]^+$ and $[M+6]^+$ for penta-BDE, $[M+6]^+$ and $[M+8]^+$ for hexa to hepta-BDE, $[M]^+$ and $[M+2]^+$ for tri-PCB, and $[M+2]^+$ and $[M+4]^+$ for tetra to deca-PCB) as described previously [28,29]. 2 μL of each sample was injected into the GC system in SRS splitless mode. The temperature of the injector was set at 280 °C. The transfer line temperature was set at 260 °C. A column with dimension 30 m × 0.25 mm i.d., 0.25 μm (DB-5 MS, J&W Scientific, Folsom, CA, USA) was coupled with GC/HRMS. Ultrapure helium (99.999%) was used as the carrier gas at a constant flow of 0.8 mL min^{-1}. The initial oven temperature 120 °C was equilibrated for 1 min. After equilibrium, the temperature was increased to 290 °C at 5 °C min^{-1} and held for 5 min. PCBs and PBDEs were quantified by isotopic dilution by using $^{13}C_{12}$-labeled PBDE and PCB internal standards.

2.7. QA/QC

QA/QC criteria were followed with USEPA Method [28,29], which were validated for the determination of PBDEs and PCBs in environmental and biological samples. In each unknown sample, retention time, intensity ratios of the monitored ions, and signal-to-noise ratio were used as the identification criteria. The retention time of the specific peaks eluted from GC column must fall within ±3 s compared to $^{13}C_{12}$-labeled standards or ±5 s compared to calibration standards. Both quantifier and qualifier ions are monitored for each analyte, with the requirement that the qualifier/quantifier ion ratio be within ±15% for confirming their presence in samples. The resulting signal-to-noise ratio from the peak responses of the two corresponding ions must be greater than three for proper quantification of the congeners. Quantification was performed by the isotopic dilution standard method relative to a multilevel calibration (0.05–10 ng mL^{-1}) for all congeners. The RSD of the relative response (RR) of the eight-point calibration levels for each compound was less than 7%, and the recoveries of the $^{13}C_{12}$-labeled surrogate analogues of each compound ranged from 67% to 120%, meeting the precision requirements of the USEPA methods (RSD < 20%) [28,29].

The limit of detection (LOD) is defined as the amount of analyte at which the signal-to-noise ratio of the peak is equal to three. Using one small spot (corresponding to 50 μL sample), the LODs of PCBs ranged from 0.002 to 0.005 ng mL^{-1}, and LODs of PBDEs ranged from 0.007 to 0.02 ng mL^{-1} and increased with the number of brominated atoms for PBDEs. For BDE153 and BDE183, the LODs were 0.017 and 0.02 ng mL^{-1}, respectively.

A procedural blank and replicate DBS filter paper blanks only for the DBS method were analyzed with each series of samples and used as the background subtraction for calculating the concentration of each congener (Fig. 1).

3. Results and discussion

3.1. DBS filter paper background

The background contamination of DBS filter paper is of great concern in qualitative and quantitative analyses of environmental chemicals in DBS [14]. The filter papers could be contaminated with the environmental pollutants in the process of manufacturing, sampling, transportation, and storage. For background study, filters from six manufacturing years (1987, 1996, 2003, 2005, 2007 and 2009) were analyzed and the results were shown in Fig. 2. Since there had been no change in sample handling procedures and storage conditions, the variations between filters with different manufacturing years (1987–2009) were more likely contaminated during the process prior to the product arrivals (i.e. manufacturing, packaging and transportation, etc.). Also, levels of PBDEs and PCBs on blank filter paper would increase along with the length of storage time if contamination source is present in storage environment. Such pattern was not observed in this study.

It is a fact that PCB was federally banned on sale and production in 1979. However, PCBs, with a diverse application in different industries, may be present in products and materials produced before the 1979 PCB ban. Furthermore, PCB congeners are highly persistent and hence it is not surprising that these compounds could still be present in the environment for long period of time. Our study also suggested that the limit of detection for some PBDE and PCB congeners might be affected by the filter paper background, but not all of them. Thus, it is feasible to use DBS technique to measure the most abundant PBDE and PCB congeners without serious concern about paper blank.

