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Research Article

Determination of some indicator polychlorinated biphenyls (I-PCBs) in foods by gas chromatography tandem mass spectrometry (GC-MS/MS)

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Abstract

Polychlorinated biphenyls (PCBs) are toxic chemicals that persist in the environment and can accumulate in the food chain. The study was conducted with the aim of developing a method to determine the content of 6 I-PCBs compounds by gas chromatography-mass spectrometry (GC-MS/MS) combined with Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) technique for sample treatment. The sample was extracted with acetonitrile (ACN) solvent and cleaned with PSA adsorbent. The cleaned extract was analyzed on a GC-MS/MS system with a DB-5MS column (30 m × 0.25 mm × 0.25 µm). The method had good specificity, the linear range was established in the range of 1.5-40 ng/g, the correlation coefficient R^2 > 0.999. The method recovery was 74.5-102.7% and the relative standard deviation (RSD%) ranged from 0.9-6.20% for milk and meat samples. The method's limit of detection (LOD) and limit of quantification (LOQ) for 6 I-PCBs were 0.5 ng/g and 1.5 ng/g, respectively. The method was applied to analyze the content of 6 I-PCBs in 40 food samples (meat and meat products, milk and dairy products) randomly purchased in Hanoi. The results showed that in all 40 analyzed samples, 1 powdered milk sample detected PCB-28 and PCB-101, the total content of I-PCBs was 13.0 ng/g fat lower than the European regulations.

Keywords: *I-PCBs, GC-MS/MS, food, meat, milk.*

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1. INTRODUCTION

PCBs are chlorinated organic compounds consisting of two benzene rings with a single carbon-carbon bond, with a general formula of $C_{12}H_{10-n}Cl_n$, where n ranges from 1 to 10 (Figure 1). One of the 10 hydrogen atoms on the biphenyl ring can be replaced by chlorine atoms, resulting in 209 different PCB compounds. The toxicity of PCBs varies depending on the number and position of chlorine atoms substituted on the two benzene rings. PCBs are hydrophobic and easily soluble in less polar organic solvents (n-hexane, isooctane, n-heptane, acetone, and alcohol), oils, and fats. Compounds with fewer chlorine atoms are more volatile than those with more chlorine atoms. PCBs exist as crystals in their pure form, but commercial PCBs are mixtures of multiple congeners in liquid or viscous form, odorless, colorless, or pale yellow [1].

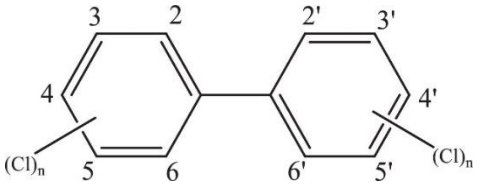

Figure 1. *Chemical structure of PCBs (n represents the number of chlorine atoms on each ring)*

PCBs have been widely used in many industries, such as transformer and capacitor insulating oils, hydraulic fluids, plasticizers in paints, inks, carbonless copy paper, synthetic rubber, and flame retardants. Although PCBs have several advantages, such as excellent insulation properties and non-flammability, they are difficult to degrade and persist in the environment, leading to their classification as Persistent Organic Pollutants (POPs). As a result, PCBs have been banned from production and use since 1979. Vietnam is a member of the Stockholm Convention [2] And the ban on the use of PCBs was enforced in 2020. However, due to past usage or improper disposal of PCB-containing products, PCBs still exist in the environment (water, soil, air). According to studies [3-5], PCB exposure often comes from consuming fish, and other products with lower PCB levels, such as meat and milk, which are regularly consumed, also contribute to human PCB exposure [6-8]. PCBs are known to be carcinogenic and negatively affect the nervous, immune, and reproductive systems [6].

Among the 209 PCB compounds, 12 compounds (PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 và 189) have physical, chemical, and toxicological properties similar to dioxins and furans, and are thus referred to as dioxin-like PCBs (dioxin-like PCBs, DL-PCBs). However, non-dioxin-like PCBs (non-dioxin-like PCBs, NDL-PCBs) which represent a significant proportion of PCBs, have also been found in human serum, adipose tissue, and breast milk [9]. According to the European Food Safety Authority (EFSA) in 2005, six NDL-PCBs, including PCB-28, 52, 101, 138, 153, and 180 account for approximately 50% of the total NDL-PCBs present in food. As a result, they are considered 'indicator' PCBs (I-PCBs) and have been selected as representatives for PCB monitoring in most countries worldwide. The European Commission (EC) regulation, dated April 25, 2023, sets the maximum limit for the total I-PCBs content in milk and dairy products, meat, and meat products at 40 ng/g of fat [10]. While Vietnam does not produce PCBs, they have

been used in equipment, transformer oils, and capacitors imported from abroad. Between 1960 and 1990, an estimated 27,000 to 30,000 tons of PCB-containing oils in electrical equipment were imported into Vietnam from the USA, Russia, China, and several other countries [3]. Therefore, analyzing PCB content in food is essential to assess the potential health risks posed by these compounds through dietary exposure.

