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Study on simultaneous determination of OCPs, PCBs and PBDEs in fish sample: application for marine fish tissues collected from Hai Phong Province

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Abstract

In this work, a GC-MS/MS-based analytical method using accelerated solvent extraction combined with multi-layer silica gel column for sample preparation was developed to simultaneously determine 20 organochlorine pesticides (OCPs), 28 polychlorinated biphenyls (PCBs) and 8 polybrominated diphenyl ethers (PBDEs) in fish tissue. The method detection limits (MDLs) were achieved in the range of 0.053 ng/g (α-chlordane) – 1.65 ng/g (δ-BHC), 0.07 ng/g (PCB-209) – 1.84 ng/g (PCB-28), 0.323 ng/g (BDE-209) – 0.796 ng/g (BDE-47) for OCPs, PCBs and PBDEs, respectively. Intra-day and inter-day repeatability of the analytical signal (peak area) were below 10.5% and 12.4%, correspondingly. The overall recovery was investigated by spiking experiments and ranged from 70.9 to 114%. The confirmation of this developed method was assessed by analysis of the standard reference material (SRM-1947) sample. The measured concentrations of target compounds were within the range of the certified values. This developed method was applied for analysis of OCPs, PCBs and PBDEs in five fish tissues collected randomly from local markets at Hai Phong province. Their concentrations (<MDL – 206 ng/g for OCPs, <MDL – 20.7 ng/g for PCBs and <MDL – 66.7 ng/g for PBDEs) were lower than the maximum residue levels permitted by European Union, Food and Agriculture Organization and World Health Organization.

Keywords: OCPs, PCBs, PBDEs, GC-MS/MS, fish tissue.

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1. INTRODUCTION

Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) are halogenated organic substances, they are classified as persistent organic pollutants (POPs) [1]. PBDEs are used as a flame retardant. PCBs are used in capacitors and transformer stations. OCPs are used to kill insects, and in the past were included in topical pharmaceuticals [1, 2]. According to the International Agency for research on cancer (IARC) classification, PCBs, OCPs and PBDEs are classified in group 1 - carcinogenic to humans, group 2A - probably carcinogenic to human and group 3 - not classified as to its carcinogenic to humans, respectively [3]. However, previous studies reported the existence of a relationship between PBDEs and OCPs exposures with the risk of cancer [4, 5]. Furthermore, these substances also cause a variety of other effects on human health [6–8]. POPs persist in the environment and easily bio-accumulate in human and animal fatty tissue through the food chain [9–12]. The Stockholm Convention 2001 has limited or eliminated the production of POPs, although no longer produced, their residues present in the environment can still pose a danger to human health [11]. The US Environmental Protection Agency (US EPA) and the International Organization for Standardization (ISO) have introduced standard methods for determining POPs in biological and environmental samples. Most of them are single analysis of a certain group of substances (US EPA 8081B for OCPs; US EPA 1668C and ISO 13876 for PCBs; US EPA 1614A and ISO 22032 for PBDEs).

In this work, an analytical method to simultaneously determine 20 OCPs, 28 PCBs and 8 PBDEs in fish samples was developed. They were extracted using accelerated solvent extraction (ASE)- a green technique with cost efficiency compared to traditional extraction one like Soxhlet extraction. Multi-layer silica gel column combined with concentrated sulfuric acid for removing a high content of lipid was applied for clean-up step. Analysis was performed by gas chromatography (GC)-tandem mass spectrometry (MS/MS). Finally, this validated method was applied for OCP, PCB and PBDE analysis in several fish tissues collected from local market in Hai Phong province.

2. MATERIALS AND METHODS

2.1. Reagents and Chemicals

The native standard (NS), labeled standard (LS) and internal standard (IS) for OCP analysis were the Pesticide 8081 Standard Mix including 20 OCPs (P/N: CRM46845, Sigma-Aldrich), d_6- γ-HCH (P/N: 684848, HPC, Germany) and 4,4'-DDT-D_8 (P/N: LM24-N-15900-1311D-100CY1, Lab Mix 24, Germany), respectively. Similarly, the WHO/NIST/NOAA Congener list including 28 PCBs (P/N: C-WNN, Accustandard, USA), ^{13}C-labeled PCB mixture, 5 µg/mL (P/N: EC-4058, ^{13}C-PCB-28/52/101/138/153/180/209,

CIL, USA) and ^{13}C-labeled PCB mixture - A, 1 µg/mL (^{13}C-PCB-77/81/123/126/169/180, CIL, USA) were produced for PCB analysis. The Congeners of Primary Interest (P/N: BDE-CSM, Accustandard, USA), PBDE surrogate standard mixture ($^{13}C_{12}$, 99%) 5 µg/mL (^{13}C – BDE-28/47/100/153/183/209) and BDE-139 C^{13} (CIL, USA) were purchased for PBDE analysis. The OCP, PCB and PBDE concentrations in seven working standard solutions were 1 – 200 ng/mL, 0.2 – 200 ng/mL and 0.5 – 200 ng/mL respectively. All of them were prepared in hexane with the range of LS concentrations (2 – 100 ng/mL) and constant IS concentration (50 ng/mL).

