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RESEARCH PAPER

Simultaneous Determination of Multiple Persistent Halogenated Compounds in Human Breast Milk

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Abstract: A method was developed for simultaneous determination of multiple persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs), hexabromocyclododecanes (HBCDs), polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs), in human breast milk. The sample pretreatment procedure included liquid-liquid extraction, gel permeation chromatography (GPC), and solid-phase extraction (SPE) cleanup. Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-tandem mass spectrometry (GC-MS/MS) were applied in the analysis of target compounds. By using GPC to remove high-molecular-weight lipids and SPE for further cleanup and separation of target compounds, the interferences from the biological matrix could be greatly reduced. The developed sample pretreatment is suitable for multi-component analysis of very low concentrations of POPs in small volume of human samples. The developed method of simultaneous determination of PCBs and OCPs by GC-MS/MS exhibited excellent sensitivity and selectivity. Spiked recoveries were 88.7%–98.8% for PBDEs, 88.5%–92.5% for HBCDs, 67.9%–82.3% for PCBs, and 81.7%–116.1% for OCPs, and limits of detection were 0.13–1.8 ng L^{-1} for PBDEs, 0.31–1.2 ng L^{-1} for HBCDs, 0.22–1.4 ng L^{-1} for PCBs, and 0.20–1.5 ng L^{-1} for OCPs. This method was applied to the analysis of POPs in 20 human breast milk samples from Weifang City. The result showed that the median concentrations of HBCDs, PBDEs, PCBs, and OCPs were 2.86, 7.76, 8.84 and 643 ng g^{-1} lipid weight, which were comparable to those in human breast milk samples from populations of other regions in China.

Key Words: Hexabromocyclododecanes; Polybrominated diphenyl ethers; Polychlorinated biphenyls; Organochlorine pesticides; Human breast milk

1 Introduction

The persistent organic pollutants (POPs), such as hexabromocyclododecanes (HBCDs), polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs), are ubiquitous contaminants in the environment, and have raised concerns about their adverse health effects for many years because of their persistence, long-range transport, bioaccumulation, and high potential toxicity. During 2001 to 2013, PCBs, dichlorodiphenyltrichloroethane (DDT), pentabromodiphenyl ethers (PentaBDEs), octabromodiphenyl ethers (OctaBDEs), lindane, hexachlorocyclohexane (HCH), and HBCDs have been listed in the Stockholm Convention on POPs. Although the use of these POPs has been forbidden in many countries, relatively high concentration levels of these compounds are still detected in environmental samples because of their high stability and wide use throughout history. Human populations, particularly those in regions still using POPs, may be exposed to these compounds via the food chain or by air inhalation and may potentially beat risk of developing health problems[1]. Therefore, it is necessary to carried long-term monitoring of

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POP levels in human population, which can provide basic data for human health risk assessments.

Many methods have been developed for determination of POPs in human samples. However, these methods usually determine compounds separately, as is the case for brominated flame retardants (BFRs) and OCPs[2–4]. Analysis of biological samples such as breast milk and serum is very difficult, because the sample volume is usually relatively small, and it is difficult to obtain sufficiently large volume of samples for different pretreatment procedures. Furthermore, POP concentrations in human samples are very low (at ng mL^{-1} level). Therefore, it is necessary to develop methods for simultaneous determination of various POPs in human matrixes. There are only few reports that have focused on development of analytical methods. Sahlström *et al*[5] developed a method for detecting multiple BFRs in human serum. Acid-resistant BFRs and acid-sensitive BFRs were separated by solid-phase extraction (SPE), and then H_2SO_4 and aminopropyl- functionalized silica column were used for clean-up. Shi *et al*[6] established a method for simultaneous determination of HBCDs, PBDEs, and tetrabromobisphenol A (TBBPA) in human serum and breast milk. Wang *et al*[7] developed a method for analysis of various persistent halogenated organic compounds, including PCBs, OCPs, PBDEs, dechlorane plus, pentabromobenzene, and pentabromotoluene, in human serum. Shi *et al*[6] and Wang *et al*[7] used gel permeation chromatography (GPC) to remove fat and then used H_2SO_4 or sulfuric acid silica for further clean-up. Due to their complicated matrixes, human samples can produce various compounds when reacting with acid during clean-up procedure with H_2SO_4 or silica functionalized with sulfuric acid; these products may interfere with the analysis of POPs. For example, cholesterol can undergo dehydration by H_2SO_4 to cholestene, resulting in ion suppression or peak distortion of BDE-99 or BDE-100 during detection by gas chromatography-mass spectrometry (GC-MS)[8].