In Vietnam, some studies have analyzed PCB compounds in seafood samples (mainly fish) [3-5], soil, water, and dust [11], but there has been very little research on food matrices that are widely consumed and pose high health risks, such as meat and milk. Therefore, in this study, the group of I-PCBs (PCB-28, 52, 101, 138, 153, 180) was selected for determination using the highly selective and accurate GC-MS/MS method, combined with the QuEChERS sample preparation technique, which has many advantages such as simplicity, efficiency, speed, and stability. The focus is on high-risk and widely consumed food samples, such as meat and milk.

2. MATERIALS AND METHODS

2.1. Research subject

The selected analytes are six compounds from the "indicator" PCBs (I-PCBs) group, including PCB-28, 52, 101, 138, 153, and 180. The analyzed sample subjects consist of 20 samples of milk and dairy products (powdered milk, liquid milk, yogurt, and drinking yogurt), and 20 samples of meat and meat products (beef, pork, pork sausage, pork pâté, and sausages) randomly collected in the Hanoi area.

2.2. Chemicals and standards

The chemicals used in the study are all analytical purity chemicals suitable for use in GC-MS/MS, including: PCB-Mix 1 standard solutions (PCB-28/52/101/138/153/180) at a concentration of 10 µg/mL in cyclohexane provided by LCG (Germany), and internal standard ^{13}C-Labeled PCB Mixture-A (PCB-77/81/123/126/169/180) at a concentration of 1 µg/mL in nonane provided by CIL (Germany). The intermediate standard solutions include analytes and internal standards at a concentration of 10 ng/mL. All PCB standard solutions are prepared in acetonitrile and stored in dark-colored plastic containers at a temperature of – 4°C. The solvents and chemicals used in the study include: methanol, acetonitrile (ACN), n-hexane, acetic acid, anhydrous magnesium sulfate, sodium chloride, hydrochloric acid, petroleum ether from Merck (Germany); trisodium citrate dehydrate from Fisher (UK); disodium hydrogen citrate sesquihydrate from Acros Organics (Spain); secondary amine powder (PSA), octadecyl silica (C18) powder from Agilent (USA); and ultrapure water filtered using a Milli-Q filtration system.

2.3. Equipment

The main equipment used in the study includes a gas chromatography-tandem mass spectrometry system (GC-MS/MS) (GC Trace 1310, MS/MS-TSQ 9000), and a Triplus RSH Autosampler from Thermo Scientific (USA); the DB-5MS chromatography column (30 m × 0.25 mm × 0.25 µm) (Agilent). Additionally, other common laboratory equipment and tools were also used in the study.

2.4. Research methods

2.4.1. Analytical method

The gas chromatography-tandem mass spectrometry (GC-MS/MS) method was used to simultaneously analyze 6 I-PCBs in food samples (meat and meat products, milk, and dairy products). This is a modern analytical method with high sensitivity, accuracy, and selectivity, suitable for the research objectives. The GC-MS/MS analytical conditions were referenced from previous studies [5, 7, 8], including: the mobile phase was high-purity helium gas (99.99%) with a constant flow rate of 1.2 mL/min; the temperature program: 70ºC for 2 minutes; then increasing 20ºC/min to 300ºC and holding for 8 minutes. The total analysis time was 23 minutes. A splitless injection mode was used with a splitless time of 3 minutes, the injection port temperature was 280°C, and the injection volume was 1 µL.

2.4.2. Sample preparation method

In this study, the QuEChERS method was used for sample preparation, with advantages such as being quick, easy to perform, low-cost, effective, stable, and safe. Based on references [7-8] the proposed sample preparation process is as follows: The sample needs to be thoroughly homogenized before analysis. Accurately weigh approximately 5.0 g of the homogenized sample on an analytical balance (accuracy of 0.1 mg) into a 50 mL centrifuge tube. Add the internal standard at a concentration of 0.5 ng/g in the sample. Add 5.0 mL of ultrapure water (liquid samples do not require water addition), and vortex for 1 minute. Add 10.0 mL of solvent (extracting solvents including ACN or ACN in 0.1% acetic acid), and vortex for 10 minutes. Add the salt mixture (extracting salt mixture is being investigated), vortex for 1 minute, and centrifuge at 6000 rpm for 5 minutes. Collect the upper extract into a 15 mL centrifuge tube, re-extract the residue with 5 mL of solvent (previously investigated and selected), collect the upper extract, and combine it into the 15 mL centrifuge tube. The sample is then frozen at ≤ – 18°C for 30 minutes to 2 hours. Proceed with dispersive solid-phase extraction (d-SPE), then bring to room temperature, and pipette 12 mL of the clear extract into a 15 mL centrifuge tube containing the cleaning powder (investigated cleaning powder mixture includes $MgSO_4$, C18, and PSA), vortex for 1 minute, and centrifuge at 13000 rpm for 5 minutes. Pipette 7.5 mL of the clear extract into a 15 mL plastic tube and evaporate to dryness using a nitrogen evaporator. Dissolve the residue in 1 mL of ACN and filter through a 0,2 µm membrane into a sample vial for analysis on the GC-MS/MS system.

