Research Article *doi: 10.47866/2615-9252/vjfc.4072*

Determination of polychlorinated biphenyls in marine fish samples by gas chromatography tandem mass spectrometry (GC-MS/MS)

Pham Thi Diem Quynh[1], Pham Thi Ngoc Mai, Hoang Quoc Anh[1], Le Minh Thuy[2], Nguyen Thi Huong[2], Vu Khanh Hoa[2], Do Thi Thu Huong[2], Nguyen Thi Xuyen[2], Nguyen Xuan Hung[2], Chu Dinh Binh[3], Vu Duc Nam[2*]

[1]Department of Chemistry, VNU-University of Science
[2]Center for Research and Technology Transfer, VAST, Hanoi, Vietnam
[3]School of Chemical Engineering, Hanoi University of Science and Technology, Hanoi, Vietnam

(Received: 28/04/2023; Accepted: 23/05/2023)

Abstract

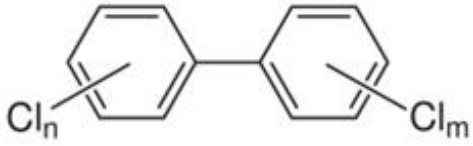
Polychlorinated biphenyls (PCBs) are a typical group of persistent organic pollutants (POPs), which have been listed under Annex A (Elimination) and Annex C (Unintentional production) of the Stockholm Convention. In this study, a gas chromatography tandem mass spectrometry method was developed and applied to analyze concentrations of 28 PCB congeners in some Vietnamese marine fish samples. PCBs in fish samples were ultrasonically extracted with an acetone/*n*-hexane (1/1, v/v) mixture. The extracts were cleaned up by using multilayer silica gel columns with dichloromethane/*n*-hexane (1/1, v/v) as elution solvent. PCBs were separated on a DB-5MS column and quantified by using a triple quadrupole mass spectrometer. The MS detector was operated in positive electron impact ionization (EI) mode and selected reaction monitoring (SRM) mode. Calibration curves of 28 PCBs exhibited good linearity ($R^2 \geq 0.9998$). Instrument detection limits (IDLs) and method detection limits (MDLs) ranged from 0.08 to 0.023 ng/mL and from 0.07 to 1.84 ng/g, respectively. Recoveries of 28 PCBs native and 7 labeled standards in matrix-spike samples ranged from 62.3 to 88.1% and from 75.5 to 91.9%, satisfying criteria proposed by AOAC (recovery 60 - 115% for 10 - 100 ppb levels). The validated method was applied to analyze 10 marine fish samples, showing levels of Σ28PCBs from 17 to 851 (mean 230) ng/g lipid. The sum of 6in-PCBs (PCB-28; -52; -101; -138; -153; -180) based on wet weight (w/w) ranged from 1.24 to 3.15 ng/g, which is lower than the maximum level recommended by The European Union (75 ng/g (w/w). Congeners PCB-126 and PCB-169 were not detected in marine fish samples.

Keywords: *POPs, PCBs, marine fish, Vietnam, GC-MS/MS.*

[*] *Corresponding author: 0989185446* *Email: vuducnam@gmail.com*

1. INTRODUCTION

Polychlorinated biphenyls (PCBs) are a group of organochlorines that consist of 209 congeners differing in the number and position of chlorine atoms on the two coupled biphenyls ring.



n + m = 10

Figure 1. *Structure of Polychlorinated biphenyls (PCBs)*

PCBs can exist as liquids or solids, are chemically inert, have good thermal conductivity and electrical insulation, decompose at temperatures above 1000^{o}C, and were produced and widely used in many countries around the world. Thanks to good dielectric properties, PCBs are used as insulating fluids in capacitors and transformers, as heat transfer agents and lubricants. PCBs are also used in a variety of products, such as glues and adhesives, combined with other compounds, such as plasticizers and flame retardants [1]. Despite having many practical applications, PCBs are persistent in the environment, which has an adverse effect on ecosystems and human health. PCBs can impair the immune system and increase the risk of certain diseases in humans, such as diabetes, liver disease, cardiovascular disease, and thyroid disease, and affect the reproductive function of men and women [2]. In 2001, The Stockholm Convention on Persistent Organic Pollutants POPs listed PCBs in Appendix A (Elimination) and Appendix C (Unintentional production) and PCBs containing equipment to eliminate PCBs in the environment completely [3]. Even though PCBs are not produced in Vietnam, they can be found in machinery, transformer oil, and capacitors imported into Vietnam. In the period 1960-1990 it is estimated that approximately 27.000 to 30.000 tons of oil containing PCBs were imported with electrical equipment from the US, Russia, China, and some other countries to Vietnam [4].

In Vietnam, there have been a number of studies on the distribution of PCBs concentration in the environment, organisms and humans since 1990 [11-12]. However, data on PCBs concentration in biological samples are limited, especially marine and mainly analyzed the concentration of 7 indicator PCBs (7in-PCBs). According to My et al (2019), fish samples taken from Cau Hai lagoon contained 7in-PCBs, with PCB-153 and PCB-138 as the majority of congeners and accounting for 57 - 87% of total 7in-PCBs. The concentrations of 7in-PCBs determined were all substantially below the recommended limit of value [13]. In another study, with fish samples also taken from Cau Hai lagoon, the total concentrations of 62 congeners of PCBs fluctuated over a wide range, ranging from 63 to 150 ng/g lipid, with mean value of 110 ng/g lipid [14].

There are some methods that can be used to analyze PCBs in fish and food, with a variety of sample processing methods and analysis methods (Table 1).

Table 1. *Methods of PCBs analysis in fish and food*

Object	*Sample preparation*	*Analytical method*	*Result*	*Total content of PCBs*	*Ref.*
18 PCBs in fish samples	ASE SPE	GC-MS	MDL: 0.4 - 1.1 (ng/g)	106.6 - 349.8 (ng/g lipid)	[5]
16 PCBs in fish samples	SPE	GC-ECD	MDL: 0.31-1.13 (ng/g)	-	[6]
7 PCBs in fish samples	m-ZrO_2@Fe_3O_4 QuEChERS	GC-MS/MS	MDL: 0.02 - 0.06 (ng/g)	-	[7]
18 PCBs in fish samples	QuEChERS	GC-MS/MS	MDL: 0.1 - 0.5 (ng/g)	-	[8]
19 PCBs in fish samples	-	-	-	4.5 - 711.6 (ng/g lipid)	[9]
15 PCBs in fish samples	Soxhlet Silica gel column	GC-MS	-	135.2 - 990.8 (ng/g fat)	[10]

Therefore, this study aimed to analyze the content of 28 PCBs in marine fish samples collected at markets in Hai Phong, Thai Binh, Nghe An, Ha Tinh and Thanh Hoa. 28 PCBs were subjected to analyze include: 7in-PCBs (-28; -52; -101; -118; -138; -153; -180), 12 dioxin-like PCBs (12dl-PCBs) (-77; -81; -105; -114; -118; -123; -126; -156; -157; -167; -169; -189), and other PCBs (-8; -18; -44; -66; -170; -187; -195; -206; -209) on GC-MS/MS. The obtained results will provide information on the PCBs contents in marine fish to assess the concentration and characteristics of PCBs accumulation in marine fish.