Solvents such as *n*-hexane, acetone, dichloromethane (DCM), methanol (MeOH) and the other chemicals such as anhydrous sodium sulfate (Na_2SO_4), silica gel, sulfuric acid (H_2SO_4 98%) with GC purities were ordered from Merck (Germany). Na_2SO_4 and silica gel were activated by baking at 450°C (4 hours) and 180°C (≥ 1 hour), respectively and kept in a precleaned glass bottle with a screwcap in a desiccator.

The standard reference material (SRM-1947) was purchased from the National Institute of Standards and Technology (Department of Commerce, US) to perform the following procedure in an individual sample batch. It includes a set of five bottles of approximately 8 grams of wet weight for individuals.

2.2. Instrumental analysis

Data was acquired by GC (GC *trace 1310*) combined with Triplus RSH liquid autosampler and electron impact (EI)-MS/MS (*TSQ9000*, Thermo Scientific).

Twenty OCPs and 28 PCBs were separated on an Agilent DB-5MS capillary column (30 m x 0.25 mm, 0.25 µm). The temperature was set at 280°C, 300°C and 280°C for inlet, transfer-line and ion source, respectively. The gradient temperature started at 100°C (1 min), linearly raised to 210°C at a rate of 15°C/min then continuously increased to 300°C at a rate of 10°C/min and kept for 15 min at this temperature.

An Agilent DB-5MS capillary column (15 m x 0.25 mm, 0.25 µm) was used for chromatographic separation of 8 PBDE analytes. The temperatures of inlet, transfer-line and ion source were at 280°C, 280°C and 250°C, respectively. The oven temperature started at 100°C for 2 min then linearly rose to 300°C at a rate of 30°C/min then kept for 7 min at this temperature.

All samples were injected with 1 µL at the splitless mode. Helium (99.9999%) was used as the carrier gas with a constant flow rate of 1 mL/min. Table 1 presents the retention time (RT) and transition with collision energy (CE) for the selected reaction monitoring (SRM) mode of detection and quantification.

***Table 1**. RT and transitions for OCP, PCB and PBDE analysis by GC-MS/MS*

Analytes	*Quantification*		*Qualification*		*RT*
	Transition	*CE (V)*	*Transition*	*CE (V)*	*(min)*
OCPs					
p,p'-DDT-d8	**243 > 173**	**40**	**243 > 206**	**20**	**13.60**
γ-BHC-d6	221.9 > 148	20	221.9 > 185	10	9.03
α-BHC	219 > 183	10	219 > 147	20	8.52
β-BHC	219 > 183	10	219 > 147	20	8.90
γ-BHC (Lindane)	219 > 183	10	219 > 147	20	9.02
δ-BHC	219 > 183	10	219 > 147	20	9.45
Heptachlor	272 > 237	15	274 > 239	15	10.14
Aldrin	263 > 191	40	263 > 228	35	10.71
Heptachlor epoxide	353 > 263	15	263 > 193	30	11.34
γ-Chlordane	373 > 266	20	373 > 264	30	11.73
Endosulfan I	195 > 159	10	195 > 125	20	11.96
α -Chlordane	373 > 266	20	373 > 264	30	11.94
4,4'-DDE	318 > 246	20	246 > 176	25	12.27
Dieldrin	263 > 191	30	263 > 228	15	12.41
Endrin	263 > 193	30	263 > 191	30	12.77
Endosulfan II	195 > 159	10	195 > 125	30	12.96
4,4'-DDD	235 > 165	20	235 > 199	15	12.99
Endrin aldehyde	345 > 317	10	185 > 121	15	13.20
Endosulfan sulfate	272 > 237	15	274 > 239	15	13.60
4,4'-DDT	235 > 165	20	235 > 199	10	13.64
Endrin ketone	317 > 101	15	317 > 281	5	14.45
Methoxychlor	227 > 212	15	227 > 169	30	14.58
PCBs					
^{13}C-PCB 77	**302 > 232**	**28**	**304 > 234**	**28**	**12.45**
^{13}C-PCB 28	270 > 198	35	270 > 163	40	9,87
PCB 8	222 > 152	22	224 > 152	22	8.55
PCB 18	256 > 186	22	258 > 188	22	9.16
PCB 28	256 > 186	22	258 > 188	22	9.90
^{13}C-PCB 52	304 > 232	45	304 > 269	10	10.38
PCB 44	289.9 > 219.9	22	289.9 > 219.9	22	10.73
PCB 52	289.9 > 219.9	22	291.9 > 221.9	22	10.42
PCB 66	289.9 > 219.9	22	291.9 > 221.9	22	11.44
PCB 77	291.9 > 221.9	22	291.9 > 221.9	22	12.45
^{13}C-PCB 81	**302 > 232**	**28**	**304 > 234**	**28**	**12.28**
PCB 81	289.9 > 219.9	22	291.9 > 221.9	22	12.28
^{13}C-PCB 123	**338 > 268**	**28**	**340 > 270**	**28**	**12.79**
^{13}C-PCB 101	338 > 268	30	338 > 303	10	11.79
PCB 101	323.9 > 253.9	22	325.9 > 255.9	22	11.81
PCB 105	323.9 > 253.9	22	325.9 > 255.9	22	13.86
PCB 114	323.9 > 253.9	22	325.9 > 255.9	22	13.29
PCB 118	323.9 > 253.9	22	325.9 > 255.9	22	13.03
PCB 123	323.9 > 253.9	22	325.9 > 255.9	22	12.79
^{13}C-PCB 126	**336 > 266**	**28**	**338 > 268**	**28**	**14.80**
PCB 126	323.9 > 253.9	22	325.9 > 255.9	22	14.80
^{13}C-PCB 169	**372 > 302**	**28**	**370 > 300**	**28**	**15.19**
^{13}C-PCB 138	372 > 302	40	372 > 337	10	13.62
PCB 128	357.9 > 287.9	22	359.9 > 289.9	22	14.12
PCB 138	357.9 > 287.9	22	359.9 > 289.9	22	13.67
^{13}C-PCB 153	372 > 302	30	372 > 337	10	13.16
PCB 153	357.9 > 287.9	22	359.9 > 289.9	22	13.20
PCB 156	357.9 > 287.9	22	359.9 > 289.9	22	14.74
PCB 157	357.9 > 287.9	22	359.9 > 289.9	22	14.62