Since PCBs are non-polar compounds and only dissolve in fats, according to European Commission regulations, the maximum allowable residue levels of NDL-PCBs are expressed as a percentage of fat in the sample. Therefore, in this study, samples in which PCBs are detected require fat content analysis following this procedure: Accurately weigh 3 g (for powdered and semi-solid milk), 20 g (for liquid milk), and 3-5 g (for meat and meat products) into a 250 mL Erlenmeyer flask. The sample is then sequentially added with double-distilled water and concentrated 37% HCl, and the sample is gently shaken. Hydrolyze in a water bath at 100°C for 2 hours. Filter the sample through filter paper and rinse the acid with hot water until pH 7. The filter paper containing the hydrolyzed sample is dried in an oven at 105°C for 2-4 hours. The filter paper is then placed into the extraction

tube of a semi-automatic fat extraction machine (VELP) and the sample is extracted with petroleum ether for 2-3 hours. The extraction beaker, which is attached to the extraction tube, must be cleaned, dried at 105°C, and weighed beforehand (m_1). After extraction, the beaker containing the lipids is dried at 105°C until a constant weight is achieved. It is then removed and cooled in a desiccator for 30 minutes, and the mass of the beaker containing the fat (m_2) is measured.

2.4.3. Method validation

The analytical method was validated according to AOAC 2016 guidelines [12] with parameters such as: specificity (based on the retention time of the standard, blank sample, spiked sample, and the number of IP points), method detection limit (LOD), method quantification limit (LOQ), matrix effect (ME), standard line development and linearity evaluation, accuracy (assessed through recovery rates), and precision (assessed through repeatability with relative standard deviation (RSD%) values).

Blank samples were used in the evaluations and validation of the method, representing two sample matrices: powdered milk and pork, which did not contain the analytes (determined by GC-MS/MS).

2.4.4. Data processing method

The concentrations of PCBs in the samples were calculated using the software provided with the GC-MS/MS system (TraceFinder 5.1). The method validation results were processed using Microsoft Excel 2016. The concentration of each I-PCB (ng/g) was calculated using the formula (1):

$$P = \frac{C \text{ x } V \text{ x } k}{n} \qquad (1)$$

In which: V is the volume of the extraction solvent (mL); C is the concentration of the extract solution calculated based on the standard line (mg/L); k is the dilution factor (volume of residue/volume before drying with nitrogen); n is the mass of the analytical sample (g); P is the concentration of each I-PCB in the test sample (ng/g).

The fat content (%) is calculated using the formula (2):

$$X = \frac{m_2 - m_1}{m} \; x \; 100 \qquad (2)$$

In which: m1 is the weight of the empty cup (g); m2 is the weight of the cup containing fat (g); m is the weight of the analyzed sample (g); X is the fat content in the tested sample (%).

The PCB content (ng/g fat) calculated based on the fat content is determined using the formula (3):

$$PCBs = \frac{P \text{ x } 100}{X} \qquad (3)$$

3. RESULTS AND DISCUSSIONS

3.1. Survey to select analysis condition on GC-MS/MS

The analysis conditions on MS/MS were automatically optimized using the device's software by injecting a standard PCB solution and internal standard at a concentration of 0.2

µg/mL into the GC-MS/MS system to select the characteristic ions for each analyte (Table 1). A full scan mode (Full scan, m/z 200-500 amu) was employed. Identification was performed by comparing the retention time results of the analytes in EI mode at 70 eV with the provided and updated mass spectral library accompanying the GC-MS/MS system (NIST MS Search 2.2). The selected parent ions were further fragmented into product ions using the SRM (Selected Reaction Monitoring) mode. Two different parent ions were selected for each PCB component, with each parent ion being fragmented to obtain one production. The production with a larger and more stable signal was used as the quantification ion, while the other ion was used for confirmation. Since PCBs have many isomers and homologues, multiple substances can have the same quantification and confirmation ions, such as PCB-153 and PCB-138. Therefore, for isomers or substances with the same molecular weight or similar fragmentation patterns, it is crucial to rely on retention time-based separation in chromatography to accurately quantify these groups of substances. Thus, in addition to relying on the fragmentation results, using the elution order of the substances and referencing the certificate of analysis from the manufacturer is essential. The retention times and analysis conditions of the selected substances are shown in Table 1.

