Determination and validation of polycyclic aromatic hydrocarbons (PAH4) in katsuobushi, plant-based food supplements, and cocoa bean shells using GC−MS/MS

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are primarily generated through the incomplete combustion or pyrolysis of organic materials in various industrial processes. Foods may become contaminated with environmental PAHs found in air, soil, or water, or through industrial food processing methods such as smoking, roasting, drying, and grilling. The Ministry of Health and Welfare in Taiwan has established maximum levels for benzo[a]pyrene (BaP) and indicative values for BaP as well as PAH4 (the sum of benz[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[a]pyrene) in foods as operational guidelines. The current study developed an analytical method for simultaneous determination of PAH4 levels in katsuobushi (dried bonito flakes), cocoa bean shells, and plant-based food supplements using gas chromatography−tandem mass spectrometry (GC−MS/MS). Sample preparation methods were assessed using cyclohexane extraction and solid-phase extraction (SPE) for purification. PAH4 levels were subsequently quantified with matrix-matched calibration curves combined with isotopic internal standards. The limit of quantitation (LOQ) for each PAH was 5 μg/kg for katsuobushi and plant-based food supplements, and 1 μg/kg for cocoa bean shells. The effectiveness of the method was validated through each PAH analyte in the matrices of katsuobushi, cocoa bean shells, and plant-based food supplements. The average recoveries of PAH4 for fortified samples in each matrix ranged from 101.1 to 115.6% with all coefficients of variation being less than 6.5%. This method is applicable for routine analysis of PAH4 in a diverse array of food products to ensure food safety.

Keywords: Cocoa bean shells, GC−MS/MS, Katsuobushi, Plant-based food supplements, Polycyclic aromatic hydrocarbons, PAH4

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of compounds characterized by two or more fused benzene rings, possessing stable chemical structures and high melting and boiling points [1]. They are primarily generated through the incomplete combustion or pyrolysis of organic materials in diverse industrial processes [2]. According to a 2002 publication by the Scientific Committee on Food (SCF), 15 PAHs have shown clear evidence of mutagenicity and genotoxicity in cellular studies [3]. In 2012, benzo[a]pyrene (BaP) was categorized as a Group 1 carcinogen by the International Agency for Research on Cancer [4]. The European Food Safety Authority examined PAH levels in high-risk foods as specified in Commission Recommendation 2005/108/EC. In an analysis of 9714 food samples from 33 categories, 30% of samples with non-detectable BaP levels still contained PAHs as listed by the SCF [5]. Although the Food and Drug Administration of the United States has not established maximum thresholds for PAH levels in foods, it has identified a range of foods—such as grilled or smoked fish, meat

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products, roasted peanuts, and tea—that are likely to contain PAHs and recommended reduced intake to minimize exposure risk [6]. The European Union has specified maximum levels for BaP and PAH4 (the sum of benz[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), and BaP) in Commission Regulation (EU) No 2015/1125 and No 2015/1933, amending Regulation (EC) No 1881/2006 regarding maximum PAH levels in foodstuffs [7–10]. Similarly, the Ministry of Health and Welfare in Taiwan has set maximum levels for BaP and also defined indicative values for both BaP and PAH4 in foods, serving as operational guidelines [11].

The environment hosts a range of hundreds to thousands of PAHs, originating from incomplete combustion or pyrolysis reactions [12]. Multiple sources contribute to atmospheric PAH levels, including volcanic eruptions, forest fires, coal combustion, and vehicle emissions [13]. Crops grown or foods processed near industrial zones with heavy traffic are especially susceptible to contamination due to elevated levels of PAHs in the local water and air [14,15]. For example, cocoa bean shells may be exposed to environmental PAHs during transit to food manufacturing facilities [16]. In addition, improper drying or roasting methods could result in increased PAH levels in cocoa bean shells [17]. Food processing techniques such as smoking, grilling, or frying contribute to the presence of PAHs due to the pyrolysis of organic compounds [18,19]. Specifically, the production of katsuobushi (dried bonito flakes) involves repeated cycles of smoking and drying, generating concerns about potential PAH contamination [20]. Such contamination may occur when fats drip onto the heating elements and interact with flames or charcoal at elevated temperatures [21]. Conversely, suboptimal drying practices have been associated with elevated PAH levels in plant-based food supplements. The concentration of plant materials during these drying processes can raise the levels of PAHs [22]. Given the need for regulatory oversight, the development of a suitable testing method for PAH4 is imperative.