Analytes	*Quantification*		*Qualification*		*RT*
	Transition	*CE (V)*	*Transition*	*CE (V)*	*(min)*
PCB 167	357.9 > 287.9	22	359.9 > 289.9	22	14.16
PCB 169	357.9 > 287.9	22	359.9 > 289.9	22	15.19
^{13}C-PCB 180	406 > 336	40	406 > 371	20	14.75
PCB 170	391.9 > 321.9	22	393.9 > 323.9	22	15.28
PCB 180	391.9 > 321.9	22	393.9 > 323.9	22	14.80
PCB 187	391.9 > 321.9	22	393.9 > 323.9	22	13.91
PCB 189	391.9 > 321.9	22	393.9 > 323.9	22	15.80
^{13}C-PCB 209	507.7 > 437.8	28	509.7 > 439.8	28	17.41
PCB 195	427.76 > 355.8	22	429.8 > 357.8	22	16.01
PCB 206	461.72 > 391.8	22	463.8 > 393.8	22	16.96
PCB 209	497.7 > 427.8	22	495.7 > 425.8	22	17.46
PBDEs					
^{13}C -BDE 99	**577.6 > 417.8**	**30**	**575.6 > 415.8**	**30**	**7.50**
^{13}C -BDE 28	417.8 > 258.0	25	419.8 > 260.0	25	6.14
BDE-28	406 > 246	20	406 > 248	20	6.14
^{13}C -BDE 47	497.7 > 337.8	20	499.7 > 339.8	20	6.84
BDE-47	486 > 326	25	486 > 328	25	6.84
^{13}C -BDE 100	577.6 > 417.8	20	575.6 > 415.8	25	7.34
BDE-100	564 > 404	25	566 > 406	20	7.34
BDE-99	564 > 404	25	566 > 406	20	7.50
^{13}C - BDE 153	655.7 > 495.8	20	657.7 > 497.8	20	8.11
BDE-154	644 > 484	25	644 > 486	25	7.89
BDE-153	644 > 484	25	644 > 486	25	8.11
^{13}C -BDE 183	735.4 > 575.6	25	735.4 > 575.6	20	8.66
BDE183	721.4 > 562	30	721.4 > 564	30	8.66
^{13}C-BDE 209	813.4 > 653.5	35	815.4 > 655.5	35	13.61
BDE-209	801.4 > 641.5	35	803.4 > 643.5	30	13.61

2.3. Sample preparation

This experiment was conducted seven times to validate the methodology, in which pooled samples, consisting of five kinds of homogenized fish tissues (HP01-HP05), were each spiked with 10 ng/mL of NSs. [14]. After that, the same experiments were performed for these individual samples to quantify investigated analytes. Five fish tissues were randomly collected from local coastal markets in Hai Phong province. Their information is given in Table 2. Fish samples were filleted, freeze-dried, homogenized, wrapped in aluminum foil, and sealed in polyethylene bags with silica gel to absorb moisture and stored at -20°C until further processing and analysis.

Table 2. *Information of collected samples*

Sample ID	*Scientific name*	*Length (cm)*	*Weight (g)*	*% Lipid/d.w*
HP01	*Mugil cephalus*	14.0	38.0	55.1
HP02	*Clupeinae*	17.0	53.0	49.7
HP03	*Lagocephalus spadiceus*	13.0	47.0	13.9
HP04	*Sciaenops ocellatus*	17.0	49.0	12.2
HP05	*Dorosomatinae*	33.0	18.0	11.8

d.w.: dry weight

The sample procedure has been referenced from the literature reported by Quynh et al. 2023 [13]. In brief, 2 g of homogenized sample was spiked with 50 ng/mL (25 µL of 1 µg/mL) in three groups of LSs and extracted by the ASE-350 instrument (Thermo Scientific). The ASE extraction was performed by a mixture of hexane: acetone (1/1, v/v) at 100°C and 2000 psi with three cycles. For each cycle, both the heating and equilibrium time were 5 min. The flush volume was 40% and the discharge time was 100 s. The extract was concentrated by the Rocket Synergy vacuum centrifuge (SP Genevac, USA) to about 3 mL. The lipid was then removed before the clean-up step. H_2SO_4 was added drop by drop into samples until they turned dark color, vortexed and centrifuged at 4500 rpm (Z32 HK). The upper layer was collected and transferred to a new falcon tube. This process was repeated until the sample did not change color. The acidic residue should be removed by adding deionized H_2O equal to the amount of acid previously given. Similarly, after being vortexed and centrifuged, the upper layer was collected and loaded on a multi-layer silica-gel column self-packed with (from the bottom to the top): anhydrous sodium sulfate (1 g), silica gel (1 g), 40% sulfuric acid-impregnated silica gel (4 g), 20% sulfuric acid impregnated silica gel (6 g), and anhydrous sodium sulfate (1 g). Then the column was eluted with a solvent mixture of 75 mL hexane: DCM (1/1, v/v). The eluent was concentrated to about 3 mL by vacuum rotary then added 50 ng/mL of ISs (25 µL of 1 µg/mL) and continuously concentrated by N_2 until the last drop. All samples were reconstituted to exactly 0.5 mL hexane and then transferred to GC-vial for the GC/MS-MS analysis.