***Table 1**. Retention times and mass spectrometry conditions used for analyzing I-PCBs by GC-MS/MS method*

Analytes	Retention time minutes)	Precursor ion (m/z)	Product ion (m/z)	CE (eV)	Note
PCB 28	11.52	256.0	186.0	20	Quantitative
		258.0	188.0	20	Qualitative
PCB 52	11.85	289.9	219.9	20	Quantitative
		291.9	221.9	20	Qualitative
PCB 101	12.69	325.9	255.9	20	Quantitative
		323.9	253.9	20	Qualitative
$^{13}C_{12}$-PCB 81	12.92	302.0	232.0	28	Quantitative
		304.0	234.0	28	Qualitative
$^{13}C_{12}$-PCB 123	13.20	338.0	268.0	28	Quantitative
		340.0	270.0	28	Qualitative
PCB 153	13.48	359.9	289.9	22	Quantitative
		357.9	287.9	22	Qualitative
PCB 138	13.75	359.9	289.9	22	Quantitative
		357.9	287.9	22	Qualitative
$^{13}C_{12}$-PCB 126	13.81	336.0	266.0	28	Quantitative
		338.0	268.0	28	Qualitative
	14.44	406.0	336.0	30	Quantitative

Analytes	Retention time minutes)	Precursor ion (m/z)	Product ion (m/z)	CE (eV)	Note
$^{13}C_{12}$-PCB 180		406.0	371.0	20	Qualitative
PCB 180	14.44	393.8	323.9	25	Quantitative
		391.8	321.9	25	Qualitative

3.2. Sample preparation condition survey

The survey was conducted on two representative blank matrices: powdered milk and pork. To evaluate the recovery, the blank samples after weighing were spiked with a standard concentration of 5 ng/g and an internal standard concentration of 0.5 ng/g. The samples were processed as described in section 2.4.2.

3.2.1. Solvent extraction survey

Through reference documents [6-8, 13-14], the QuEChERS method was selected for sample processing to analyze PCB compounds. The extraction solvent is one of the most important factors that determine the efficiency of extracting analytes. A commonly used solvent in the QuEChERS method is acetonitrile (ACN) due to its ability to dissolve many substances with varying polarities. This is also the primary solvent used in analytical studies of PCBs employing the QuEChERS method, consistently yielding high recovery rates [13]. Two extraction solvents selected for investigation were ACN and ACN with an additional 0.1% acetic acid, as acetic acid enhances the salting-out effect. The recovery results of the 6 I-PCBs on the two blank matrices (pork and milk) are shown in Figure 2. The analysis results in Figure 2 indicate that the recovery efficiency for both milk and meat matrices for PCBs ranged from 40.8% to 69.5% with ACN containing 0.1% acetic acid and from 70.9% to 97.5% with ACN. The recovery efficiency of PCBs with the extraction solvent ACN on both milk and meat matrices was comparable and consistent, ranging from 89.1% to 97.5% for PCB-28, 52, 101, 153, and 138, except for PCB 180, which had a lower recovery rate of 79.2% on the milk matrix and 70.9% on the meat matrix. Therefore, ACN was chosen as the solvent for subsequent investigations.

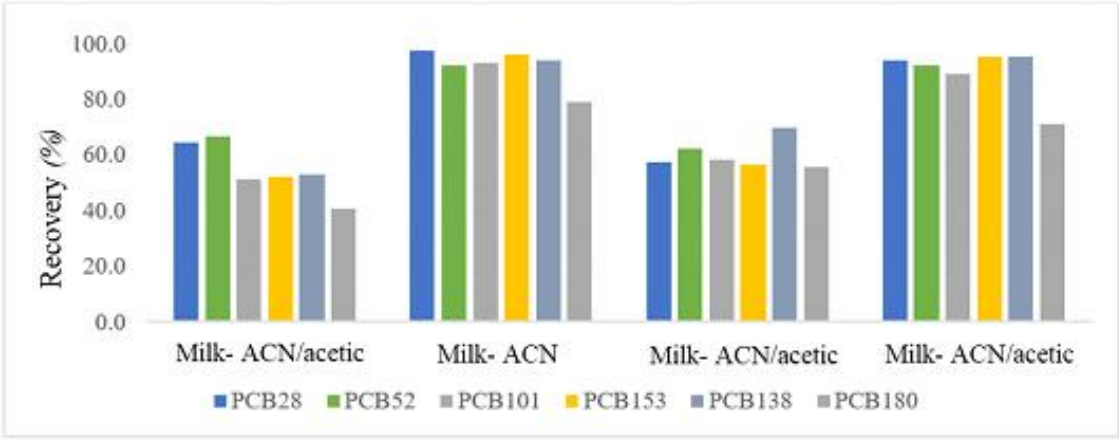

***Figure 2**. The recovery efficiency of 6 I-PCBs in two investigated extraction solvents*

3.2.2. Investigation of extraction salt composition

In the QuEChERS extraction process, in addition to the extraction solvent, the salt mixture plays an important role in stabilizing the pH and increasing the polarity of the aqueous phase. Two salt mixtures were selected for investigation: the citrate buffer salt mixture (1) 4.0 g $MgSO_4$ + 1.0 g NaCl + 1.0 g trisodium citrate dihydrate + 0.5 g disodium hydrogen citrate sesquihydrate and the non-buffer salt mixture (2) 4.0 g $MgSO_4$ + 1.0 g NaCl. The recovery results of the 6 I-PCBs on two blank matrices (pork and milk) using different salt mixtures are shown in Figure 3. The results in Figure 3 indicate that for both milk and meat matrices, the recovery efficiency of the I-PCBs ranged from 80% to 103% when using the non-citrate buffer salt mixture (2), while the recovery efficiency of the I-PCBs using the citrate buffer salt mixture was from 70.9% to 103%. Overall, the use of the non-buffer salt mixture resulted in higher recovery efficiency for the I-PCBs compared to the citrate buffer, although the difference was not significant. To simplify the sample extraction process and save on chemicals, the salt mixture of $MgSO_4$ and NaCl was chosen for further investigations.