Commonly employed methods for PAH analysis include liquid chromatography and gas chromatography [23]. Among these, gas chromatography–tandem mass spectrometry (GC–MS/MS) is the most frequently used method because of its superior selectivity and effectiveness in detecting PAHs compared to liquid chromatography–tandem mass spectrometry [24]. Various methods have been suggested for detecting PAHs in an array of foodstuffs, such as fats, milk, bread, biscuits, barbecued meats, aquatic animal products, infant and toddler formulas, smoked meat products, and dried spices [25–27]. Different sample preparation techniques, including liquid–liquid extraction, Soxhlet extraction, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), and solid-phase extraction, are utilized depending on the matrix under examination [28,29]. However, limited methods have been established for the determination of PAHs in katsuobushi, cocoa bean shells, and plant-based food supplements. Therefore, the present study employed GC–MS/MS in conjunction with multiple reaction monitoring (MRM) for the simultaneous quantification of PAH4 (BaP, CHR, BaA, and BbF) in katsuobushi, cocoa bean shells, and plant-based food supplements. The analytical method outlined in the current study is readily adaptable for routine laboratory applications aimed at regulatory monitoring.

2. Materials and methods

2.1. Reagents and chemicals

All reagents and chemicals were of analytical grade and ultra-pure grade. Ultra-pure water (H_2O; 18.2 MΩ cm^{-1}) was obtained by de-ionization with a Millipore water purification system (Bedford, MA, USA). n-Hexane (purity ≥95 %) was purchased from J.T. Baker (Center Valley, PA, USA). Cyclohexane was purchased from Merck Ltd. (Darmstadt, Germany). The Sep-Pak® silica solid phase extraction (SPE) cartridge (1 g/6 mL) was purchased from Waters (Milford, MA, USA). Analytical standards of benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[a]pyrene (BaP) and chrysene (CHR) were obtained from Agilent Technologies (Santa Clara, CA, USA). Deuterated PAHs, including Benz[a]anthracene-d_{12}, Benzo[b]fluoranthene-d_{12}, Benzo[a]pyrene-d_{12}, and Chrysene-d_{12}, were used as internal standards (IS) and were purchased from Chiron.no (Trondheim, Norway).

2.2. Sampling and sample preparation

The four katsuobushi samples analyzed in this study were procured from supermarkets and online stores in Taipei, Taiwan, between January and March 2021. The single cocoa bean shell sample was collected from Pingtung, Taiwan, in March 2021. The four plant-based food supplement samples were acquired in January 2020 from supermarkets in Taipei, Taiwan. For control purposes, blank katsuobushi samples were prepared from freeze-dried bonito that was homogenized in a blender and stored in a freezer at −20 °C prior to extraction. Both the cocoa bean shell and the plant-based food supplements were stored at room temperature until they were used for analysis.

2.3. Sample extraction and clean-up

The optimal extraction procedure was as follows. Initially, 0.5 g of katsuobushi or plant-based food supplement sample, or 1 g of cocoa bean shell sample, was weighed into a 15-mL centrifuge tube. The sample was then spiked with 5 μL of an IS solution at a concentration of 10 μg mL^{-1}. Subsequently, 4 mL of cyclohexane was added, followed by vortex mixing and ultrasonic extraction for a duration of 10 min. After centrifugation at 5000 ×*g* for 5 min, the supernatant was collected. The residue was subjected to the addition of another 4 mL of cyclohexane, and the extraction process was repeated twice. The supernatants were then combined and evaporated to approximately 5 mL by gently purging with a stream of nitrogen at 40 °C. This solution was transferred into a Sep-pak® Silica SPE cartridge (Milford, MA, USA), and the eluent was collected. An additional 10 mL of cyclohexane was passed through the cartridge, and the eluents were combined. The eluate was evaporated to dryness using a gentle nitrogen stream at 40 °C and the remaining residue was reconstituted with 1 mL of n-hexane. Finally, the solution was filtered through a 0.2-μm polytetrafluoroethylene (PTFE) membrane and transferred to a vial for GC−MS/MS analysis (Fig. 1).