2. MATERIALS AND METHOD

2.1. Chemicals

2.1.1. Chemicals

Gasses: He gas with a purity of 99.999%, N_2 gas with a purity of 99.99%. Solvents including: n-hexane; acetone; methanol with chromatographic purity from Merck (Germany); dichloromethane (DCM) with chromatographic purity from Fisher Scientific (USA). Other chemicals including anhydrous sodium sulfate Na_2SO_4 which is calcined at 450^{o}C for 3 hours ; silica gel (particle size 0.063 - 0.200 mm) and acid sulfuric H_2SO_4 (98%) were obtained from Merck (Germany).

2.1.2. Standards

Standards include: a mixture of 28 congeners (WHO/NIST/NOAA) Congener list (28 PCBs) with a concentration of 10 µg/mL in n-hexane were purchased from Accustandard (USA, P/N: C-WNN); mixture of 7 labeled standards (13C12 LABELED PCB MIXTURE

including PCB-28; -52; -101; -138; -153; -180; -209) with a concentration of 5 µg/mL in n-hexane were obtained from CIL, (Germany, P/N: EC-4058) and mixture of 5 internal standards (13C12 LABELED PCB MIXTURE-A including PCB-77; -81; -123; -126; 169) with a concentration of 1 µg/mL in n-hexane were supplied by LGC (Germany, P/N: EC-4938).

2.2. Collection and preparation of samples

Fish samples were collected at several local coastal markets in Hai Phong, Thai Binh, Nghe An, Ha Tinh and Thanh Hoa (Table 2).

Table 2. *List of fish samples collected*

Num	*Fish name*	*Science name*	*Source*	*Code*
1	Blunthead puffer	*Sphoeroides pachygaster*	Hai Phong	HP01
2	White sardine	*Escualosa thoracata*	Hai Phong	HP02
3	Scomber	*Scomber spp*	Thai Binh	TB01
4	Torpedo scad	*Megalaspis cordyla*	Thai Binh	TB02
5	Yellow croaker	*Larimichthys polyactis*	Thanh Hoa	TH01
6	Crescent grunter	*Terapon jarbua*	Nghe An	NA01
7	Snubnose pompano	*Trachinotus blochii*	Nghe An	NA02
8	Scatophagus argus	*Scatophagus argus*	Nghe An	NA03
9	Nuchequula nuchalis	*Nuchequula nuchalis*	Nghe An	NA04
10	Idian mackerel	*Rastrelliger kanagurta*	Ha Tinh	HT01

Fish samples collected at markets were cleaned and coded as in Table 2. In this study, the fillet samples were analyzed. Samples were freeze-dried, homogenized by a blender to determine water content, and then finely ground. Samples were wrapped in aluminum foil and sealed in PE bags with silica gel to absorb moisture and stored at -20°C until analysis.

2.3. Instruments

GC-MS/MS system (GC-Trace 1310, MS/MS-TSQ 9000) using a DB-5MS capillary column (30m × 0.25mm × 0.25µm), the automatic sample pump Triplus RSH Autosampler, all from Thermo Scientific (USA). Some other equipment includes: ultrasonic tank Powersonic 410 (Hwashin, Korea); centrifugator Z32HK (Germany); nitrogen evaporator MGS-2200D (Eyela, Japan), freeze dryers FDU-2110 (Eyela, Japan).

Analytical conditions for PCBs were referenced from [15-16], whereas PCBs were determined on the GC-MS/MS system (Thermo Scientific, USA). Carrier gas was helium (99.999%) at a flow rate of 1mL/min. The column oven temperature program was initially set at 100°C (keep 1 min), increased to 210°C (15°C/min), and finally increased to 300°C (10°C/min, keep 15 min). The sample injection volume was 1 µL (splitless). Mass

spectrometer detector was operated in electron collision ionization (EI) and set at positive ionization mode, with the electron energy of 70 eV, the ionization source temperature of 280°C, and the interface temperature of 300°C. The mass sweep range was adjusted from 50 to 500 amu. Spectral data were observed and collected in selected reaction monitoring (SRM) mode.

2.4. Fish sample analysis

The fish samples processing procedure (Figure 2) is referenced from [17]. Homogenized sample was weighed about 1.000 g ± 0.001 g into a 15 mL centrifuge tube, spiked with 27.8 µL PCBs labeled standards 13C12-PCB-28/52/101/138/153/180/209 (1 µg/mL). Samples were ultrasonically extracted using mixture of 10 mL of acetone/ n-hexane (1/1, v/v) in an ultrasonic water bath containing dry ice for 20 min, repeatedly 3 times. The extracts were then pooled and concentrated under a gentle stream of nitrogen to a volume less than 5 mL and later reconstituted to 5 mL with n-hexane solvent.

For determining the lipid content, accurately aspirate 0.5 mL of the extraction solution into aluminum weighing pam (m1). The pans containing the extraction were later dried at 65°C for 5 hours. After drying, reweigh the aluminum pan containing the extraction solution (m2).

The remaining extract (4.5 mL) was treated with sulfuric acid (98%). Acid was added dropwise to the sample until the upper layer did not change color. After vortexing for 1 min, sample was centrifuged at 5,000 rpm for 5 min before transferring the organic phase to a 15 mL centrifuge tube. To remove excess acid, the extraction solution after treatment was mixed with H2O, vortexed for 1 min and centrifuged at 5,000 rpm for 5 min. The organic phase was later cleaned with a multi layer silica gel column containing glass wool, 1 g sodium sulfate, 3 g silica gel impregnated with 40% acid, 3 g silica gel impregnated with 20% acid, 1 g sodium sulfate. The clean-up column was activated with 50 mL methanol and 50 mL n-hexane. PCBs were eluted by 75 mL of mixture of n-hexane/DCM (1/1, v/v). The eluate was concentrated under a gentle nitrogen stream and spiked with 50 µL of internal standards (13C 12 -PCB-77/81/123/126/169) (500 ng/mL). Reconstituted the eluate with 0.5 mL n-hexane by a micro pipette, centrifuged to remove residue before transferring to the analytical vial before GC-MS/MS.