Moreover, the SRM-1947, which is the Lake Michigan fish tissue, was analyzed to confirm the accuracy of the validated method. They were analyzed in each sample batch, following as mentioned preparation procedure and analysis.

3. RESULTS AND DISSCUSION

3.1. Chromatographic separation of target analytes

The capillary column of 5MS with (5%-phenyl)-methyl polysiloxane in the stationary phase has been chosen for chromatographic separation in terms of separation efficiency and peak shape. A standard solution of 500 ng/mL was analyzed in spectral scanning mode and compared with the reference from the NIST spectrum library to determine the RT and select the ion transition in SRM mode. The RT and SRM transitions of individual analytes are given in Table 1. To qualify and quantify analytes in standard solutions and real samples, SRM mode was performed based on corresponding RT and ion fragments of analytes. Figure 1 shows the chromatograms of (a) OCPs and PCBs and (b) PBDEs at SRM mode under operating conditions.

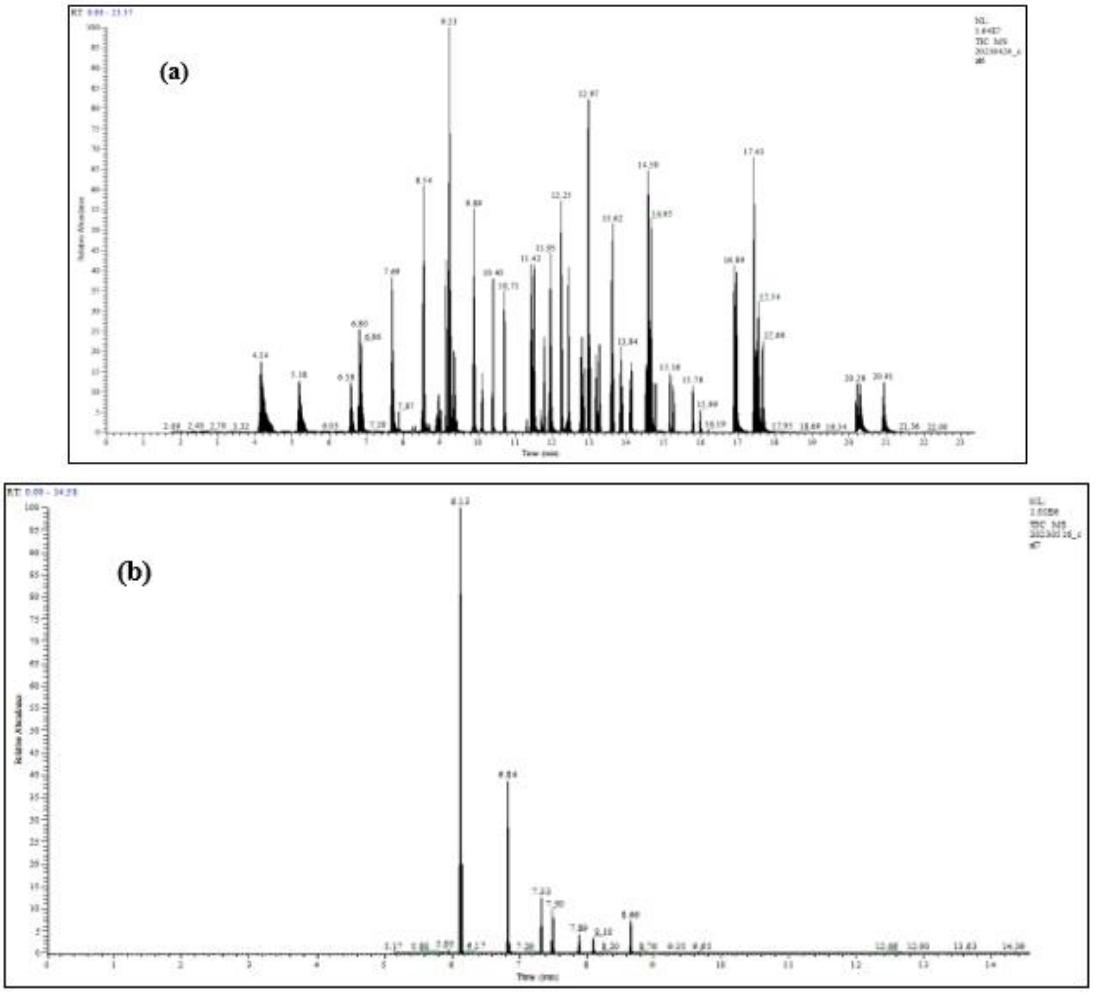

Figure 1. *Chromatograms of OCPs, PCBs (a) and PBDEs (b) at 100 ng/mL standard solution*

3.2. Methodology validation

3.2.1. Stability of the Analytical signal

The stability of analytical signal plays an important role in measurement uncertainty of the developed analytical method. This parameter was measured by the relative standard deviation (RSD%) of peak area in short-term and long-term analysis. In this work, five solutions containing all target analytes at 50 ng/mL concentration were prepared in hexane and injected into the GC/MS-MS system under the mentioned conditions. The short-term and long-term stabilities were measured for the same sample batch and three continuous sample batches, respectively. As can be seen from Table 3, the good repeatability of the analytical signal was achieved with RSD for both short-term and long-term less than 10.5% and 12.4%, respectively.