In this study, potential contamination of DBS filter papers due to short term (30 days) exposure to air during sample handling, transportation and storage, were also investigated. Representative filter papers were washed with a mixture of hexane and dichloromethane to reduce the effects on the experimental results from the filter paper's inherent background and fluctuation. The DBS filter paper was then air dried and wrapped in aluminum foil for storage at laboratory temperature (~25 °C) and humidity (~55%). These papers were sampled at intervals of 0, 2, 7, 15 and 30 days and compared to the concentrations measured at day 0. Our results showed that the variation (%) of the filter paper blank concentration for inter-day deviation was within ±20% and therefore indicated that passive adsorption of PBDEs and PCBs on filter papers were insignificant. Our results also suggested that the background PCBs and PBDEs were independent on the transportation and storage time of DBS specimens within a short period of time. Therefore, the current sample handling/storage procedure should be sufficient to maintain low background of PBDEs and PCBs. However, this stability test should be extended to longer scale of time frame.

With the aim of using DBS filter paper archives from Newborn Screening Program, the DBS filters were used as the forms as received without washing. Therefore, background subtraction was used to estimate the levels of PBDEs and PCBs in dried blood spots. In order to perform background correction, homogeneity of individual paper lot was studied. The dispersion pattern of target analytes on the filter paper during the spotting was studied as well. To assess the background homogeneity of a single lot of paper, five