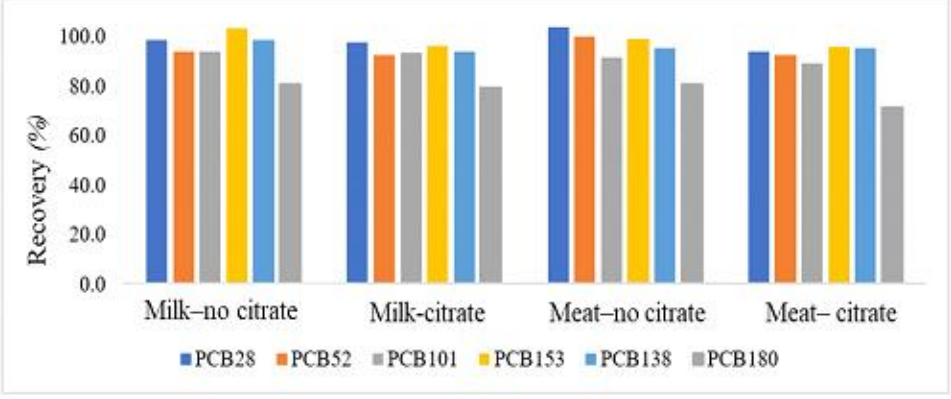

***Figure 3**. The recovery efficiency of 6 I-PCBs using different extraction salt mixtures*

3.2.3. Investigation of freezing conditions and the composition of d-SPE adsorbents

The adsorbent in QuEChERS plays a crucial role in removing impurities that are co-extracted with the analyte, helping to purify the sample matrix and reduce the influence of the sample matrix on the detection of the analyte. To eliminate non-polar impurities from the extract, such as fats, the composition of the adsorbent in d-SPE typically includes C18 powder as a hydrophobic reverse-phase adsorbent. Additionally, fats can also be easily removed from the extract when frozen at temperatures below -18°C. Therefore, to optimize the removal of non-polar impurities in the extract, the selected conditions for investigation include:

- To freeze the extract: temperature ≤ -18°C for 2 hours, with two d-SPE mixtures
    - d-SPE 1 mixture: 1.8 g $MgSO_4$ + 1.0 g PSA
    - d-SPE 2 mixture: 1.8 g $MgSO_4$ + 1.0 g PSA + 0.5 g C18
- Without freezing the extract:

- d-SPE 2 mixture: 1.8 g $MgSO_4$ + 1.0 g PSA + 0.5 g C18

The results of the investigation on two blank sample matrices (pork and milk) are shown in Figures 4 and 5.

The results obtained in Figures 4 and 5 show that the sample processing procedure for freezing the extract and not using C18 adsorbent in d-SPE yields the highest recovery efficiency. All substances have a recovery efficiency above 80.0%. In contrast, when using C18 adsorbent in d-SPE, both processes-with and without freezing the extract—result in decreased recovery efficiency for the substances. Notably, for PCB-153, 138, and 180, which are less polar than PCB-28 and 52, the recovery efficiency tends to decrease more significantly. This could be due to the inherently low polarity of these PCBs, leading to their adsorption onto C18. Furthermore, to eliminate non-polar impurities like fats without affecting the recovery efficiency of PCBs, C18 adsorbent should not be used; instead, freezing the extract and removing fats before cleaning with d-SPE is recommended. Therefore, the procedure involving freezing the extract and not using C18 in the d-SPE mixture was chosen for subsequent investigations.

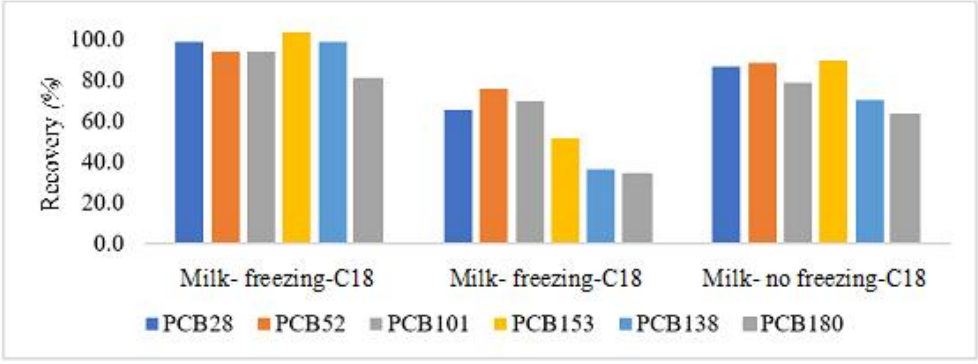

Figure 4. *The recovery efficiency of 6 I-PCBs in the milk matrix with different adsorbent compositions*