3.2.2. Linearity, MDL and Recovery

As shown in Table 3, excellent correlations between analytical signal and concentration of analytes were obtained, with R^2 >0.9990. The internal calibration equations

and correlation coefficients of all target analytes are also listed in Table 3. The method detection limit (MDL) is equal to three times the standard deviation (MDL= 3*SD) and is given in Table 3. These values ranged from 0.053 ng/g (α-chlordane) – 1.65 ng/g (δ-BHC), 0.07 ng/g (PCB-209) – 1.84 ng/g (PCB-28) and 0.323 ng/g (BDE-209) – 0.796 ng/g (BDE-47) for OCPs, PCBs and PBDEs, respectively. Besides, the overall recovery (Re) of all target analytes in the pooled sample is also shown in Table 3. It can be seen that the mean overall recovery of all analytes was within an acceptable range (60 – 115% at 10 ng/mL) [15].

Table 3. *Internal equations, MDL, stability of analytical signal and Re% of analytes*

Analytes	***Equation, R^2***	***MDL (ng/g)***	***Re ± RSD (%)***	***RSD (%)***	
				Intra-day (n=5)	***Inter-day (n=3)***
OCPs					
α-BHC	Y= (0.0058±5.41E-05)x + (0.0039±2.48E-03); R^2 = 0.9997	1.28	92.7 ± 2.97	6.9	9.7
β-BHC	Y= (0.0059±2.61E-05)x + (0.0043±1.20E-03); R^2 = 0.9999	1.57	77.9 ± 4.31	7.6	8.9
γ-BHC	Y= (0.0053±4.76E-05)x + (0.0023±2.18E-03); R^2 = 0.9997	1.20	97.2 ± 3.15	8.0	8.5
δ-BHC	Y= (0.0017±8.66E-06)x + (-0.0013±3.98E-04); R^2 = 0.9999	1.65	77.3 ± 4.54	7.7	9.8
Heptachlor	Y= (0.0143±1.21E-04)x + (0.0055±5.57E-03); R^2 = 0.9997	0.118	82.4 ± 4.40	7.5	6.9
Aldrin	Y= (0.0091±5.79E-05)x + (-0.0681±4.72E-02); R^2 = 0.9998	1.60	78.1 ± 4.71	5.2	4.5
Heptachlor epoxide	Y= (0.0063±4.44E-05)x + (0.0010±2.04E-03); R^2 = 0.9994	0.063	81.8 ± 4.96	4.2	12.0
γ-Chlordane	Y= (0.0019±3.02E-05)x + (0.0012±1.39E-03); R^2 = 0.9999	0.080	77.4 ± 3.15	3.4	8.2
α-chlordane	Y= (0.0048±2.44E-05)x + (0.0030±1.12E-03); R^2 = 0.9999	0.053	95.7 ± 3.14	8.0	5.1
α-Endosulfan	Y= (0.0019±1.56E-05)x + (-0.0004±7.17E-04); R^2 = 0.9997	0.249	98.5 ± 3.79	7.5	6.8
p,p'-DDE	Y= (0.0637±7.90E-04)x + (0.0280±3.36E-02); R^2 = 0.9994	1.06	81.0 ± 4.04	8.4	10.8
Dieldrin	Y= (0.0018±1.17E-05)x + (0.0033±5.36E-04); R^2 = 0.9998	0.076	90.0 ± 4.49	6.3	8.6
Endrin	Y= (0.0019±2.30E-05)x + (0.0011±1.06E-03); R^2 = 0.9994	0.648	80.0 ± 1.48	5.2	9.4
p,p'-DDD	Y= (0.0782±4.19E-04)x + (-0.0070±1.92E-02); R^2 = 0.9999	1.01	77.9 ± 2.80	5.8	12.4
β-Endosulfan	Y= (0.0017±1.65E-05)x + (-0.0016±7.56E-04); R^2 = 0.9996	0.545	72.2 ± 3.14	6.6	7.3
Endrin aldehyde	Y= (0.0008±6.06E-06)x + (-0.0001±2.78E-04); R^2 = 0.9997	0.686	70.9 ± 1.48	6.3	7.8
p,p'-DDT	Y= (0.0426±1.61E-04)x + (-0.0113±7.37E-03); R^2 = 0.9999	0.434	96.8 ± 2.62	6.1	6.7
Endosulfan sulfate	Y= (0.0029±1.53E-05)x + (-0.0015±7.04E-04); R^2 = 0.9999	0.355	77.9 ± 2.26	7.3	9.5
Methoxychlor	Y= (0.0153±1.25E-04)x + (-0.0021±5.75E-03); R^2 = 0.9997	0.852	86.9 ± 1.81	7.2	7.5
Endrin ketone	Y= (0.0148±6.13E-05)x + (-0.0772±4.99E-02); R^2 = 0.9999	0.339	77.0 ± 1.14	4.9	10.0
PCBs					
PCB 8	Y= (0.0420±6.23E-04)x + (0.0015±2.89E-02); R^2 = 0.9991	1.73	82.6 ± 1.87	7.7	10.1
PCB 18	Y= (0.0259±1.24E-04)x + (0.0005±5.73E-03); R^2 = 0.9999	1.49	90.0 ± 1.14	8.4	6.7