2.4. GC−MS/MS analysis

Gas chromatographic separation was conducted on an Agilent 7890B GC system, which was coupled with an Agilent 7693 autosampler and an Agilent 7000D triple quadrupole mass spectrometer. A 2-μL volume of the sample was injected into an ultra-inert inlet liner fitted with deactivated glass wool (P/N 5190-2295) provided by Agilent Technologies, CA, USA. The injector temperature was maintained at 320 °C and the injection was carried out in splitless mode. Helium, with a purity exceeding 99.999%, served as the carrier gas at a constant flow rate of 1 mL/min. The specifications for the capillary column and oven temperature program are detailed in Supplementary Table 1 [https://doi.org/10.38212/2224-6614.3505]. For mass spectrometric analysis, the transfer line temperature was set at 280 °C. The electron impact (EI) ion source operated at an energy level of 70 eV and a temperature of 340 °C, while the quadrupole temperature was set at 180 °C (Supplementary Table 2 [https://doi.org/10.38212/2224-6614.3505]). Quantification of PAH4 was performed using multiple reaction monitoring (MRM) mode. The optimal MRM transitions and collision energy of ion pairs for each PAH, as described in Table 1, were established using reference standards. Perfluorotributylamine (PFTBA) of mass spectrometric-grade was utilized for daily mass calibration. Data analysis was conducted using the Agilent MassHunter Quantitation software.

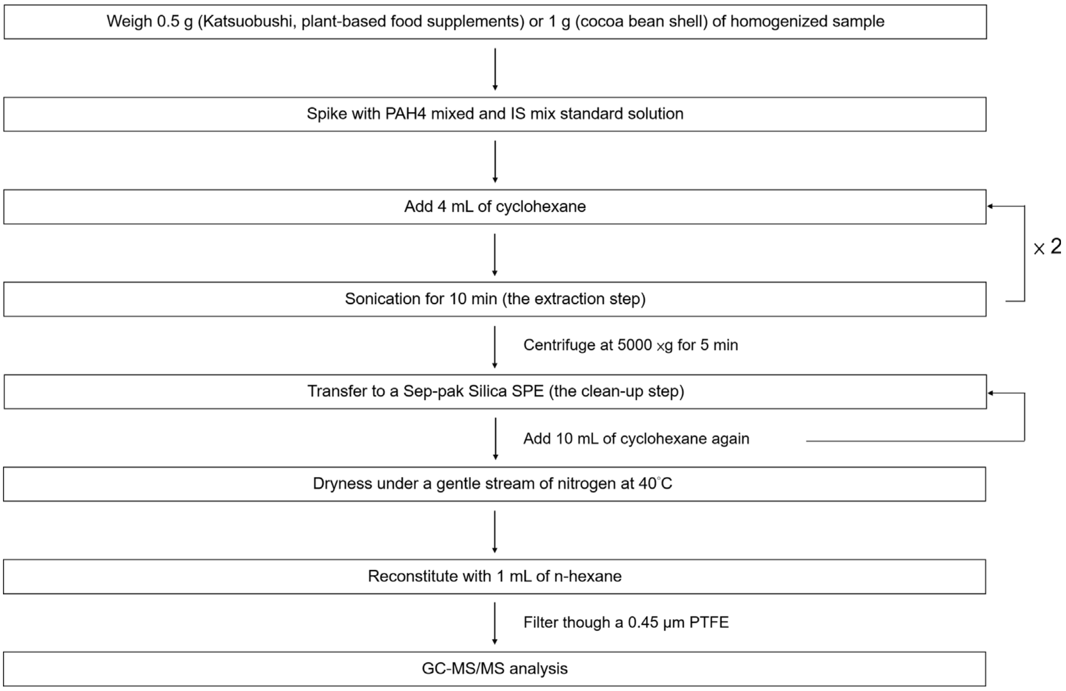

Fig. 1. Scheme of the sample preparation procedure for the katsuobushi, cocoa shells, and plant-based food supplement products.