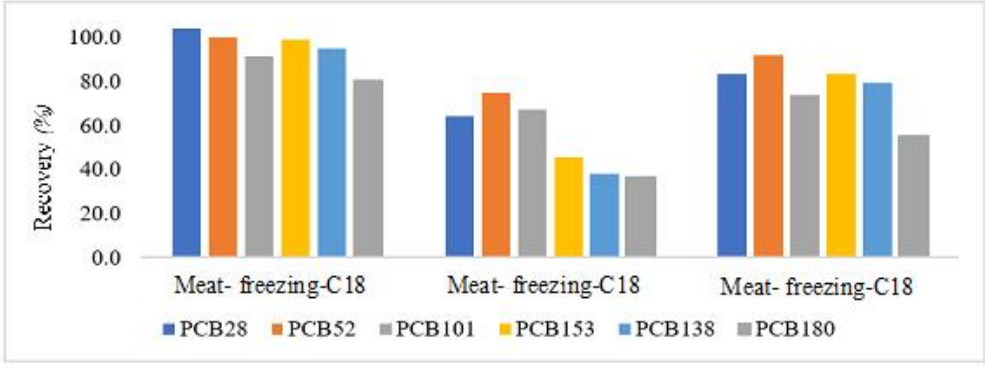

Figure 5. *The recovery efficiency of 6 I-PCBs in the meat matrix with different adsorbent compositions*

3.2.4. Investigation of PSA mass in d-SPE

PSA is a primary and secondary amine adsorbent that can remove polar substances such as fatty acids and organic acids. To optimize the cleaning capacity for polar impurities

of PSA, the amounts of PSA chosen for investigation in the d-SPE mixture were 0.75 g, 1.00 g, and 1.25 g on both sample matrices (milk and pork). The results obtained from the investigation show that the recovery efficiency of I-PCBs on both sample matrices increased when the amount of PSA was raised from 0.75 g to 1.00 g. However, the efficiency did not change significantly when increasing from 1.00 g to 1.25 g of PSA. On the other hand, using a mass of 1.00 g of PSA yielded recovery rates for the I-PCBs that were all ≥ 80.0%. Therefore, in this study, the selected amount of PSA for use is 1.0 g.

3.3. Method validation

3.3.1. Specificity

The specificity of the method was confirmed through the analysis of blank samples, standard samples, and blank samples (pork and milk) spiked with 6 I-PCBs at the same concentration of 3 ng/mL. The results showed that the blank samples did not exhibit any signals for the analytes, and the retention times of the 6 analytes in the standards and spiked samples did not differ significantly (≤ 2%). The results illustrating the assessment of the specificity of PCB-28 are shown in Figure 6.

In the chromatography method using mass spectrometry (MS) detection, the identification point (IP) count is an important value for evaluating specificity. The calculation of the IP count in the GC-MS/MS method is as follows: 1 point for the GC separation technique, 1 point for each parent ion, and 1.5 points for each product ion. According to the results in Table 2, each analyte has 1 parent ion and 2 product ions. Therefore, all analytes have an IP score ≥ 5, meeting the requirement for the IP score (IP ≥ 4) [12].

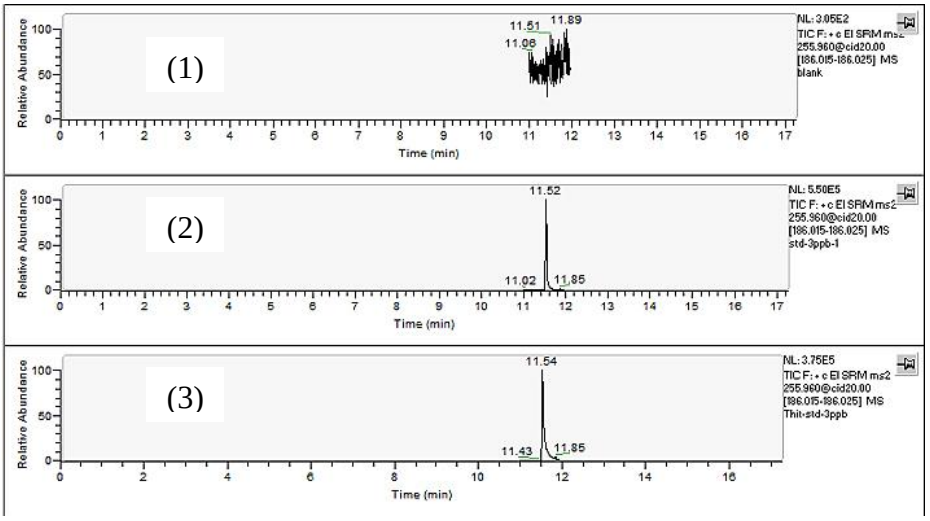

Figure 6. *Chromatogram assessing the specificity of PCB-28 ((1) Blank sample; (2) Standard sample in solvent; (3) Blank sample spiked with standard)*