The purpose of this work was to establish a method for simultaneous determination of POPs including HBCDs, PBDEs, PCBs, and OCPs in human breast milk. Liquid-liquid extraction, GPC, and SPE cleanup were used in sample preparation. PCBs and OCPs were then analyzed by gas chromatography-tandem mass spectrometry (GC-MS/MS) with high sensitivity and selectivity.

2 Experimental

2.1 Instruments and reagents

Standard solutions of PBDEs (IUPAC Nos. BDE-28, 47, 99, 100, 153, 154, 183 and 209), PCBs (IUPAC Nos. PCB-28, 52, 101, 118, 138, 153 and 180), and individual congeners of PBDE (BDE-77, 128, 181, 196, 197, 201, 202, 203, 206, 207, 208 and 209) were purchased from AccuStandard Inc. (New Haven, CT, USA). Native, $^{13}C_{12}$-labeled and d_{18}-labeled α-, β-, and γ-HBCD; $^{13}C_{10}$-labeled PCB-141; $^{13}C_{10}$-labeled PCB-208; standard solutions of ^{13}C-labeled OCPs including $^{13}C_6$-labeled β-HCH, $^{13}C_{12}$-labeled 4,4'-dichlorodiphenyldichloroethylene (p,p'-DDE) and ^{13}C-labeled p,p'-DDT were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Standard solution of OCPs including α-, β-, γ- and δ-HCH, p,p-DDE, 1,1-(2,2-dichloroethylidene)bis(4-chlorobenzene) (p,p-DDD), and p,p-DDT were purchased from Supelco (Bellefonte, PA, USA).

Dichloromethane (pesticide grade) was purchased from CNW Technologies (Düsseldorf, Germany). Methyl *tert*-butyl ether, *n*-hexane, methanol, and ethanol (LC grade) were obtained from Merck (Darmstadt, Germany). Potassium oxalate monohydrate (99% pure) was purchased from Alfa Aesar (Ward Hill, MA, USA). Anhydrous Na_2SO_4 (analytical grade) was purchased from Enox (Shanghai, China). Bio-Beads S-X3 (Bio-Rad Laboratories, Hercules, CA, USA) and silica gel (63–200 μm, Merck, Germany) were used in this study.

The instruments including a BS210Ss electronic balance (Sartorius, Goettingen, Germany), a TDL-5A centrifuge (Fichal, Shanghai, China), an R-210 rotary evaporator (Buchi, Flawil, Switzerland), and polytetrafluoroethylene centrifugal tubes (Oak Ridge, TN, USA) were used in this study. Chromatography systems used in this study included Agilent 7890A gas chromatograph coupled to Agilent 5975C mass spectrometer (Agilent Technologies, Palo Alto, CA, USA), an Agilent 7890A gas chromatograph coupled to an Agilent 7000 triple quadrupole mass spectrometer (Agilent Technologies), and an Agilent 1100 series liquid chromatograph (Agilent Technologies) coupled to an Applied Biosystems/Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA).

2.2 Preparation of experimental apparatus

All glassware were washed with hot chromic acid (5% (*m/V*) $K_2Cr_2O_7$ in sulfuric acid solution). After dipped for 5 h, the glassware were rinsed with deionized water, dried at 110 °C, and heated at 450 °C for 5 h. Anhydrous Na_2SO_4 was stored in sealed containers after it was baked at 450 °C for 5 h. The silica gel was activated at 150 °C for 12 h and then deactivated with distilled water (2.5%, *w/w*) before use.

2.3 Sample collection

Twenty human breast milk samples were collected between November 2010 and March 2011 from a hospital in Weifang City, Shandong Province. Before collection, all volunteers were informed about the objectives of the study, and all agreed to participate in the study. For each donor, about 20 mL of breast milk was collected and then stored at −80 °C

until analysis. Information such as age, parity, and occupation were obtained at the same time.