2.5. Data processing

2.5.1 Lipid content in fish samples

Lipid content was calculated as follow:

$$m_L = (m_2 - m_1) \times 10$$

Where:

m_L: lipid weight in fish samples (g)
m_1: weight of aluminum weighing pan (g)
m_2: weight of pan containing samples after drying (g)

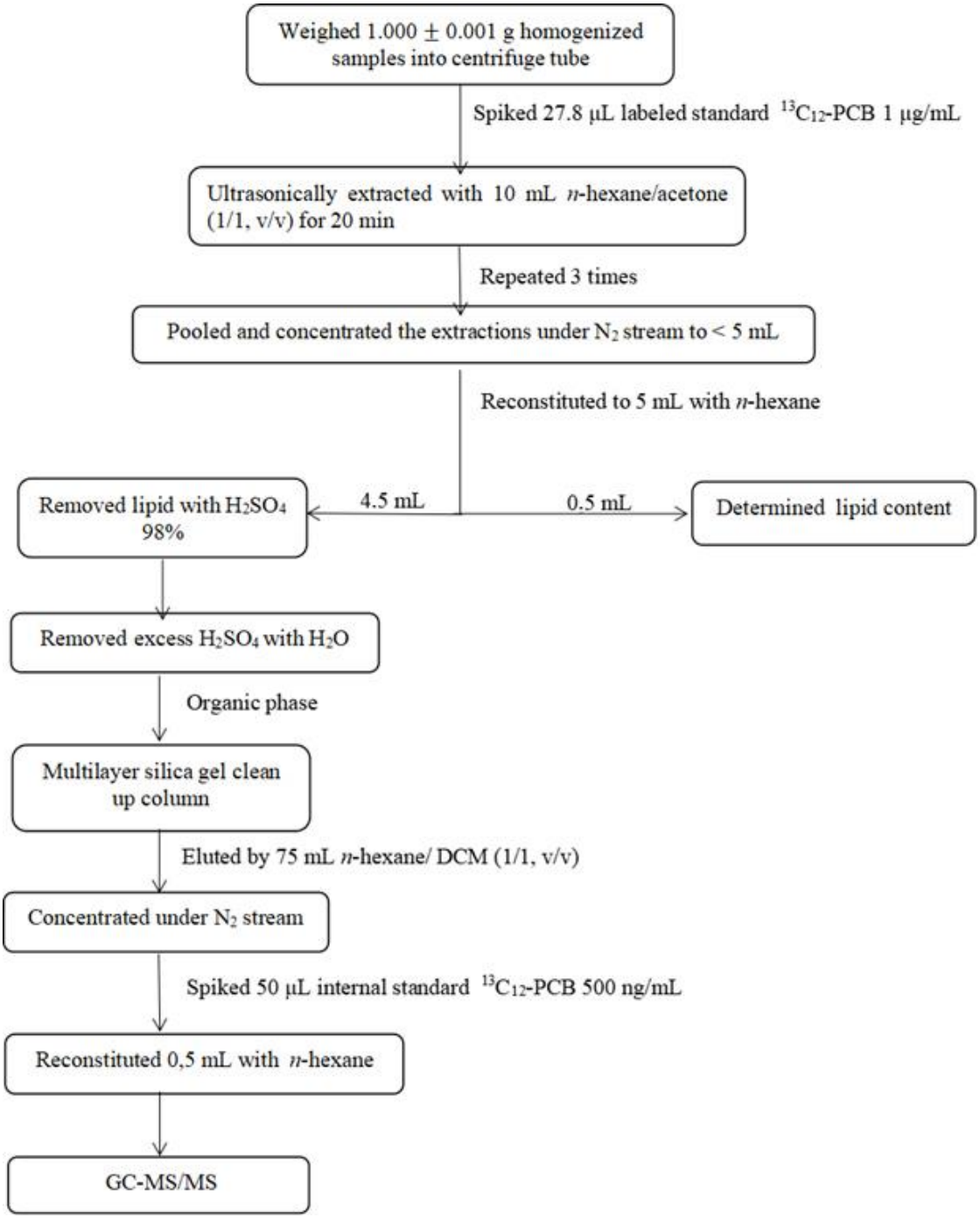


Figure 2. *Flowchart of fish sample processing*

The percentage of lipids in fish samples was determined according to the following equation:

$$\% \text{ lipid} = \frac{m_L}{m} \times 100$$

Where: m: weight of fish samples (g)

2.5.2 Content of PCBs based on lipid weight in fish samples

Content of PCBs was determined as follow:

$$C = (C_{sample} - C_{blank}) \times \frac{V}{m_L}$$

Where:

C: content of PCBs based on lipid weight (ng/g lipid)

C_{sample}: concentrations of PCBs in samples (ng/mL)

C_{blank}: concentrations of PCBs in blank samples (ng/mL)

V: Reconstituted volume (mL)

m_L: lipid weight in fish samples (g)

2.5.3 Moisture content of fish samples

The moisture content of fish samples was determined as follow:

$$H\% = \frac{(\text{weight of samples before freeze drying} - \text{weight of samples after freeze drying})}{\text{weight of samples before freeze drying}} \times 100$$

2.5.4 Content of PCBs based on wet weight

Content of PCBs based on wet weight was determined as follow:

$$C = (C_{sample} - C_{blank}) \times \frac{V}{m: (100\% - H\%)}$$

where:

C: content of PCBs based on wet weight (ng/g wet weight)

C_{sample}: concentration of PCBs in samples (ng/mL)

C_{blank} : concentration of PCBs in blank samples (ng/mL)

V: reconstituted volume (mL)

m: weight of fish samples (g)

H%: moisture content of fish samples

3. RESULTS AND DISCUSSION

3.1. Retention time and mass spectrometry conditions

Analyzed standard solution at concentration of 100 ng/mL in spectral scanning mode and compared with reference from NIST spectrum library to determine the retention time of each substance, select measurement transitions in SRM mode. The retention times and mass spectrometry conditions to analyze 28 PCBs are presented in Table 3.

Each peak in the chromatogram represents 28 PCBs native, 7 labeled and 5 internal standards. Detector MS/MS is highly selective because the ions are fragmented MS1 and MS2, so it can accurately determine the peaks of substances, for isomeric components have the same qualitative and quantitative SRM fragments were determined based on retention time.