3.3.2. Detection limit and quantitative limit of the method

The detection limit (LOD) and the Quantitative limit (LOQ) of the method were determined based on the signal-to-noise ratio (S/N). Blank samples spiked with progressively smaller concentrations of the 06 I-PCBs mixture were analyzed until a signal-to-noise ratio (S/N) of 3 was obtained to determine the LOD. The LOQ was determined after

establishing the LOD by multiplying the LOD by 10/3 (LOQ = 10 x LOD/3). The results showed that all I-PCBs had an LOD value of 0.5 ng/g and an LOQ of 1.5 ng/g, which is suitable for determining the concentration of 6 I-PCBs in actual samples.

3.3.3. Surveying the influence of sample matrix

In the chromatography method using mass spectrometry detectors, the sample matrix significantly affects the analysis results, especially when the samples are not well processed. The QuEChERS procedure is straightforward and involves few steps for impurity removal, so it is highly susceptible to matrix effects. To assess the influence of the sample matrix (ME%), two standard lines with five points at 3.0, 6.0, 10.0, 20.0, and 40.0 ng/mL were constructed on the solvent matrix and on the blank sample matrix (the blank sample was extracted following the procedure to obtain the extract, which was then prepared for analysis). The results of the slopes of the two standard lines were compared to evaluate this influence. The influence of the sample matrix is calculated using the following formula:

$$ME\ (\%) = \frac{a\ \ blank\ sample\ matrix\ \ - a\ solvent\ matrix}{a\ solvent\ matrix}\ x\ 100\%$$

The results in Figure 7 show that the ME% values differ among the analytes and across each sample matrix. Most of the compounds exhibit ME < 20.0% on both the milk and meat matrices. However, some compounds have ME > 20.0%, such as PCB 180 on the milk matrix and PCB 101 and 138 on the meat matrix. Therefore, standard lines on the sample matrix are used to quantify the compounds to exclude matrix effects.

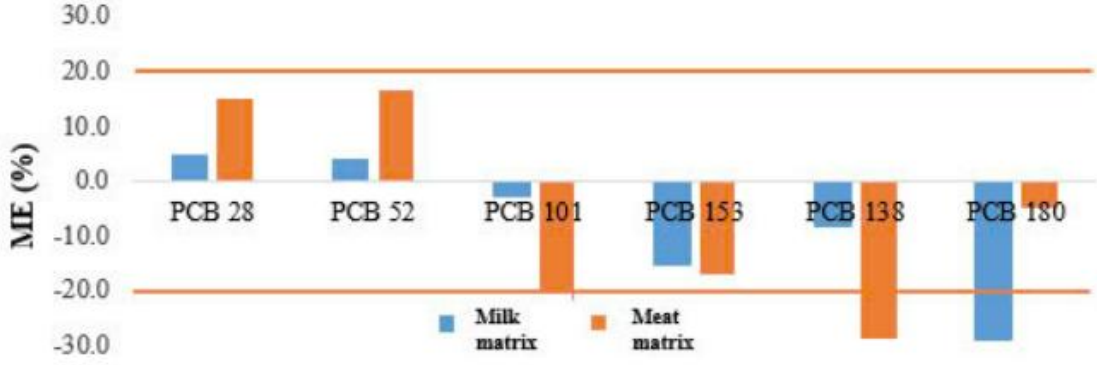

***Figure 7**. Results of the matrix effect investigation*

3.3.4. Standard line construction

Based on the analyzed and selected conditions, a standard line was constructed showing the linear dependence between the peak area ratio of the analyte with the internal standard and the concentration of the analytes, ranging from 1.5 to 40.0 ng/g. The results of the calibration equations for the six I-PCB compounds on the blank matrices, along with their correlation coefficients, are presented in Table 2.

The results indicate that the calibration equations for all six compounds have correlation coefficients $R^2 > 0.999$. Therefore, within the investigated concentration range, there is a linear dependence between the peak area ratio of the analytes and the

corresponding concentrations of the analytes. The deviation at all concentration points does not exceed ± 15%, meeting the AOAC requirements [12].

3.3.5. Accuracy and precision of the method

To evaluate accuracy (through recovery efficiency (H%)) and precision (through repeatability with the relative standard deviation (RSD%)), spiked blank samples at three concentration levels of 1.5, 3.0, and 15.0 ng/g were analyzed and assessed. Each concentration level was analyzed in duplicate six times following the selected sample preparation procedure. The results of the assessment of precision and accuracy are summarized in Table 2.