Analytes	*Equation, R^2*	*MDL (ng/g)*	*Re ± RSD (%)*	*RSD (%)*	
				Intra-day (n=5)	*Inter-day (n=3)*
PCB 28	Y= (0.0352±1.01E-04)x + (-2.66E-07±4.71E-03); R^2 = 1.0000	1.84	97.1 ± 1.48	4.7	5.8
PCB 44	Y= (0.0211±4.06E-05)x + (0.017±1.88E-03); R^2 = 1.0000	1.57	97.3 ± 1.81	4.3	9.6
PCB 52	Y= (0.0229±1.23E-04)x + (0.0016E-07±5.69E-03); R^2 = 0.9999	1.49	97.6 ± 2.26	8.0	9.8
PCB 66	Y= (0.0265±3.05E-04)x + (-0.0094±1.41E-02); R^2 = 0.9995	0.680	95.7 ± 2.62	10.2	8.3
PCB 77	Y= (0.0244±9.72E-05)x + (-0.0017±4.51E-03); R^2 = 0.9999	0.850	90.5 ± 3.14	7.8	7.7
PCB 81	Y= (0.0250±8.061E-05)x + (-0.0021±3.74E-03); R^2 = 1.0000	0.580	93.7 ± 2.8	7.0	6.4
PCB 101	Y= (0.0234±8.61E-05)x + (-0.0027±3.99E-03); R^2 = 0.9999	0.660	102 ± 4.04	5.1	7.2
PCB 123	Y= (0.0242±8.09E-05)x + (0.0011±3.75E-03); R^2 = 1.0000	1.44	103± 4.49	8.7	9.0
PCB 118	Y= (0.0243±3.72E-05)x + (0.0005±4.72E-03); R^2 = 1.0000	1.19	103± 4.03	6.7	7.8
PCB 114	Y= (0.0258±1.24E-04)x + (-0.0028±5.76E-03); R^2 = 0.9999	1.57	103 ± 4.90	7.9	7.7
PCB 105	Y= (0.0271±1.77E-04)x + (0.0003±8.21E-03); R^2 = 0.9998	0.590	76.1 ± 7.55	4.9	7.6
PCB 126	Y= (0.0315±2.30E-04)x + (-0.0096±1.07E-02); R^2 = 0.9998	1.10	83.7 ± 7.48	5.7	6.7
PCB 153	Y= (0.0222±2.45E-04)x + (0.0016±1.14E-02); R^2 = 0.9995	0.830	102.9 ± 1.48	4.2	7.8
PCB 138	Y= (0.0204±8.34E-05)x + (-0.0013±3.87E-03); R^2 = 0.9999	1.20	97.9 ± 5.10	4.7	8.2
PCB 156	Y= (0.0201±9.21E-05)x + (-0.0002±4.27E-03); R^2 = 0.9999	0.860	98.8 ± 3.61	7.9	8.4
PCB 128	Y= (0.0153±1.01E-04)x + (0.0004±4.69E-03); R^2 = 0.9998	0.350	103 ± 4.04	7.7	7.5
PCB 167	Y= (0.0211±2.21E-04)x + (-0.0003±1.02E-02); R^2 = 0.9996	0.320	100 ± 5.87	7.1	6.0
PCB 157	Y= (0.0221±4.02E-05)x + (0.0010±1.86E-03); R^2 = 1.0000	0.310	97.8 ± 7.18	7.2	6.7
PCB 169	Y= (0.0171±2.42E-04)x + (0.0015±1.12E-02); R^2 = 0.9992	0.330	99.3 ± 9.93	8.4	9.6
PCB 187	Y= (0.0114±5.06E-05)x + (0.0002±2.35E-03); R^2 = 0.9999	0.270	101 ± 5.59	7.8	7.9
PCB 170	Y= (0.0113±4.56E-05)x + (-2.01E-05±2.12E-03); R^2 = 0.9999	0.300	103 ± 3.79	4.7	7.0
PCB 180	Y= (0.0116±5.92E-05)x + (0.0015±2.74E-03); R^2 = 0.9999	0.140	99.2 ± 3.00	8.2	6.7
PCB 189	Y= (0.0170±6.91E-05)x + (0.0014±3.20E-03); R^2 = 0.9998	0.260	98.3 ± 3.62	6.2	6.4
PCB 195	Y= (0.0043±3.98E-05)x + (0.0010±1.85E-03); R^2 = 0.9997	0.080	106 ± 1.48	8.2	7.7
PCB 206	Y= (0.0096±1.47E-04)x + (0.0007±6.80E-03); R^2 = 0.9991	0.440	112 ± 2.19	6.8	7.3
PCB 209	Y= (0.0229±1.78E-04)x + (0.0140±8.90E-03); R^2 = 0.9998	0.070	100.8 ± 4.95	5.5	7.8
PBDEs					
BDE 28	Y= (0.0013±1.13E-05)x + (-0.0143±9.22E-03); R^2 = 0.9995	0.387	85.4 ± 2.21	10.3	6.5
BDE 47	Y= (0.0051±3.84E-05)x + (-0.0409±3.12E-02); R^2 = 0.9997	0.796	84.9 ± 2.28	8.2	7.0
BDE 100	Y= (0.0077±5.96E-05)x + (-0.0680±4.86E-02); R^2 = 0.9996	0.560	86.6 ± 1.48	9.4	9.4
BDE 99	Y= (0.0013±6.42E-06)x + (-0.0078±5.23E-03);	0.426	87.8 ± 4.74	8.6	7.7