2.4 Sample extraction and cleanup

On the basis of our previous study for PBDE determination in human breast milk[9], a new pretreatment procedure was developed in this study. The procedure mainly included extraction and lipid determination, lipid removal, and cleanup.

2.4.1 Extraction of lipid

Each milk sample was thawed in a refrigerator (4 °C). After a 5 mL portion was spiked with indicators (BDE-77, 128 and 181; $^{13}C_6$-labeled β-HCH; $^{13}C_{12}$-labeled 4,4-DDE and 4,4-DDT; $^{13}C_{10}$-labeled PCB-141; and $^{13}C_{12}$-labeled α-, β- and γ-HBCD), the milk sample was homogenized in a Teflon tube and stored in the dark overnight. Before extraction, 5 mL of water was added into the sample which was then denatured using 1 mL of saturated potassium oxalate solution and 10 mL of ethanol. Subsequently, the milk was extracted once with 10 mL of mixture of methyl *tert*-butyl ether and *n*-hexane (1:1, *V/V*), and twice with *n*-hexane (5 mL each time). The organic phase was combined and rotate evaporated to about 1 mL, and then blew to dry with high-purity nitrogen. The lipid was then determined gravimetrically.

2.4.2 Clean-up and solid phase extraction

After the lipid content was determined by weighing, the extract was redissolved in 1 mL of mixture of *n*-hexane and dichloromethane (1:1, *V/V*) and cleaned up in a GPC system with a mixture of *n*-hexane and dichloromethane (1:1, *V/V*) as the mobile phase (at a flow rate of 2 mL min^{-1}). The first 55 mL of the eluate was discarded, and subsequent 120 mL of fraction which contained the target compounds was collected. The GPC fraction was concentrated to 1.0 mL, and further cleaned up on a SiO_2 SPE column (containing 0.5 g of silica deactivated with 2.5% (*m/m*) H_2O) to separate polar and non-polar fractions. Fraction I, e.g., non-polar fraction, which contained PBDEs, PCBs and DDTs, was eluted with 10 mL of *n*-hexane. Fraction II, e.g. polar fraction, which contained HCHs and HBCDs, was eluted with 7 mL of mixture of *n*-hexane and dichloromethane (1:1, *V/V*). Fraction I and II were blew to dry under a gentle stream of nitrogen gas, and dissolved in 20 μL of *iso*-octane. Then $^{13}C_{10}$-labeled PCB-208 was added as internal standard for PBDEs, PCBs and OCPs. After OCPs contents in fraction II were analyzed by GC-MS/MS, the solvent was exchanged to 200 μL of methanol, and d_{18}-labeled α-, β-, γ-HBCD was added as internal standard for determination of HBCDs by LC-MS/MS.

2.5 Instrumental analysis

2.5.1 Analysis of PBDE by GC-NCI-MS

A nonpolar DB-5HT column (15 m × 250 μm i.d., 0.10 μm film, J&W Scientific, Folsom, CA, USA) was used for the separation of PBDE congeners. High-purity helium, introduced at a flow rate of 1.2 mL/min, was used as the carrier gas. The injection volume was 1 μL. The GC injection port was held at 290 °C. The GC oven temperature program was set as follows: the initial temperature was 110°C and held for 5 min, raised to 200 °C at 20 °C min^{-1}, and held for 4.5 min; then raised to 300 °C at 7.5 °C min^{-1} and held for 10 min. Selected ion monitoring in negative chemical ionization (NCI) mode was used for quantification. Methane was used as a chemical ionization moderating gas. The transfer line, ion source, and quadrupole temperatures were set at 300, 250 and 150 °C, respectively. The following ions were monitored: *m/z* 475.7 and *m/z* 473.7 for $^{13}C_{10}$-labeled PCB-208, *m/z* 79 and *m/z* 81 for tri- to octaBDEs, *m/z* 486.7 and *m/z* 488.7 for nona-BDEs and decaBDEs.