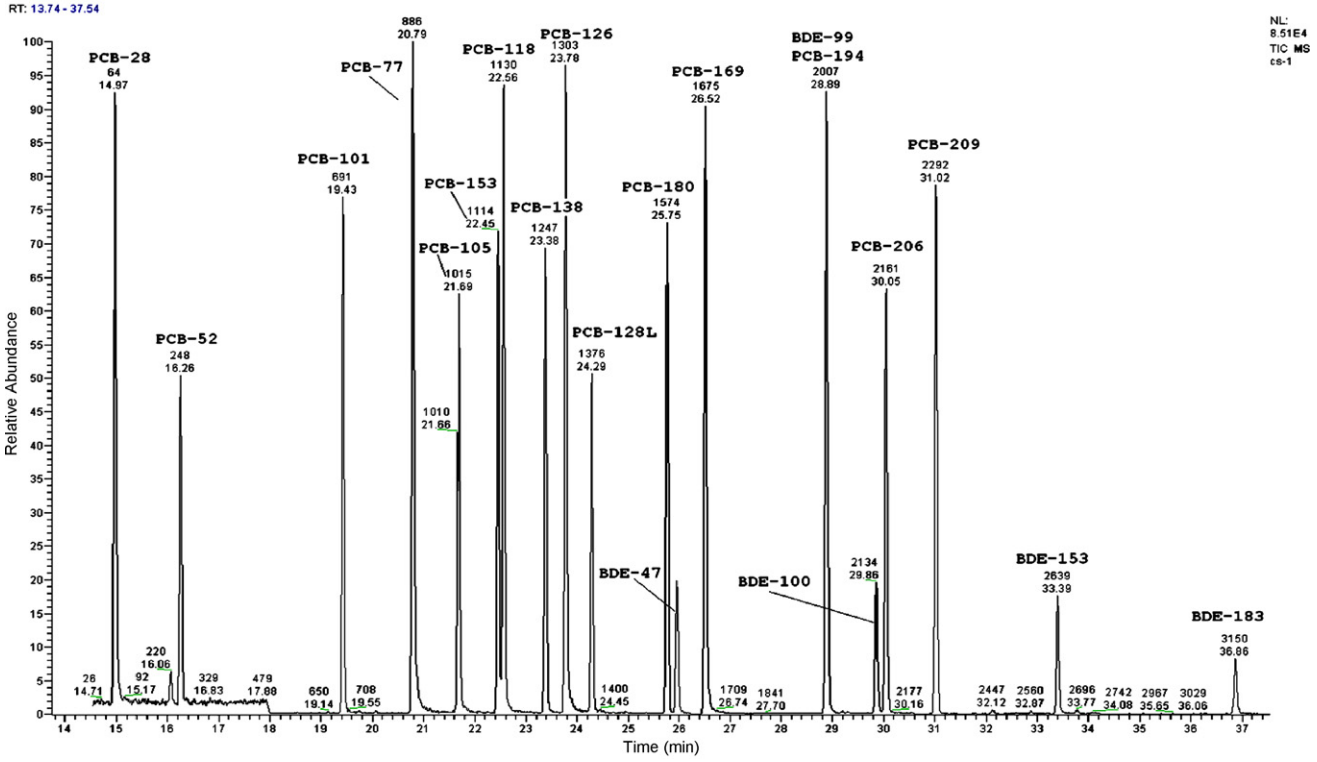

Fig. 1. Total ion chromatogram (TIC) of a spiked blood sample by GC/HRMS.

different regions located diagonally across one paper (21 cm (L) × 15 cm (W)) were cut for GC/HRMS analysis. 12 congeners were detected (All except for PCB77, PCB126, PCB169, PCB206, and PCB209, and BDE153 and BDE183). Statistical analysis was conducted in SPSS 17.0. Based on the one-way analysis of variance (ANOVA), the results showed that there was no significant difference in levels of detected congeners between the five regions ($p = 0.05$), meaning that there was no obvious background difference across the paper. Moreover, the background variation (%) between the five sampling points was within ±22%. Thus, the concentration of the targeted analytes can be determined by subtracting the background value from the detected value.

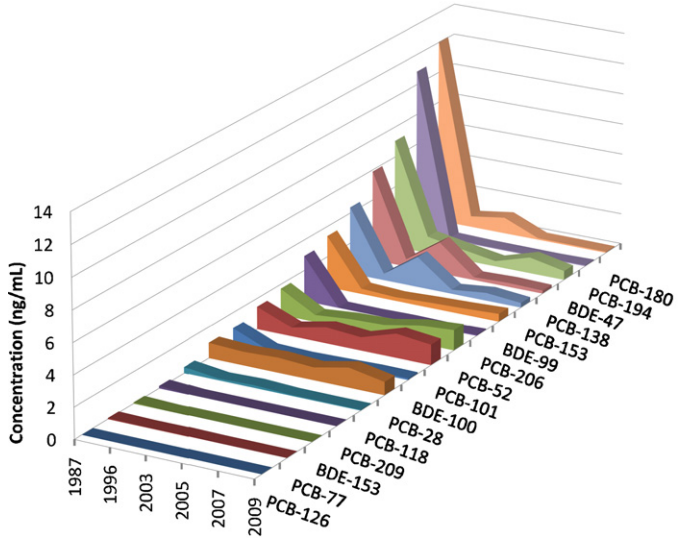

Fig. 2. Background PBDE and PCB levels in the filter paper manufactured in 1987, 1996, 2003, 2005, 2007 and 2009.

Since subtracting the background of filter paper is an important step due to inherent background contamination, the background levels must be reasonably far from the blood spot. During the spotting of blood, the filter paper may exhibit a paper chromatographic phenomenon, with bound water as the stationary phase and serum as the mobile phase. Since PCBs and PBDEs have a high retention factor (R_f) due to their lesser interaction with the stationary phase (water), these analytes could be dispersed outside the dried spots. Thus, the potential dispersion of analytes could cause significant underestimation for the target analyte if only partial filter was cut out for analysis. To study this, the blank filter papers were sampled and tested at 0, 1, 2, 3, and 4 mm from the margin of spiked DBS. The results showed no obvious difference between samples at distances of 1, 2, 3 and 4 mm. However, at zero distance, PCB126, PCB 169 and PBDE153 were detected. Since these compounds were usually undetectable in blank filter paper, the presence of these analytes at zero distance indicated that these analytes were likely originated from the DBS. Thus, our result suggests that the blank should be cut at least 1 mm away from the edge of the blood spot for background information; on the other hand, an extra 1 mm around the blood spot should be cut with the DBS for chemical analysis to minimize analyte losses on the filter paper.