Analytes	*Equation, R^2*	*MDL (ng/g)*	*Re ± RSD (%)*	*RSD (%)*	
				Intra-day (n=5)	*Inter-day (n=3)*
BDE 154	R^2 = 0.9999 Y= (0.0011±1.29E-05)x + (-0.0158±1.50E-02); R^2 = 0.9992	0.515	81.5 ± 1.48	7.0	8.1
BDE 153	Y= (0.0018±2.01E-05)x + (-0.0366±1.63E-02); R^2 = 0.9992	0.667	100 ± 1.87	7.4	9.7
BDE 183	Y= (0.0113±9.20E-05)x + (-0.2145±7.49E-02); R^2 = 0.9996	0.586	92.3 ± 7.00	10.5	7.1
BDE 209	Y= (0.0120±9.49E-05)x + (-0.1730±7.73E-02); R^2 = 0.9996	0.323	80.1 ± 4.49	8.8	7.5

Besides, the average values of PBDE, PCB and OCP concentrations measured in the SRM-1947 sample are given in Table 4 (Experimental value, Exp. value). It can be clearly seen that the measured concentrations for several OCP, PCB, PBDE congeners were within the expected range reported in the SRM-1947 certificate. Some results were near the upper or lower bounds due to the complexity of the sample matrix, especially with high lipid content. Therefore, it is necessary to further optimize the clean-up step to improve the purification efficiency.

***Table 4**. The results of experimental measurement and assigned range values of SRM-1947 report*

OCPs			***PCBs***		
Analytes	***Exp. value***	***Assigned range value***	***Analytes***	***Exp. value***	***Assigned range value***
α-BHC	0.99	0.94 – 1.18	PCB 28	14.8	13.1 – 15.1
γ-BHC	0.450	0.26 – 0.45	PCB 44	21.8	18.7 – 22.1
Heptachlor epoxide	12.7	12.6 – 14.2	PCB 52	37.7	32.1 – 40.7
γ-Chlordane	12.7	11.6 – 14	PCB 66	74.4	64.1 – 74.7
α-chlordane	43.5	43.5 – 54.5	PCB 101	90.6	90.5 – 91.1
p,p-DDE	683	677 – 763	PCB 118	117	106 – 118
Dieldrin	77.5	77 – 84.6	PCB 105	52.8	46.6 – 54
p,p'-DDD	42.6	42.3 – 49.5	PCB 153	200	198 – 204
p,p'-DDT	15.4	14.81 – 16.59	PCB 138	155	155.1 – 168.9
			PCB 156	14.1	12.4 – 14.2
PBDEs			PCB 128	33.0	29.5 – 33.7
BDE 28	2.12	1.8 – 2.72	PCB 157	3.85	3.31 – 4.85
BDE 47	71.4	70.4 – 76.2	PCB 170	31.0	26.8 – 31.6
BDE 100	17.3	16.5 – 17.7	PCB 180	85.1	75.8 – 85.8
BDE 99	18.6	18.4 – 20	PCB 195	4.65	4.18 – 5.72
BDE 154	7.31	6.36 – 7.4	PCB 206	6.07	5.36 – 7.12
BDE 153	3.93	3.79 – 3.87	PCB 209	2.38	1.77 – 3.13

3.3. Residue concentration in fish samples

The validated method was used to determine the concentrations of OCPs, PCBs and PBDEs in five fish tissues collected from Hai Phong province. In this work, concentrations of all target analytes in real samples were calculated by internal calibration curves calibrated using the recoveries of corresponding labeled compounds and reported in wet weight and labeled ng/g. The decreasing order of the concentrations of target analytes found in investigated fish samples was Σ_{20}OCPs (175 ng/g) > Σ_{28}PCBs (30.9 ng/g) > Σ_{8}PBDEs (23.4 ng/g). Among them, the content of OCPs was in the range of <MDL – 206 ng/g with the highest contribution of *p,p'*-DDE congener (24.9%), followed by *p,p'*-DDD (14.1 %) and p,p'-DDT (11.7%). The levels of PCBs were from <MDL to 20.7 ng/g, in which, PCB-138 and PCB-153 congeners were predominant with individual proportion of their concentrations higher than 15.6%, followed by PCB-118 (11.9%) and PCB-101 (8.2%). The observed PBDE concentrations fluctuated from <MDL – 66.7 ng/g, this was due to the presence of BDE-209, which accounted for 59.1% Σ_{8}PBDEs. On the other hand, the concentration of almost all target analytes tended to contribute similarly to all samples. However, there were special cases with remarkable contributions, such as Dieldrin in HP03 (50.8%); PCB-8 and PCB-209 in sample HP05 (10.7% and 29.9%, respectively) and BDE 209 in samples HP03 and HP05 (89.4% and 84%, respectively). These tendencies can be seen in Figure 2.