2.5.2 Determination of PCB and OCP by GC-MS/MS

A nonpolar DB-5MS column (30 m × 250 μm i.d., 0.25 μm, J&W Scientific) was used to analyze OCPs and PCBs. High-purity helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. The injection volume was set as 1 μL. The GC injection port was held at 250 °C. The GC oven temperature program was set as follows: initial temperature at 70 °C (held for 1 min), raised to 160 °C at 10 °C min^{-1}, raised to 280 °C at 5 °C min^{-1} and held for 5 min, and then raised to 300 °C at 20 °C min^{-1} and held for 5 min. Multiple reaction monitoring (MRM) mode using an electron impact ion source was adopted in the quantification. High-purity helium and nitrogen were used as collision gases. The transfer line, ion source, and quadrupole temperatures were set at 300, 230 and 150 °C, respectively.

2.5.3 Analysis of HBCD by LC-MS/MS

A Zorbax SB-C_{18} reversed-phase column (250 mm × 4.6 mm i.d., 5 μm, Agilent Technologies) was used to separate HBCD diastereoisomers. The injection volume was 20 μL. The mobile phase for LC analysis was a mixture of water (A), methanol (B), and acetonitrile (C). The flow rate was set at 0.5 mL min. The gradient elution was started at an initial A/B/C composition of 10:80:10 (*V/V*). Subsequently, the composition was changed to A/B/C = 10:50:40 (*V/V*) in 18 min and to B/C = 30:70 (*V/V*) at 23 min, held for 7 min. Finally, the elution was carried out at A/B/C = 10:80:10 (*V/V*) for 5 min and then the column was equilibrated for another 10 min. Electrospray ionization negative ion detection in MRM mode was used for quantification. High-purity nitrogen was used as auxiliary gas, curtain gas and collision gas. The ion pairs monitored were

m/*z* 640.7/78.8 for native HBCDs, *m*/*z* 658.7/78.8 for d_{18}-labeled HBCDs, and *m*/*z* 652.7/78.8 for $^{13}C_{12}$-labeled HBCDs.

3 Results and discussion

3.1 Optimization of separation and clean-up method

Sample cleanup is very important for the removal of interferences from biological matrix in analysis of trace POP. In this study, we first used a GPC column to remove most of the biomacromolecules in human breast milk, such as lipids and proteins, and then the small-molecule interferences in the matrix were further separated on a SiO_2 SPE column

After optimization, the final SPE procedure could effectively remove the interferences in biological matrix. By changing polarity of solvent, the analytes were eluted into two fractions in the SPE procedure: fraction I which contained PCBs, PBDEs and DDTs, and fraction II which contained HCHs and HBCDs. Non-polar fraction I was eluted with *n*-hexane. The polarity of the eluent was then increased by using a mixture of *n*-hexane and dichloromethane (1:1, *V*/*V*) to elute polar fraction II. Whereas small molecules with stronger polarity in the matrix were retained in the SPE cartridge.

3.2 Optimization of GC-MS/MS for analysis of PCBs and OCPs

After sample pretreatment, both PCBs and most of OCPs (except for HCHs) were eluted in fraction I. Because of low concentration levels of PCBs and OCPs in human serum and milk, GC-MS/MS with high sensitivity was used for determination. A simultaneous determination of DDTs and PCBs could decrease injection times and increase detection efficiency. Three steps were used for optimization of instrumental parameters. Firstly, precursor ions were determined by analyzing an authentic standard under MS full scan mode (Scan range, *m*/*z* 50–500). Secondly, product ions were further determined by analyzing a standard in product ion scan mode to obtain the quantification and qualification ion pairs. Thirdly, the collision energy (CE) for each MRM transition was optimized by analyzing standards at different CEs in MRM mode. The final optimized quantitative and qualitative ion pairs, as well as CEs in MRM mode in GC-MS/MS analysis of OCPs and PCBs, are listed in Table 1.