3.2. Optimization of DBS extraction

Generally, formic acid is added to blood or serum to denature proteins before solvent extraction [19,20]. In this study, the addition of formic acid was found to effectively remove the dry blood spot from the filter paper and dispersed the solid blood spot by destroying the adhesion of dried blood with the filter paper. We found that this greatly improved the extraction efficiency of PCBs and PBDEs in DBS sample. Fig. 3 shows the concentration of all congeners with and without formic acid during the extraction procedure. Fig. 3 showed that the addition of formic acid enhanced the

Table 1
Percentage recoveries (Rec) and relative standard deviations (RSD) for measurement of PCB and PBDE congeners in high (0.16 ng mL^{-1}) and low (0.05 ng mL^{-1}) spiked levels (n = 8).

Congeners	Low spiked level (0.05 ng mL^{-1})				High spiked level (0.16 ng mL^{-1})			
	DBSV		DBS		DBSV		DBS	
	Rec.%	RSD, %	Rec.%	RSD, %	Rec.%	RSD, %	Rec.%	RSD, %
PCB-28	71	12	132	13	106	5	105	10
PCB-52	111	20	103	47	127	8	81	11
PCB-101	83	20	158	30	126	10	98	15
PCB-153	74	12	84	11	119	8	114	10
PCB-138	99	11	74	7	125	7	117	8
PCB-180	86	9	68	7	115	8	119	17
PCB-77	80	9	68	5	107	6	118	6
PCB-105	83	16	86	14	123	5	118	10
PCB-118	84	9	77	8	119	5	124	6
PCB-126	76	11	67	4	108	4	120	10
PCB-169	78	10	70	10	117	5	131	9
PCB-194	81	9	76	7	111	7	115	22
PCB-206	88	16	81	7	105	9	117	25
PCB-209	75	9	68	5	93	14	106	26
BDE-47	74	19	107	16	121	13	93	8
BDE-100	71	18	75	18	111	5	115	10
BDE-99	99	20	108	18	104	5	106	12
BDE-153	94	12	109	11	110	8	140	14
BDE-183	88	13	120	9	109	7	115	14

extraction efficiency of all congeners except for PCB209. In addition to formic acid assisted extraction, ultrasonication and vortex could also be applied for facilitating the extraction action of formic acid. Optimal extractions were completed in only 20 min, as opposed to overnight static soaking.

The extraction of PCBs and PBDEs from biological samples is typically carried out using hexane, methyl-tertiarybutyl ether (MTBE), dichloromethane (DCM), or a mixture of hexane with acetone, MTBE or DCM. Given the trace amount of analytes available in one drop of blood, the extraction solvent must be strong enough to extract all the analytes binding with protein in the blood or with filter paper. Extraction performance of different solvent compositions was evaluated for PBDEs and PCBs. Good recoveries for all PBDEs and PCBs were observed for both hexane/MTBE (1:1, v/v) and hexane/DCM (4/1, v/v) extraction solvents. However, the use of MTBE has been reported to be problematic previously due to its solubility in aqueous phase [27]. Therefore, hexane/DCM was selected as extraction solvent attributable to its compatible properties with the plasma blood and dried blood spot to its compatible properties with the plasma blood and dried blood spot. The ratio of DCM to hexane is typically chosen to maximize the DCM fraction during extraction of PCBs and PBDEs [27]. Our experiments showed that the recoveries of isotopic internal standards increased by more than 5% for 10 congeners, especially PCB77, with up to a 20% increase, when the ratio of DCM to hexane is equal to or greater than the ratio of 1–4. To maintain a lower density of the organic phase compared to the aqueous/formic acid phase, the ratio of DCM to hexane at 1–4 was optimal.