Table 3. *Retention time and mass spectrometry conditions*

Num	*Analyte*	*Ret. time (min)*	*Quatitative m/z*	*Collision energy (eV)*	*Qualitative m/z*	*Collision energy (eV)*
^{1}IS	*$^{13}C_{12}$-PCB-77*	*12.45*	*302.0 > 232.0*	*28*	*304.0 > 234.0*	*28*
^{2}LS	*$^{13}C_{12}$-PCB-28*	*9.87*	*270.0 > 198.0*	*35*	*270.0 > 163.0*	*40*
1	PCB-8	8.55	222.0 > 152.0	22	224.0 > 152.0	22
2	PCB-18	9.16	256.0 > 186.0	22	258.0 > 188.0	22
3	PCB-28	9.90	256.0 > 186.0	22	258.0 > 188.0	22
^{2}LS	*$^{13}C_{12}$-PCB-52*	*10.38*	*304.0 > 232.0*	*45*	*304.0 > 269.0*	*10*
4	PCB-44	10.73	289.9 > 219.9	22	291.9 > 221.9	22
5	PCB-52	10.42	289.9 > 219.9	22	291.9 > 221.9	22
6	PCB-66	11.44	289.9 > 219.9	22	291.9 > 221.9	22
7	PCB-77	12.45	289.9 > 219.9	22	291.9 > 221.9	22
^{1}IS	*$^{13}C_{12}$-PCB-81*	*1228*	*302.0 > 232.0*	*28*	*304.0 > 234.0*	*28*
8	PCB-81	12.28	289.9 > 219.9	22	291.9 > 221.9	22
^{1}IS	*$^{13}C_{12}$-PCB-123*	*12.79*	*338.0 > 268.0*	*28*	*340.0 > 270.0*	*28*
^{2}LS	*$^{13}C_{12}$-PCB-101*	*11.79*	*338.0 > 268.0*	*30*	*338.0 > 303.0*	*10*
9	PCB-101	11.81	323.9 > 253.9	22	325.9 > 255.9	22
10	PCB-105	13.86	323.9 > 253.9	22	325.9 > 255.9	22
11	PCB-114	13.29	323.9 > 253.9	22	325.9 > 255.9	22
12	PCB-118	13.03	323.9 > 253.9	22	325.9 > 255.9	22
13	PCB-123	12.79	323.9 > 253.9	22	325.9 > 255.9	22
^{1}IS	*$^{13}C_{12}$-PCB-126*	*14.80*	*336.0 > 266.0*	*28*	*338.0 > 268.0*	*28*
14	PCB-126	14.80	323.9 > 253.9	22	325.9 > 255.9	22
^{1}IS	*$^{13}C_{12}$-PCB-169*	*15.19*	*372.0 > 302.0*	*28*	*370.0 > 300.0*	*28*
^{2}LS	*$^{13}C_{12}$-PCB-138*	*13.62*	*372.0 > 302.0*	*40*	*372.0 > 337.0*	*10*
15	PCB-128	14.12	357.9 > 287.9	22	359.9 > 289.9	22
16	PCB-138	13.67	357.9 > 287.9	22	359.9 > 289.9	22
^{2}LS	*$^{13}C_{12}$-PCB-153*	*13.16*	*372.0 > 302.0*	*30*	*372.0 > 337.0*	*10*
17	PCB-153	13.20	357.9 > 287.9	22	359.9 > 289.9	22
18	PCB-156	14.74	357.9 > 287.9	22	359.9 > 289.9	22
19	PCB-157	14.62	357.9 > 287.9	22	359.9 > 289.9	22
20	PCB-167	14.16	357.9 > 287.9	22	359.9 > 289.9	22
21	PCB-169	15.19	357.9 > 287.9	22	359.9 > 289.9	22

Num	*Analyte*	*Ret. time (min)*	*Quatitative m/z*	*Collision energy (eV)*	*Qualitative m/z*	*Collision energy (eV)*
[2]LS	*$^{13}C_{12}$-PCB-180*	*14.75*	*406.0 > 336.0*	*40*	*406.0 > 371.0*	*20*
22	PCB-170	15.28	391.9 > 321.9	22	393.9 > 323.9	22
23	PCB-180	14.80	391.9 > 321.9	22	393.9 > 323.9	22
24	PCB-187	13.91	391.9 > 321.9	22	393.9 > 323.9	22
25	PCB-189	15.80	391.9 > 321.9	22	393.9 > 323.9	22
[2]LS	*$^{13}C_{12}$-PCB-209*	*17.41*	*507.7 > 437.8*	*28*	*509.7 > 439.8*	*28*
26	PCB-195	16.01	427.8 > 355.8	22	429.8 > 357.8	22
27	PCB-206	16.96	461.7 > 391.8	22	463.8 > 393.8	22
28	PCB-209	17.46	497.7 > 427.8	22	495.7 > 425.8	22

[1]IS: internal standard
[2]LS: labeled standard

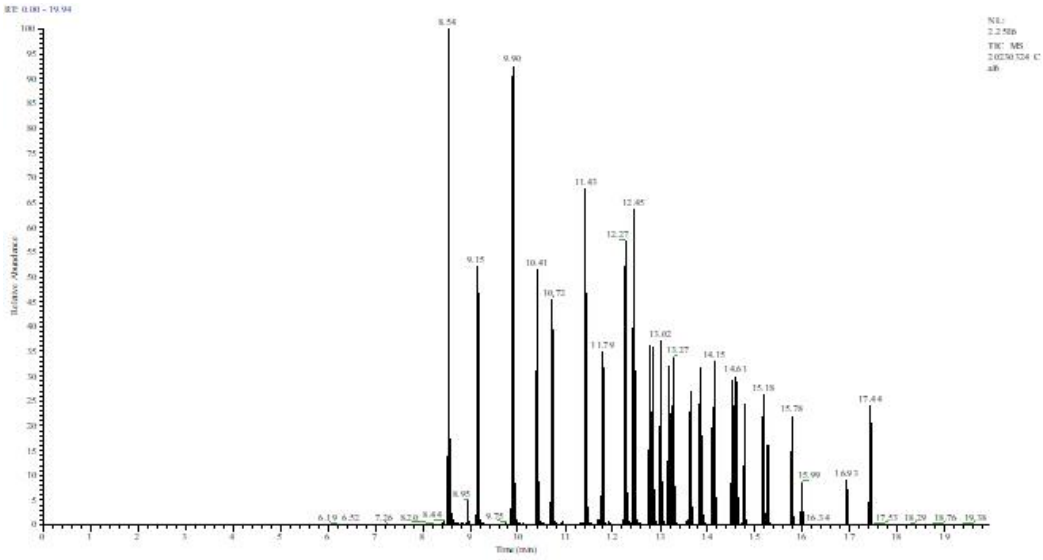


Figure 3. *Total ion chromatograms (TICs) of 28 PCBs, 7 labeled standard and 5 internal standards at concentration of 100 ng/mL*