3.3 Limits of detection, recoveries and stability

A series of standard solutions at concentration levels of 2.5, 5, 10, 25, 50, 100, 200 and 400 ng mL^{-1}, were diluted from

Table 1 Instrument parameters for determination of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBS) by gas chromatography-tandem mass spectrometry (GC-MS/MS)

Compounds	Quantitative ion pair (*m*/*z*)	Collision energy (eV)	Qualitative ion pair (*m*/*z*)	Collision energy (eV)
α-HCH	219>183	10	256>183	10
β-HCH	219>183	10	256>183	10
γ-HCH	219>183	10	256>183	10
4,4'-DDE	246>176	45	318>248	20
4,4'-DDD	235>165	15	318>235	15
4,4'-DDT	235>165	10	354>235	5
$^{13}C_6$-*β*-HCH	225>189	10	223>187	10
$^{13}C_{12}$-4,4'-DDE	258>188	45	332>262	20
$^{13}C_{12}$-4,4'-DDT	247>177	10	368>247	5
PCB-28	256>186	15	258>186	15
PCB-52	290>220	15	292>222	15
PCB-101	324>254	16	326>256	16
PCB-118	324>254	16	326>256	16
PCB-138	360>290	20	362>292	20
PCB-153	360>290	20	362>292	20
PCB-180	394>324	20	392>322	20
$^{13}C_{10}$-PCB-141	372>302	20	374>304	20
$^{13}C_{10}$-PCB-208	474>404	35	476>406	35

α-, *β*-, and *γ*-HCH: *α*-, *β*- and *γ*-hexachlorocyclohexane; 4,4'-DDE: 4,4'-dichlorodiphenyldichloroethylene; 4,4'-DDD: 1,1'-(2,2-dichloroethylidene)bis(4-chlorobenzene); 4,4'-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; $^{13}C_6$-*β*-HCH: $^{13}C_6$-labeled *β*-hexachlorocyclohexane; $^{13}C_{12}$-4,4'-DDE: $^{13}C_{12}$-labeled 4,4'-dichlorodiphenyldichloroethylene; $^{13}C_{12}$-4,4'-DDT: $^{13}C_{12}$-labeled 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; PCB-28: 2,4,4'-trichlorobiphenyl; PCB-52: 2,2'5,5'-tetrachlorobiphenyl; PCB-101: 2,2'4,5,5'-pentachlorobiphenyl; PCB-118: 2,3',4,4'5-pentachlorobiphenyl; PCB-138: 2,2'3,4,4',5'-hexachlorobiphenyl; PCB-153: 2,2'4,4'5,5'-hexachlorobiphenyl; PCB-180: 2,2',3,4,4'5,5'-heptachlorobiphenyl; $^{13}C_{10}$-PCB-141: $^{13}C_{10}$-labeled 2,2',3,4,5,5'-hexachlorobiphenyl; $^{13}C_{10}$-PCB-208: $^{13}C_{10}$-labeled 2,2',3,3',4,5,5',6,6'-nonachlorobiphenyl.

stock solution. A seven-point calibration curve for individual compounds (concentration range of 5–400 ng mL^{-1} for OCPs, and 2.5–200 ng mL^{-1} for other POPs), with concentration ratio of standard to internal substance as *Y* axis and peak area ratio of standard to internal substance as *X* axis, was established. The standard curves exhibited good linearity ($R^2 \geq 0.998$) in respective linear concentration range. The standard deviation (*S*) was obtained by six repetitive determinations using the standard solution at a low concentration level. According to the formula for the limit of detection (LOD = 3.36*S*), the instrument LODs for PBDEs, HBCDs, PCB and OCPs were 0.06–0.25 pg μL^{-1}, 0.08–0.19 pg μL^{-1}, 0.07–0.28 pg μL^{-1}, and 0.07–0.18 pg μL^{-1}, respectively. Based on the low concentrations of the analytes in human breast milk (5 mL), the method LODs for PBDEs, HBCDs, PCB, and OCPs were 0.13–1.76 pg mL^{-1}, 0.31–1.18 pg mL^{-1}, 0.22–1.37 pg mL^{-1} and 0.20–1.5 pg mL^{-1}, respectively.