Table 2. *Results of method validation for the 6 I-PCB compounds on meat and milk matrices*

Matrix	Analyte	Calibration equations	Correlation coefficients (R^2)	RSD (%)	R (%)
Milk	PCB 28	y = 0.617751 + 1.3618x	0.9994	3.60-5.10	89.1-102.1
Meat		y = -0.180048 + 1.24019x	0.9999	1.40-4.30	93.6-102.2
Milk	PCB 52	y = 0.168925 + 0.767509x	0.9996	2.60-6.20	87.9-101.9
Meat		y = 0.0822204 + 0.783734x	0.9996	1.00-2.00	96.9-102.1
Milk	PCB 101	y = 0.380453 + 0.5235x	0.9999	0.90-5.10	85.6-98.5
Meat		y = 0.306016 + 0.771732x	0.9997	2.50-5.90	81.5-101.6
Milk	PCB 153	y = 0.168925 + 0.767509x	0.9991	1.40-2.70	93.5-102.7
Meat		y = 0.336516 + 0.783965x	0.9999	3.80-5.90	82.5-101.6
Milk	PCB 138	y= 0.581184 + 0.684437x	0.9997	1.50-2.00	96.9-101.9
Meat		y = 0.00723747 + 0.883479x	0.9993	3.00-5.40	83.8-99.0
Milk	PCB 180	y = 0.113902 + 0.930975x	0.9996	1.50-3.00	79.2-86.8
Meat		y = 0.201077 + 0.69445x	0.9995	2.30-6.20	74.5-87.2

The results obtained in Table 2 show that the recovery efficiency of the 6 I-PCBs is relatively high (>74%) and the RSD% does not exceed 10%. This demonstrates that the analytical method has met the requirements for accuracy and repeatability according to AOAC standards [12]. At concentration levels of 1 - 10 ng/g, the required recovery is 60 - 115%, and the required repeatability RSD is less than 21%. Therefore, this method can be applied to analyze I-PCBs in real samples.

3.4. Analysis of real samples

The validated method that meets the requirements was applied to analyze the content of 6 I-PCBs in 20 meat and meat product samples (including pork, beef, and sausages) and 20 milk and dairy product samples (including liquid milk, powdered milk, and yogurt) collected in Hanoi. The results showed that no analytes were detected in 39 out of 40 meat and meat product samples (with a LOD of 0.5 ng/g). However, two substances, PCB-28 and PCB-101, were detected in one powdered milk sample, with concentrations of 6.40 and 6.55 ng/g fat, respectively (with a fat content of 19.8%). The chromatograms of PCB-28 and PCB-101 in the powdered milk sample are shown in Figure 8. Consequently, the total content of the 6 I-PCBs was 13.0 ng/g fat, which is lower than the maximum allowable limit set by European regulations (40 ng/g fat) [10]. These analytical results are quite similar to some previous studies [14].

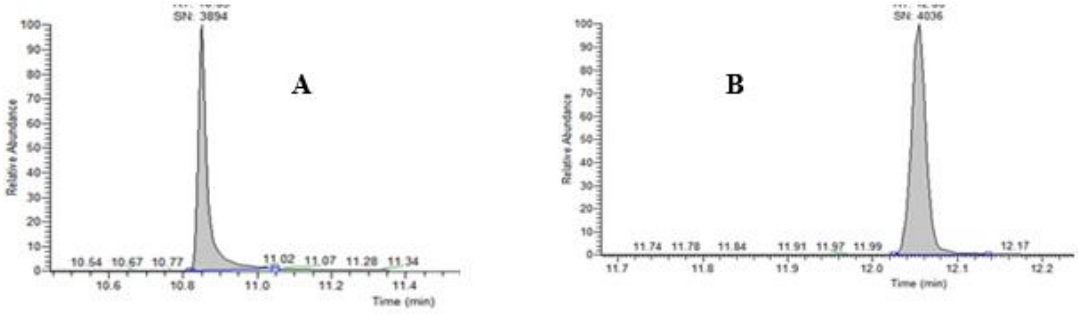

Figure 8. *Chromatogram of PCB Analysis in Powdered Milk (A) PCB-28 (B) PCB-101*

4. CONCLUSION

The study successfully developed a gas chromatography-tandem mass spectrometry (GC-MS/MS) method combined with sample preparation using QuEChERS to determine the levels of 6 I-PCBs in food. The advantage of this method is its quick and simple sample processing, which can be applied to various sample matrices. The method has been validated to meet AOAC requirements and has been used to determine the levels of 6 I-PCBs in 40 randomly collected samples (meat and meat products, milk and dairy products) from the Hanoi market. Preliminary results show that 1 out of 40 samples contained PCB-28 and PCB-101, with a total concentration of 6 I-PCBs at 13.0 ng/g fat, which is below the European regulatory limit. Meat and milk are two types of food that have a high and widespread demand in the Vietnamese market. Therefore, controlling PCB levels is essential to ensure the quality of these foods and protect consumer health.

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