3.2. Instrument detection limit

The instrument detection limit is considered to be the lowest concentration of the analyte for which the instrument will give an analyte signal that is significantly different from the background signal. The instrument detection limit was determined by 10 times repeated injection of the 0.2 ng/mL standard solution and calculation based on the standard deviation of the 10 times measured values. The formula for calculating IDL is referenced from [18].

$$IDL = 3 \times SD$$

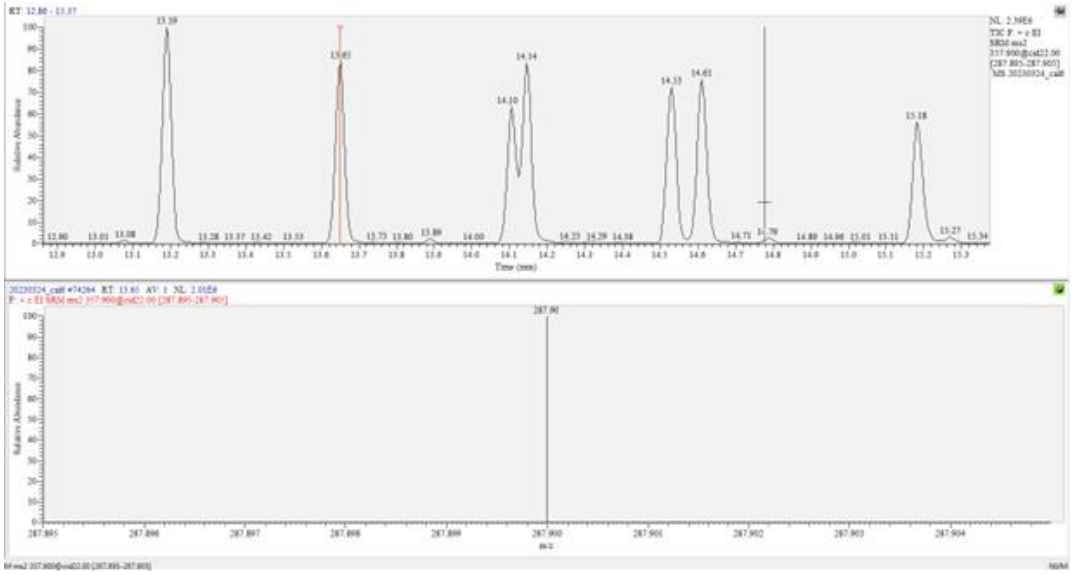


Figure 4. *Chromatogram of group ions with 6 chlorine atoms around biphenyls (6-Cl) at concentration of 100 ng/mL*

Table 4. *Instrument detection limit of 28 PCBs*

Analyte	***IDL (ng/mL)***	***Analyte***	***IDL (ng/mL)***	***Analyte***	***IDL (ng/mL)***
PCB-8	0.014	PCB-114	0.013	PCB-169	0.011
PCB-18	0.016	PCB-118	0.014	PCB-170	0.009
PCB-28	0.016	PCB-123	0.011	PCB-180	0.017
PCB-44	0.021	PCB-126	0.017	PCB-187	0.011
PCB-52	0.018	PCB-128	0.017	PCB-189	0.008
PCB-66	0.023	PCB-138	0.011	PCB-195	0.019
PCB-77	0.023	PCB-153	0.012	PCB-206	0.015
PCB-81	0.016	PCB-156	0.017	PCB-209	0.021
PCB-101	0.019	PCB-157	0.013		
PCB-105	0.021	PCB-167	0.011		

Instrument detection limits ranged from 0.008 to 0.023 ng/mL, whereas the minimum value belongs to PCB-189 (0.008 ng/mL) and the maximum value belongs to PCB-66, PCB-77 (0.023 ng/mL). Method has similar IDLs values to other studies [19-20].

3.3 Calibration curve and linearity

Calibration curves were constructed at concentrations 0.2; 0.5; 2; 20; 50; 100; 200 ng/mL for native standards and 50 ng/mL for internal standard in *n*-hexan solvent. The calibration curves represent the dependence between $S_{peak\ of\ native\ standard}/S_{peak\ of\ internal\ standard}$ and the analyte concentration.

Table 5. *Calibration curves of 28 PCBs (concentration ranged 0.2 to 200 ng/mL)*

Congeners	*Linear regression equations*	R^2	*Congeners*	*Linear regression equations*	R^2
PCB-8	Y = 0.063X + 0.03	0.9999	PCB-128	Y = 0.023X + 0.0001	1.0000
PCB-18	Y = 0.033X + 0.0003	1.0000	PCB-138	Y = 0.022X + 0.002	1.0000
PCB-28	Y = 0.045X + 0.001	0.9999	PCB-153	Y = 0.026X + 0.005	1.0000
PCB-44	Y = 0.025X - 0.0002	1.0000	PCB-156	Y = 0.021X + 0.001	1.0000
PCB-52	Y = 0.028X + 0.002	0.9998	PCB-157	Y = 0.022X + 0.001	1.0000
PCB-66	Y = 0.030X - 0.001	1.0000	PCB-167	Y = 0.026X + 0.005	1.0000
PCB-77	Y = 0.025X - 0.001	0.9998	PCB-169	Y = 0.017X + 0.001	1.0000
PCB-81	Y = 0.026X + 0.001	1.0000	PCB-170	Y = 0.012X + 0.001	1.0000
PCB-101	Y = 0.025X + 0.001	0.9998	PCB-180	Y = 0.014X + 0.003	0.9999
PCB-105	Y = 0.028X + 0.00001	1.0000	PCB-187	Y = 0.014X + 0.002	1.0000
PCB-114	Y = 0.026X - 0.001	1.0000	PCB-189	Y = 0.012X + 0.001	1.0000
PCB-118	Y = 0.025X - 0.002	1.0000	PCB-195	Y = 0.006X - 0.0001	0.9998
PCB-123	Y = 0.025X - 0.002	1.0000	PCB-206	Y = 0.012X + 0.001	0.9999
PCB-126	Y = 0.032X + 0.0005	1.0000	PCB-209	Y = 0.023X + 0.03	0.9999

where Y is $S_{peak\ of\ native\ standard}$ / $S_{peak\ of\ internal\ standard}$ and X is concentration of analyte

Calibration curves of 28 PCBs exhibited good linearity with correlation coefficient higher than 0.9998, demonstrating a linear relationship between the signal and the analyte concentration.