Because of the difficulties in sampling and the relatively high concentrations of DDTs and HCHs (the lowest concentrations of which are 149 and 52 ng g^{-1} lipid weight (lw), respectively[10]) in human breast milk, it is not feasible for spiking test using human breast milk sample in the method development. Therefore, cow's milk were usually chosen as biological matrix for assessment of recoveries[10–12]. In the method established by Shi *et al*[6] for determination of HBCDs, PBDEs, and TBBPA in human serum and breast milk, both fetal bovine serum and cow milk were used in the matrix spiking test. The recoveries of analytes in cow milk and fetal bovine serum were similar. However, the relative standard deviations (RSDs) of recoveries in cow milk were significantly higher than those in bovine serum, especially for *α*-HBCD (13.9% *vs* 2.7%) and *β*-HBCDs (14.0% *vs* 1.7%). Similar result was also observed in the present study. Method performance was poor when cow milk was used as the matrix. Nevertheless, the recoveries for the spiked indicator and the corresponding RSDs for fetal bovine serum and in human breast milk were similar (Table 2), indicating that fetal bovine serum can replace human breast milk as the matrix in the spiking test. Therefore, fetal bovine serum (5 mL) was used in matrix spiking test in our study. As shown in Table 3, the recoveries of PBDEs, HBCDs, PCBs, and OCPs in the spiked fetal bovine serum were 88.7%–98.8%, 88.5%–92.5%, 67.9%–82.3%, and 81.7%–116.1%, respectively. The corresponding RSDs were 8.7%–11.7%, 3.6%–4.0%, 0.7%–5.5%, and 7.5%–11.1%, respectively. These results indicated that the developed method had good recoveries and high repeatability.

In comparison with the method for simultaneous detection of multiple contaminants in published studies, this method has good recoveries and a lower LOD (Table 4) in determination of POPs in human breast milk.

3.4 Application to POPs detection of human breast milk

The developed method was applied to the determination of PBDEs, HBCDs, PCBs, and OCPs concentrations in 20 human breast milk samples from Weifang City. Typical total ion chromatograms for POPs in human breast milk (Fig.1) indicate that higher brominated BDEs (octa- to deca-BDEs) are the major PBDE congeners in this study (Fig.1A). Similar results were obtained with human breast milk samples from Shanghai City in our previous study[9]. *α*-HBCD was the dominant diastereomer in all samples (Fig.1B), similar to a finding in a study on human breast milk samples from 12 provinces in China[15]. PCB-28 and PCB-153 were the primary PCB congeners (Fig.1C), which was consistent with those found in food in a study in China[16]. 4,4'-DDE and *β*-HCH were the dominant DDTs and HCHs, respectively (Fig.1C and 1D), similar to the dominant compounds in human breast milk from general populations in other regions in China[10]. The POP concentrations are shown in Fig.2. Median concentrations of HBCDs, PBDEs, PCBs, HCHs, and DDTs are 2.86, 7.76, 8.84, 140 and 503 ng g^{-1} (lw), respectively. These

Table 2 Recoveries for the spiked indicator in fetal bovine serum and Human breast milk samples

Compound	Concentration (ng mL^{-1})	Fetal bovine serum (n = 6)		Human milk (n = 6)	
		Recovery	Stability	Recovery (%)	Stability (RSD, %)
BDE-77	20	98.7	6.1	103.0	2.7
BDE-128	20	91.3	7.5	94.7	5.5
BDE-181	20	90.9	7.1	94.1	4.7
$^{13}C_{12}$-*α*-HBCD	10	90.2	4.0	93.7	5.1
$^{13}C_{12}$-*β*-HBCD	10	92.5	3.7	93.1	3.7
$^{13}C_{12}$-*γ*-HBCD	10	88.5	3.6	90.2	2.1
$^{13}C_{6}$-*β*-HCH	50	95.6	5.1	100.7	4.8
$^{13}C_{12}$-4,4'-DDE	125	82.8	4.8	85.5	5.0
$^{13}C_{12}$-4,4'-DDT	50	91.3	6.8	98.4	6.5
$^{13}C_{10}$-PCB-141	20	75.4	3.2	78.3	3.4

BDE-77: 3,3',4,4'-tetrabromodiphenyl ether; BDE-128: 2,2',3,3',4,4'-hexabromodiphenyl ether; BDE-181: 2,2',3,4,4′,5,6-heptabromodiphenyl ether; $^{13}C_{12}$-*α*-HBCD: $^{13}C_{12}$-labeled *α*-hexabromocyclododecane; $^{13}C_{12}$-*β*-HBCD: $^{13}C_{12}$-labeled *β*-hexabromocyclododecane; $^{13}C_{12}$-*γ*-HBCD: $^{13}C_{12}$-labeled *γ*-hexabromocyclododecane.