3.3. Stability test

The DBS requires less demanding storage conditions compared to other biological samples, such as the absence of refrigeration in some cases. DBS may delay the degradation process or even

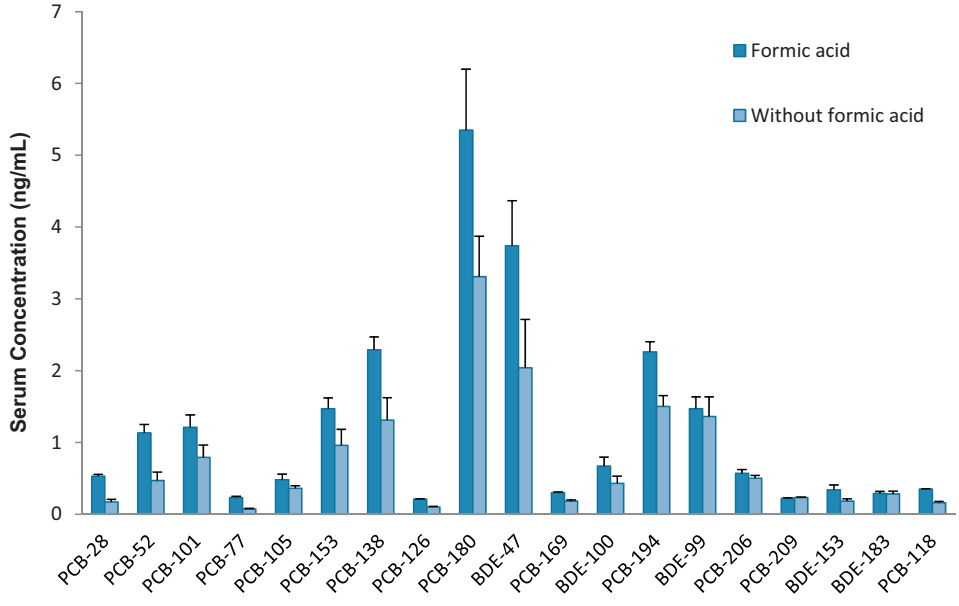

Fig. 3. Concentrations of 19 PCB and PBDE congeners in the final analytical solutions from extraction of samples with and without formic acid.

Table 2
Proficiency test was performed by using spiked DBS and DBS volume (DBSV) of blood (50 μL) against certified reference materials.

Congeners	DBS, ng kg^{-1}	DBSV, ng kg^{-1}	Certified value, ng kg^{-1}
PCB-28	11.5	9	9.3 ± 1.2
PCB-52	N.D.[a]	N.D.[a]	/
PCB-101	N.D.[a]	N.D.[a]	/
PCB-138	59	64	57.2 ± 3.3
PCB-153	38.5	44	36.9 ± 5.4
PCB-180	57	54	54.4 ± 1.3
PCB-77	N.D.[a]	N.D.[a]	/
PCB-105	8.5	5	4.1 ± 3.1
PCB-118	14	13	18.5 ± 2.7
PCB-126	N.D.[a]	N.D.[a]	/
PCB-169	N.D.[a]	N.D.[a]	/
PCB-194	13	12	12 ± 0.5
PCB-206	8	9	7.51 ± 0.4
PCB-209	3	4	3.58 ± 0.63
BDE-47	268.5	286	272 ± 14
BDE-100	55	52	50.5 ± 2.5
BDE-99	76.5	71	77.8 ± 1.7
BDE-153	65	59	62.1 ± 3
BDE-183	N.D.[a]	6	3.4 ± 2.3

[a] N.D., not detected.

preserve unstable analytes from degradation [30]. To study the stability of the target analytes, DBS which were made using duplicate spiked blood samples were wrapped in aluminum foil and stored at room temperature. The concentration of all congeners was determined by sampling at 0, 2, 7, 15, and 30 day intervals. Compared to the analyte concentrations measured at day 0, the analyte concentrations from all intervals were within ±15% of the nominal values, indicating that in DBS, analytes were stable for at least 30 days even at room temperature.