3.4. Analysis of blank samples and Method Detection Limit

To determine the method detection limit (MDL), 3 blank samples containing *n*-hexane solvent were analyzed following the procedure shown in Figure 2. MDLs were calculated based on the mean of the blank and the standard deviation of the blank:

$$MDL = \overline{BL} + 3SD$$

Where MDL: method detection limit; $\overline{BL}$: mean of the blank; SD: the standard deviation of the blank

Table 6. *Method detection limit of 28 PCBs*

Congeners	*MDL (ng/g)*	*Congeners*	*MDL (ng/g)*	*Congeners*	*MDL (ng/g)*
PCB-8	1.73	PCB-114	1.57	PCB-169	0.33
PCB-18	1.49	PCB-118	1.19	PCB-170	0.30
PCB-28	1.84	PCB-123	1.44	PCB-180	0.14
PCB-44	1.57	PCB-126	1.10	PCB-187	0.27
PCB-52	1.49	PCB-128	0.35	PCB-189	0.26
PCB-66	0.68	PCB-138	1.20	PCB-195	0.08
PCB-77	0.85	PCB-153	0.83	PCB-206	0.44
PCB-81	0.58	PCB-156	0.86	PCB-209	0.07
PCB-101	0.66	PCB-157	0.31		
PCB-105	0.59	PCB-167	0.32		

Method detection limits (MDLs) of 28 PCBs ranged from 0.07 to 1.84 ng/g. These MDL values are similar and even lower than those reported in other studies [5-6], which proved that the analytical method completely satisfied the requirements of PCBs analysis in marine fish samples.

3.5. Recovery of labeled standard and analysis matrix-spike samples

PCBs labeled standard $^{13}C_{12}$-PCB-28/52/101/138/153/180/209 at concentration of 50 ng/mL were added to all samples before the extraction, separation.

Recovery of PCBs labeled standard in matrix-spiked samples (sample HT01):

$$R\ (\%) = \frac{\text{Concentration of surrogate}}{\text{Concentration of surrogate spiked}} \times 100$$

Recovery of PCBs native standard in matrix-spiked samples (HT01):

$$R\ (\%) = \frac{C_{spike} - C_{blank}}{C_0} \times 100\%$$

Where: C_{spike} : concentration of matrix-spike samples

C_{blank}: concentration of the background sample

C_0 : concentration of standard was added into sample

Table 7. *Recovery of native and labeled standards in matrix-spiked samples*

Congeners	*Recovery (%)*	*Congeners*	*Recovery(%)*	*Congeners*	*Recovery(%)*
PCB-8	68.0	PCB-123	77.4	PCB-189	76.2
PCB-18	68.0	PCB-126	74.6	PCB-195	77.8
PCB-28	74.8	PCB-128	68.5	PCB-206	76.4
PCB-44	64.2	PCB-138	73.7	PCB-209	71.9
PCB-52	62.3	PCB-153	74.3	$^{13}C_{12}$-PCB-28	91.9
PCB-66	71.3	PCB-156	85.6	$^{13}C_{12}$-PCB-52	88.9
PCB-77	73.3	PCB-157	88.1	$^{13}C_{12}$-PCB-101	84.3
PCB-81	73.4	PCB-167	76.9	$^{13}C_{12}$-PCB-138	89.6
PCB-101	71.7	PCB-169	86.7	$^{13}C_{12}$-PCB-153	80.0
PCB-105	76.3	PCB-170	75.7	$^{13}C_{12}$-PCB-180	77.4
PCB-114	79.8	PCB-180	79.9	$^{13}C_{12}$-PCB-209	75.5
PCB-118	75.2	PCB-187	68.3		

The recoveries of 28 PCBs native and 7 PCBs labeled standards ranged from 62.3 to 88.1%, and from 75.5 to 91.9%, respectively. These values satisfying criteria proposed by AOAC (recovery 60 - 115% for 10 - 100 ppb levels), suitable for analyzing marine fish samples.

3.6. Concentration of PCBs in fish sample

The validated method was applied to analyze 10 marine fish samples collected from Hai Phong, Thai Binh, Nghe An, Ha Tinh and Thanh Hoa (Figure 5). Levels of ∑28PCBs range from 17 to 851 (mean 230) ng/g lipid, with the highest level found in white sardine (Hai Phong) and torpedo scad (Thai Binh), and the lowest level found in scatophagus argus (Nghe An).

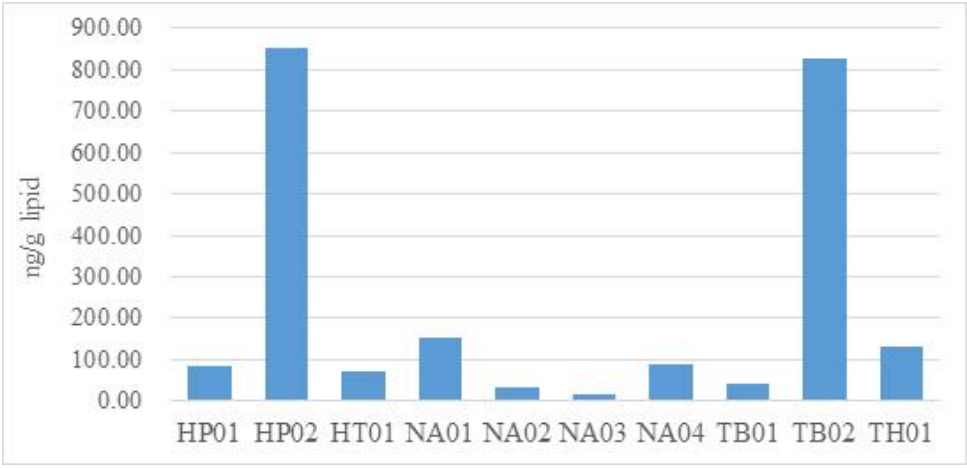


Figure 5. *Total content of 28 PCBs on lipid weight in marine fish samples*

The cumulative of 28 PCBs in marine fish samples in this study were shown as the percentage (Figure 6) of 9 isomer groups with the number of chlorine atoms ranging from 2 to 10. In all 10 fish samples, the 5-Cl and 6-Cl groups were the main isomer groups, accounting for 5 -32%, and 17 - 45%, respectively. PCB-153 and PCB-138 were the dominant congeners, with content of 4.23 - 45.90 ng/g lipid and 1.29 - 39.66 ng/g lipid, respectively, accounting for 4 - 23% and 4 - 19% of ∑28PCBs. This finding agrees well to another research by Yo. Uekusa et al. [21], which reported that the 5-Cl and 6-Cl groups were also major isomer groups, with the percentage of the 5-Cl group being 21 - 39% and the 6-Cl group being 23 - 43%. PCB-153 was also found to be the main congener with a percentage of about 7.2 - 18% of ∑209PCBs. In another study, that analysed 36 PCBs in marine fish samples, PCB-153 and PCB-138 were also the dominant PCBs [22].