Table 3 Recoveries of persistent organic pollutants in spiked fetal bovine serum samples (n = 6)

Compound	Concentration (ng mL^{-1})	Recovery (%)	Stability (RSD, %)	Compound	Concentration (ng mL^{-1})	Recovery (%)	Stability (RSD, %)
PCBs				PBDEs			
PCB-28	20	69.0	0.7	BDE-28	20	95.4	8.7
PCB-52	20	67.9	3.4	BDE-47	20	97.3	9.2
PCB-101	20	69.2	3.6	BDE-99	20	98.8	10.0
PCB-118	20	76.8	5.5	BDE-100	20	93.0	9.4
PCB-153	20	75.0	3.9	BDE-153	20	97.3	10.3
PCB-138	20	77.9	4.5	BDE-154	20	94.2	10.8
PCB-180	20	82.3	5.1	BDE-183	20	88.7	10.3
OCPs				BDE-209	100	97.2	11.7
α-HCH	20	112.3	9.8	HBCDs			
β-HCH	20	107.5	9.2	α-HBCD	10	90.2	4.0
γ-HCH	20	116.1	11.1	β-HBCD	10	92.5	3.7
4,4'-DDE	20	81.7	8.5	γ-HBCD	10	88.5	3.6
4,4'-DDD	20	98.5	10.8				
4,4'-DDT	20	100.3	7.5				

BDE-28: 2,4,4'-tribromodiphenyl ether; BDE-47: 2,2',4,4'-tetrabromodiphenyl ether; BDE-99: 2,2',4,4',5-pentabromodiphenyl ether; BDE-100: 2,2',4,4',6-pentabromodiphenyl ether; BDE-153: 2,2',4,4',5,5'-hexabromodiphenyl ether; BDE-154: 2,2',4,4',5,6'-hexabromodiphenyl ether; BDE-183: 2,2',3,4,4',5',6-heptabromodiphenyl ether; BDE-209: 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether; α-HBCD: α-hexabromocyclododecane; β-HBCD: β-hexabromocyclododecane; γ-HBCD: γ-hexabromocyclododecane.

Table 4 Comparison of detection results by this method and combined multi-component detection methods in the literature

Samples	PBDEs		PCBs		OCPs		HBCDs		Reference
	LOD* (pg mL^{-1})	Recovery (%)	LOD* (pg mL^{-1})	Recovery (%)	LOD* (pg mL^{-1})	Recovery (%)	LOD* (pg mL^{-1})	Recovery (%)	
Human serum	0.5–4.1	64–82	—	—	—	—	0.06–0.1	48–101	[5]
	—	72–110	—	55–61	—	81–130	—	—	[7]
	—	—	0.7–5.1	44–83	0.1–6.6	—	—	—	[13]
Human milk	3.3–333	99–113	0.33–3.3	60–68	—	—	—	—	[14]
	2.5–40	81–109	—	—	—	—	20–50	95–102	[6]
	0.1–1.8	89–99	0.2–1.4	68–82	0.2–1.5	82–116	0.3–1.2	89–93	This study

*: Limit of detection.