3.4. Comparison between the DBS and DBSV in recovery experiments and proficiency test

The LLE and C18 SPE are the workhorses of PBDEs and PCBs analysis of plasma or serum, largely because of the widespread availability of the technology and the ready compatibility of subsequent clean-up methods [13,27]. Generally, the volume of sample in LLE is more than 1 mL [19], and therefore, a large amount of interfering matrix was extracted along with the target analytes. As

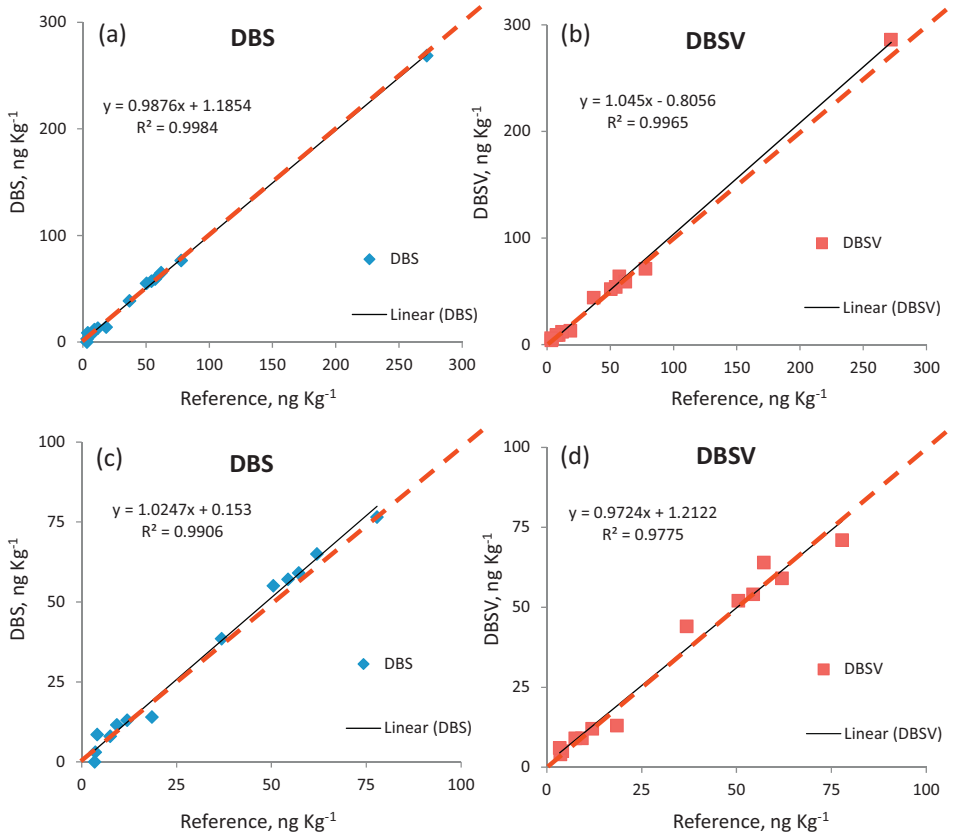

Fig. 4. Comparison of measured values of all PCB and PBDE congeners against reference values from NIST. (a) DBS; (b) DBSV; (c) DBS excluding the highest point from (a); (d) DBSV excluding the highest point in plot (c). The red dashed line represents an ideal slope (1:1) between two values. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