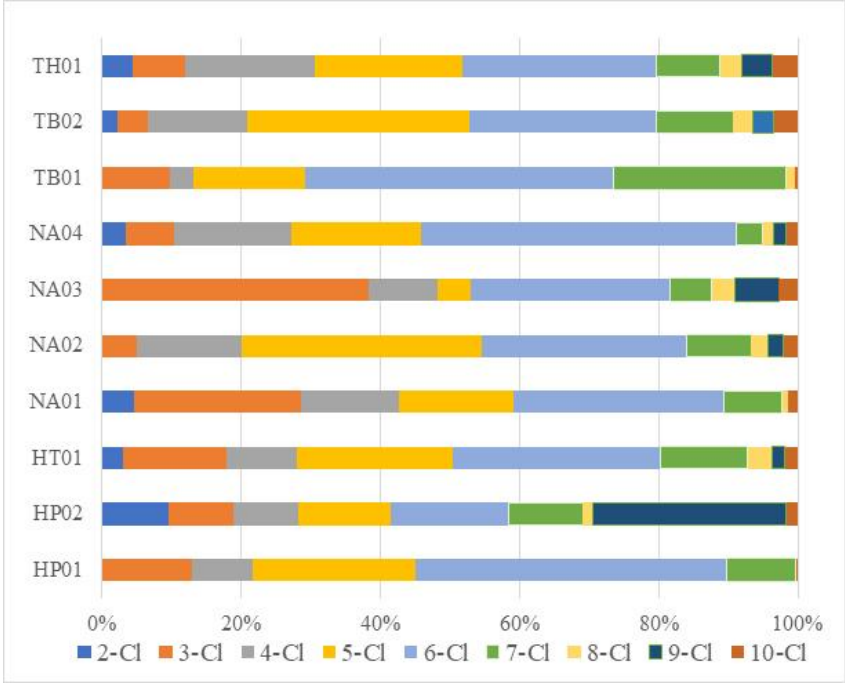


Figure 6. *Percentage of PCBs isomer groups on lipid weight in marine fish samples*

The sum of 6in-PCBs (PCB-28; -52; -101; -138; -153; -180) of 10 marine fish samples on wet weight were calculated to compare with the maximum level recommended by The European Union. The obtained values for 10 fish samples from 1.24 to 3.15 ng/g wet weight, much lower than the recommended value (75 ng/g ww) [23]. Congeners PCB-126 and PCB-169, which are two highly toxic substances belonging to the group of 12dl-PCBs, were not detected in marine fish samples.

4. CONCLUSIONS

This study analyzed 28 PCBs in marine fish samples on the GC-MS/MS system. The method was validated, which allow to to estimate the content and features of PCBs in marine fish with high accuracy. In all samples were collected, PCB-153 and PCB-138 were the dominant congeners at concentration ranging from 4.23 to 45.90 ng/g lipid, and 1.29 to 39.66 ng/g lipid, accounting for 4 - 23% and 4 - 19% ∑28PCBs, respectively, due to lipophilic properties and environmental stability. The sum of 6in-PCBs (PCB-28; -52; -101; -138; -153; -180) on wet weight in all fish samples were lower than the maximum level recommended by The European Union. And the two highly toxic congeners PCB-126, PCB-169 were not detected in all fish samples. This study was preliminary assessment of the PCBs levels in marine fish samples due to the small sample size (n = 10), however it may be used as a basis for further research with other fish samples, as well as larger sample size.

ACKNOWLEDGEMENT

The study was funded by Component 3: “Building a profile of common toxic substances (Pb, As, Hg, Pesticide etc.) in some marine foods, code TĐĐTB0.03/21-23" under the key science and technology project of the Vietnam Academy of Science and Technology: "Developing procedures for determining toxins in some marine species in Vietnam", code TĐĐTB0.00/21-23.

REFERENCES

[1]. M. D. Erickson and R. G. Kaley, "Applications of polychlorinated biphenyls," *Environmental Science and Pollution Research*, vol. 18, pp. 135-151, 2011.

[2]. D. O. Carpenter, "Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health," *Reviews on environmental health*, vol. 21, no. 1, pp. 1-24, 2006.

[3]. P. L. Lallas, "The Stockholm Convention on persistent organic pollutants," *American Journal of International Law*, vol. 95, no. 3, pp. 692-708, 2001.

[4]. N. Sinh, L. Thuy, N. Kinh, and L. Thang, "The persistent organic pollutants and their management in Vietnam," in *Proceedings of the Regional Workshop on the Management of Persistent Organic Pollutants-POPs, UNEP, Hanoi, Vietnam*, pp. 16-19, 2019.

[5]. Ottonello, Giuliana, Ferrari, Angelo Magi, Emanuele, "Determination of polychlorinated biphenyls in fish: Optimisation and validation of a method based on accelerated solvent extraction and gas chromatography-mass spectrometry," *Food Chemistry*, vol. 142, pp. 327-333, 2014.

[6]. Abdurrahman Aksoy; Yavuz Kursad Das; Oguzhan Yavuz; Dilek Guvenc; Enes Atmaca; Sema Agaoglu "Organochlorine Pesticide and Polychlorinated Biphenyls Levels in Fish and Mussel in Van Region, Turkey," *Toxicology letters*, vol 196, pp. 65-69, 2011.

[7]. X.-T. Peng, L. Jiang, Y. Gong, X.-Z. Hu, L.-J. Peng, and Y.-Q. Feng, "Preparation of mesoporous ZrO2-coated magnetic microsphere and its application in the multi-residue analysis of pesticides and PCBs in fish by GC–MS/MS," *Talanta*, vol. 132, pp. 118-125, 2015.

[8]. K. Kalachova, J. Cajka. Pulkrabova, L. Drabova, M. Stupak, and J. Hajslova, "Gas chromatography-triple quadrupole tandem mass spectrometry: a powerful tool for the (ultra) trace analysis of multiclass environmental contaminants in fish and fish feed," *Analytical and Bioanalytical Chemistry*, vol 405, no. 24, pp. 7803-7815, 2013.

[9]. T.-T. Tran-Lam, T. C. Quan, P. T. Pham, A.-T. T. Phung, M. Q. Bui, and Y. H. Dao, "Occurrence, distribution, and risk assessment of halogenated organic pollutants (HOPs) in marine fish muscle: The case study of Vietnam," *Marine Pollution Bulletin*, vol. 192, pp. 114986, 2023.

[10]. Stancheva. M, Georgieva, S, & Makedonski. L, " Organochlorine pollutants in fish from the Bulgarian region of the Black Sea," *Quality Assurance and Safety of Crops & Foods*, vol. 5, no. 3, pp. 243-251, 2013.