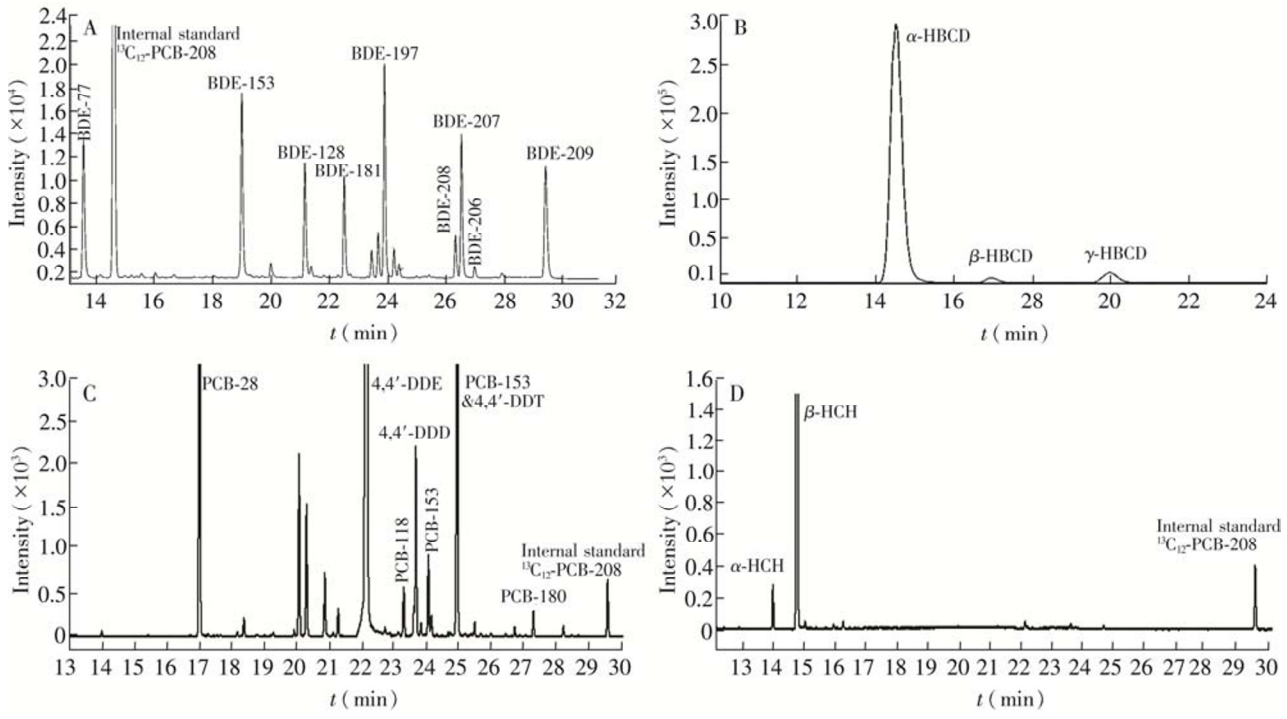

Fig.1 Typical total ion chromatograms for POPs in human breast milk, obtained by (A) GC-NCI-MS(for PBDEs), (B) LC-MS/MS(for HBCDs), (C) GC-MS/MS (for PCBs and DDTs), and (D) GC-MS/MS(for HCHs)

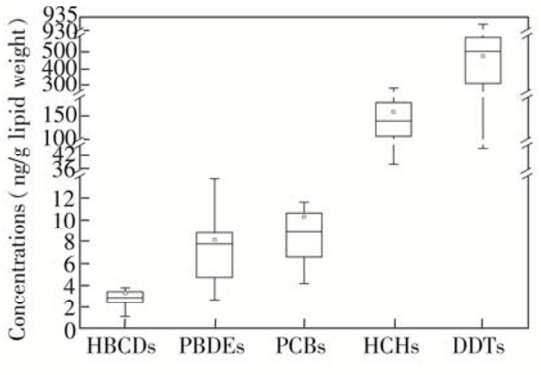

Fig.2 POPs levels in Human breast milk from Weifang (n = 20)

concentrations are identical to those in human breast milk samples from other regions in China[9,10,12,17]. POPs at the highest concentrations in this study were DDTs, followed by HCHs, in agreement with survey results for POPs in human breast milk samples from 12 provinces in China[10,15,17]. These results indicate that DDTs and HCHs are the most abundant POPs in the general population in China. Their abundance may be related to their resistance to degradation in biota and in the environment, as well as the widespread use of DDTs and HCHs throughout the history of China.

4 Conclusions

A new method was developed for the simultaneous determination of HBCDs, PBDEs, PCBs, and OCPs in human breast milk. This method used GPC instead of H_2SO_4 to remove lipid to avoid interferences from the matrix produced during lipid removal. Here, interferences from the biomatrix were reduced to a great extent via SPE column cleanup. OCP and PCB detection by GC-MS/MS further decreased the matrix interference. This method has good recovery and stability, and is suitable for POPs detection in human breast milk.

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