this affected chromatographic separation and peak shape, it may cause matrix induced response enhancement and target analyte loss. It is therefore necessary to perform clean-up with multilayer silica gel or other analogous chromatographic methods. In this study, our GC/HRMS system was calibrated with 20 fg μL^{-1} of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) with signal to noise ratio is greater or equal to 3. Given the high sensitivity of GC/HRMS and the small volume of applied plasma (50 μL), further clean-up after the extraction of DBS and DBSV is not necessary. The developed procedure was validated by analyzing DBSV samples spiked at two concentration levels (0.05 ng mL^{-1} and 0.16 ng mL^{-1}). The mean values and RSDs of each congener obtained in the recovery experiment of eight spiked samples at both low (0.05 ng mL^{-1}) and high concentrations (0.16 ng mL^{-1}) are listed in Table 1. For DBSV, the recoveries ranged from 71 to 111% at low level and varied from 93 to 127% at high level for targeted PCB and BDE congeners. Our results suggested that the analytical method is accurate even with small volume (50 μL) of blood.

Compared to DBSV, the recoveries for DBS varied to a wider range of 67–132% at low level and 81–140% at high level. The precision of the measurements for DBS were also affected, since RSDs ranged from 4 to 47% at low level and 6 to 26% at high level, compared to the RSD ranging from 9 to 20% at low level and 4 to 14% at high level for DBSV. It indicated that our results were interfered by the high background of filter. In particular, the recoveries for two congeners PCB28 and PCB101 that commonly associated with high background were 132% and 158%, respectively. At high level, DBS measurements were less affected by the filter paper background and hence both recoveries and RSD were restored. The blank, recoveries and precision studies indicated that the background of the filter remains one of the challenges for the measurements of PCBs and PBDEs in DBS samples. In this study, our results suggested that the DBS sampling technique is feasible for PCB and PBDE measurement with the current type of filter paper by GC/HRMS. However, the method LOD would be improved substantially if a more specialized filter paper can be used.

3.5. Proficiency test

In this study, our analytical method was validated by certified reference materials from NIST. All PBDEs and PCBs congeners except BDE-183 were within 95% confidence interval of reference values against the certified material (NIST SRM 1957). The result was shown in Table 2. BDE-183 could not be clearly separated by gas chromatography due to the interference by a large peak nearby in DBS, not DBSV. Thus, BDE-183 was not reported in the DBS analysis. Fig. 4 shows the comparison of measured values of all PCB and PBDE congeners against the certified values from the reference materials. PCB52, PCB101, PCB77, PCB123 and PCB169 were excluded in these plots, because these compounds were not included in the reference material. Linear correlation coefficients were 0.9985 and 0.9968 for DBS and DBDV analysis, respectively. Also, the slopes for DBS and DBSV against reference values were close to 1, which indicated that our measured values were in good agreement with reference values. Therefore, our method was validated to be effective in PCB and PBDE congener analysis by using small volume of blood and GC/HRMS. Applications of this effective approach could be extended to other biomarkers in the future study.

4. Conclusions

A novel analytical method for PBDEs and PCBs in DBSV and DBS by GC/HRMS has been developed and validated. Using one small spot (corresponding 50 μL sample), the LODs of PCBs ranged from 0.002 to 0.005 ng mL^{-1}, and LODs of PBDEs ranged from 0.007 to 0.02 ng mL^{-1}. Precision, robustness, DBS stability and accuracy were performed for method evaluation. Our study suggested that the analysis of PCBs and PBDEs can be performed in a small drop of blood and these target analytes are stable in DBS storage form. Thus, it is feasible to use DBS sample collecting technique for PCB and PBDE analysis. The use of DBS provides a cost effective alternative for sample collection by reducing the sample size in transportation and storage. We expect that it will eventually enable us to extend the biomonitoring network by using DBS in the United States. However, the filter paper background remains the major limitation for widespread application. We hope that with the advancement of filter paper materials and filter paper pretreatment technique, the filter paper background would be resolved in the near future. Therefore, application of DBS will soon play a much more important role in biomonitoring program.

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