[11]. D. D. Nhan, N. M. Am, N. C. Hoi, L. V. Dieu, F. P. Carvalho, J-P Villeneuve, and C. Cattini, "Organochlorine pesticides and PCBs in the Red river delta, North Vietnam," *Marine Pollution Bulletin*, vol. 36, no. 9, pp. 742-749, 1998.

[12]. D. N. Dang, M. A. Nguyen, F. P. Carvalho, J. P. Villeneuve, and C. Cattini, "Organochlorine pesticides and PCBs along the coast of North Vietnam," Science of The Total Environment, vol. 237, pp. 363-371, 1999.

[13]. T. A. M. Tran, G. Malarvannan, T. L. Hoang, A. Covaci, and M. Elskens, "Occurrence of organochlorine pesticides and polychlorinated biphenyls in sediment and fish in Cau Hai lagoon of Central Vietnam: Human health risk assessment," *Marine Pollution Bulletin*, vol. 141, pp. 521-528, 2019.

[14]. P. T. Hien, N. M. Tue, G. Suzuki, S. Takahashi, and S. Tanabe, "Polychlorinated biphenyls and polybrominated diphenyl ethers in fishes collected from Tam Giang-Cau Hai Lagoon, Vietnam," *Interdisciplinary studies on environmental chemistry - Environmental pollution and ecotoxicology*, pp. 221-227, 2012.

[15]. E. US, "Method 8270E (SW-846): semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS)," *Washington, DC*, 2014.

[16]. E. US EPA, "Method 1668C. "Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS, " *Washington, DC*, 2010.

[17]. U. US EPA, "Method 3550C. Ultrasonic extraction," Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, vol. 3, pp. 1-17, 2007.

[18]. Thermo Scientific, "Practical Determination and Validation of Instrument Detection Limit for the ISQ 7000 Single Quadrupole GC-MS System with Advanced Electron Ionization Source". [Online]. Available: https://www.thermofisher.com/vn/en/home/global/forms/instrument-detection-limit-isq-7000-single-quad-gc-ms-system-aei-source.html.

[19]. W. Li et al, "Matrix solid-phase dispersion combined with GC–MS/MS for the determination of organochlorine pesticides and polychlorinated biphenyls in marketed seafood," *Chromatographia*, vol. 80, no. 5, pp. 813-824, 2017.

[20]. P. Castells, J. Parera, F. Santos, and M. J. C. Galceran, "Occurrence of polychlorinated naphthalenes, polychlorinated biphenyls and short-chain chlorinated paraffins in marine sediments from Barcelona (Spain)," *Chemosphere*, vol. 70, no. 9, pp. 1552-1562, 2008.

[21]. Y. Uekusa et al., "Determination of polychlorinated biphenyls in marine fish obtained from tsunami-stricken areas of Japan," *PLoS One*, vol. 12, no. 4, pp. e0174961, 2017.

[22]. S. Georgieva, M. Stancheva, and L. Makedonski, "Organochlorine pesticides and PCBs in marine fish," *Ovidius University Annals of Chemistry*, vol. 23, no. 1, pp. 92-98, 2012.

[23]. EU, "Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs," *Official Journal*, vol 320, pp 18-23, 2011.

Phân tích polychlorinated biphenyls trong mẫu cá biển bằng phương pháp sắc ký khí khối phổ hai lần (GC-MS/MS)

Phạm Thị Diễm Quỳnh[1], Phạm Thị Ngọc Mai, Hoàng Quốc Anh[1], Lê Minh Thùy[2], Nguyễn Thị Hương[2], Vũ Khánh Hòa[2], Đỗ Thị Thu Hương[2], Nguyễn Thị Xuyên[2], Nguyễn Xuân Hưng[2], Chu Đình Bính[3], Vũ Đức Nam[2*]

[1]Khoa Hoá học, Trường Đại học Khoa học Tự nhiên, Đại học Quốc gia Hà Nội, Hà Nội, Việt Nam

[2]Trung tâm Nghiên cứu và Chuyển giao Công nghệ, Viện Hàn lâm Khoa học và Công nghệ Việt Nam, Hà Nội, Việt Nam

[3]Viện Kỹ thuật Hóa học, Đại học Bách Khoa Hà Nội, Hà Nội, Việt Nam

Tóm tắt

Polychlorinated biphenyls (PCBs) là nhóm chất ô nhiễm hữu cơ khó phân hủy (POPs) điển hình được liệt kê trong Phụ lục A (các chất cần loại bỏ) và Phụ lục C (các chất phát sinh không chủ định) theo Công ước Stockholm. Trong nghiên cứu này, phương pháp sắc ký khí ghép nối khối phổ hai lần (GC-MS/MS) được nghiên cứu và áp dụng để phân tích hàm lượng 28 cấu tử PCBs trong một số mẫu cá biển tại Việt Nam. PCBs trong cá biển được chiết siêu âm với hỗn hợp dung môi acetone/*n*-hexane (1/1, v/v). Dịch chiết mẫu được làm sạch bằng cột silica gel đa lớp với dung môi rửa giải dichloromethane/*n*-hexane (1/1, v/v). PCBs được tách trên cột mao quản DB- 5MS và được xác định bằng detector khối phổ ba tứ cực. Detector được vận hành ở chế độ ion hóa va chạm electron (EI) và chế độ quan sát phản ứng chọn lọc SRM (selected reaction monitoring). Đường chuẩn của 28 PCBs có độ tuyến tính cao $R^2 \geq$ 0,9998. Giới hạn phát hiện của thiết bị (IDL) và giới hạn phát hiện của phương pháp (MDL) dao động trong khoảng 0,08 - 0,023 ng/mL và 0,07 - 1,84 ng/g. Độ thu hồi của 28 chất chuẩn PCBs và 7 chất chuẩn đồng hành trong mẫu thêm chuẩn dao động trong khoảng 62,3 - 88,1% và 75,9 - 91,9%, đáp ứng yêu cầu của AOAC (độ thu hồi 60 - 115% với khoảng nồng độ 10 - 100 ppb). Phương pháp sau khi thẩm định đã được áp dụng để phân tích 10 mẫu cá biển, cho thấy hàm lượng Σ28PCBs dao động từ 17 - 851 ng/g lipid. Kết quả tổng nồng độ 6in-PCBs (PCB-28; -52; -101; -138; -153; -180) theo khối lượng ướt dao động từ 1,24 - 3,15 ng/g, giá trị này thấp hơn hàm lượng tối đa cho phép theo quy định của Châu Âu (75 ng/g wet weight). Hai cấu tử PCB-126 và PCB-169 đều không được phát hiện trong tất cả mẫu cá nghiên cứu.

Từ khóa: *POPs, PCBs, cá biển, Việt Nam, GC-MS/MS.*