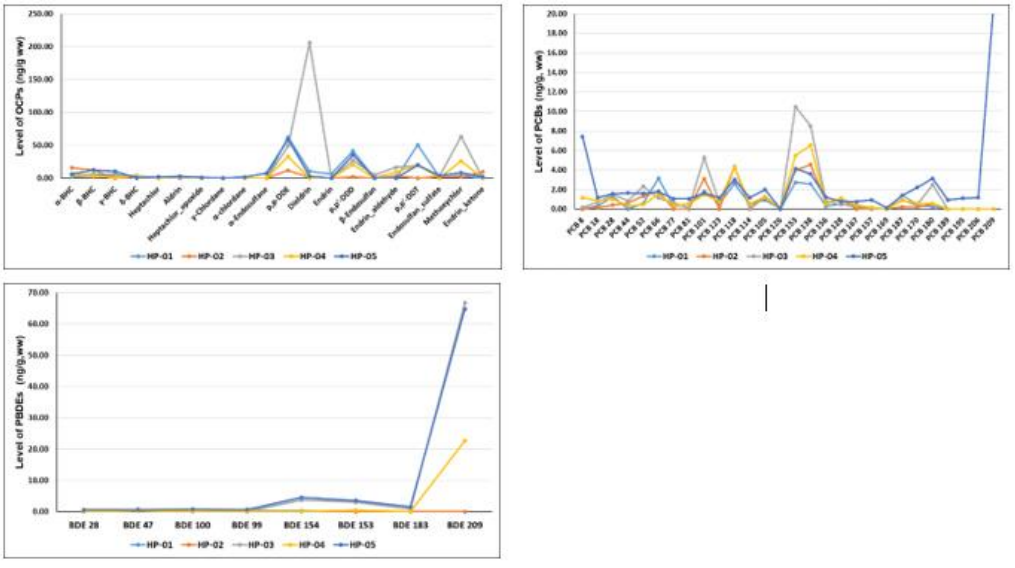

***Figure 2**. Contribution trends of OCPs, PCBs, and PBDEs in collected samples*

4. CONCLUSION

This work has validated a method for the analysis of 20 OCPs, 28 PCBs, and 8 PBDEs in fish samples on the GC-MS/MS system using ASE extraction and a multi-layer silica gel column for sample preparation. The MDLs of OCPs, PCBs and PBDEs were 0.053 – 1.65

ng/g, 0.07 – 1.84 ng/g and 0.323 – 0.796 ng/g, respectively. The repeatability of analytical signals was lower than 10.5% and 12.4% for intra-day analysis and inter-day analysis, respectively. The recovery values ranged from 70.9 to 114%. This developed method was also confirmed by analysis of SRM-1947 samples, and the measured concentrations of the investigated analytes were within the range of certified values. Finally, this method was applied to determine OCPs (<MDL – 206 ng/g), PCBs (<MDL – 20.7 ng/g) and PBDEs (<MDL – 66.7 ng/g) in five marine samples collected from a local market in Hai Phong province. The obtained results have indicated that the contamination levels were low.

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Nghiên cứu phương pháp xác định đồng thời OCPs, PCBs và PBDEs trong nền mẫu cá: Áp dụng phân tích một số mẫu cá biển thu thập tại Hải Phòng

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Tóm tắt

Nghiên cứu đã sử dụng phương pháp sắc ký khí ghép nối khối phổ tứ cực (GC-MS/MS) kết hợp với các kĩ thuật xử lý mẫu bao gồm chiết gia tốc dung môi và làm sạch trên cột silicagel đa lớp để phân tích đồng thời 20 hợp chất hữu cơ cơ clo (OCPs), 28 hợp chất polyclobiphenyl (PCBs) và 8 hợp chất polybrominated diphenyl ethers (PBDEs) trong mẫu cá. Các thí nghiệm với mẫu thêm chuẩn được thực hiện để xác nhận giá trị sử dụng của phương pháp. Phương pháp đã xây dựng được giới hạn phát hiện (MDL) nằm trong khoảng từ 0,053 ng/g ww (α-chlordane) – 1,65 ng/g ww (δ-BHC) đối với các OCPs, 0,07 ng/g ww (PCB-209) – 1,84 ng/g ww (PCB-28) đối với các PCBs và 0,323 ng/g ww (BDE-209) – 0,796 ng/g ww (BDE-47) đối với các PBDEs. Độ lặp lại và tái lặp của tín hiệu phân tích lần lượt là 10,5% và 12,4%. Hiệu suất thu hồi của quá trình xử lý mẫu nằm trong khoảng từ 70,9 – 114%. Phương pháp đã được xác nhận thông qua phân tích mẫu CRM-1947 với kết quả giá trị trung bình giữa các lần đo đều nằm trong khoảng chứng nhận. Cũng trong nghiên cứu này, phương pháp được áp dụng để phân tích OCPs, PCBs và PBDEs trong 5 mẫu cá thu mua ngẫu nhiên ở chợ ven biển tỉnh Hải Phòng. Nồng độ các chất được phát hiện (OCPs: <MDL – 206 ng/g, PCBs: <MDL – 20,7 ng/g và PBDEs: <MDL – 66,7 ng/g) đều nằm dưới ngưỡng khuyến cáo của Châu Âu và Tổ chức Y tế thế giới.

Từ khóa: *OCPs, PCBs, PBDEs, GC-MS/MS, cá.*