Table 1. *Retention time and MRM parameters of PAH4 and isotopic internal standards analyzed by GC–MS/MS.*

Analyte	Retention time (min)			Transition for quantitative ion (*m/z*)	Collision energy (eV)	Transition for qualitative ion (*m/z*)	Collision energy (eV)
	DB-EUPAH	Select PAH	ZB-PAH-SeleCT				
Benz[a]anthracene (BaA)	24.00	29.30	20.59	228 > 226	35	113 > 112 228 > 227	15 20
Benzo[b]fluoranthene (BbF)	30.70	38.43	27.64	252 > 250	40	125 > 124 126 > 113	15 15
Benzo[a]pyrene (BaP)	33.91	42.54	30.33	252 > 250	40	125 > 124 126 > 113	15 15
Chrysene	24.34	29.90	20.84	228 > 226	35	228 > 227 113 > 112	20 15
Benz[a]anthracene-d_{12} (I.S.)	23.90	29.10	20.43	240 > 236	35		
Benzo[b]fluoranthene-d_{12} (I.S.)	30.47	38.18	27.45	264 > 260	35		
Benzo[a]pyrene-d_{12} (I.S.)	33.75	42.22	30.11	264 > 260	40		
Chrysene-d_{12} (I.S.)	24.21	29.66	20.66	240 > 236	35		

2.5. *Method validation*

Stock solutions for PAH4 and deuterated ISs were prepared at concentrations of 100 and 1000 μg mL^{-1} respectively, using n-hexane as the solvent. These solutions were stored in a dark environment at −20 °C. Prior to use, the stock solutions were diluted to a concentration of 0.5 μg mL^{-1} using n-hexane. Method validation for GC–MS/MS analysis was conducted in accordance with reference guidelines outlined in European Union documents, specifically Commission Regulation (EU) No 836/2011 [30]. Matrix-matched calibration curves were constructed by adding known concentrations of standard solutions to blank sample extracts, as described in Section 2.3. For cocoa bean shell samples, final concentrations in the range of 1–50 ng mL^{-1} were used, whereas for katsuobushi and plant-based food supplements, concentrations ranged from 2.5 to 50 ng mL^{-1}. The limit of quantification (LOQ) was determined based on signal-to-noise (S/N) ratios of 10, with recoveries of concentrations falling between 75% and 120%. The accuracy and precision of the method were assessed through both intra-day and inter-day analyses. Intra-day recovery and precision were evaluated using five replicates of PAH4-fortified samples at two different concentration levels. Inter-day precision and accuracy were evaluated through analyses conducted on separate days. The coefficient of variation (CV) was used to define both intra-day and inter-day precisions. For real sample analysis, the LOQ was verified to meet the criteria for accuracy and precision at the lowest concentration of the validated method.

3. Results and discussion

3.1. *Optimization of GC chromatographic conditions*

PAHs are compounds characterized by the presence of two or more fused benzene rings. The optimized MS/MS parameters and retention time for PAH4 are summarized in Table 1. Some PAHs with the same molecular formula can have several structural isomers, complicating their differentiation through MS [31]. For instance, triphenylene (TRP) and CHR are structural isomers commonly found in katsuobushi [20]. If both isomers coexist in the sample without chromatographic separation, the concentration of CHR could be inaccurately elevated. Therefore, separation of critical pairs such as "TRP vs. CHR" and "benzo[k]fluoranthene (BkF) vs. BbF" is essential for accurate PAH4 quantification.

Previous literature has indicated that the Select PAH column (30 m) can improve the separation of these critical pairs [32]. In our study, we used the Select PAH column (30 m × 0.25 mm i.d., 0.15 μm) and adjusted the temperature program described in Supplementary Table 1 [https://doi.org/10.38212/2224-6614.3505]. The results indicate that this column can distinguish TRP and CHR, and achieve satisfactory separation of BkF from BbF (Figs. 2B and 3A).

Additionally, a ZB–PAH–SeleCT column (40 m × 0.18 mm i.d., 0.14 μm) was employed for subsequent tests. The column oven temperature program is detailed in Supplementary Table 1 [https://doi.org/10.38212/2224-6614.3505]. The results reveal that the chromatographic conditions for

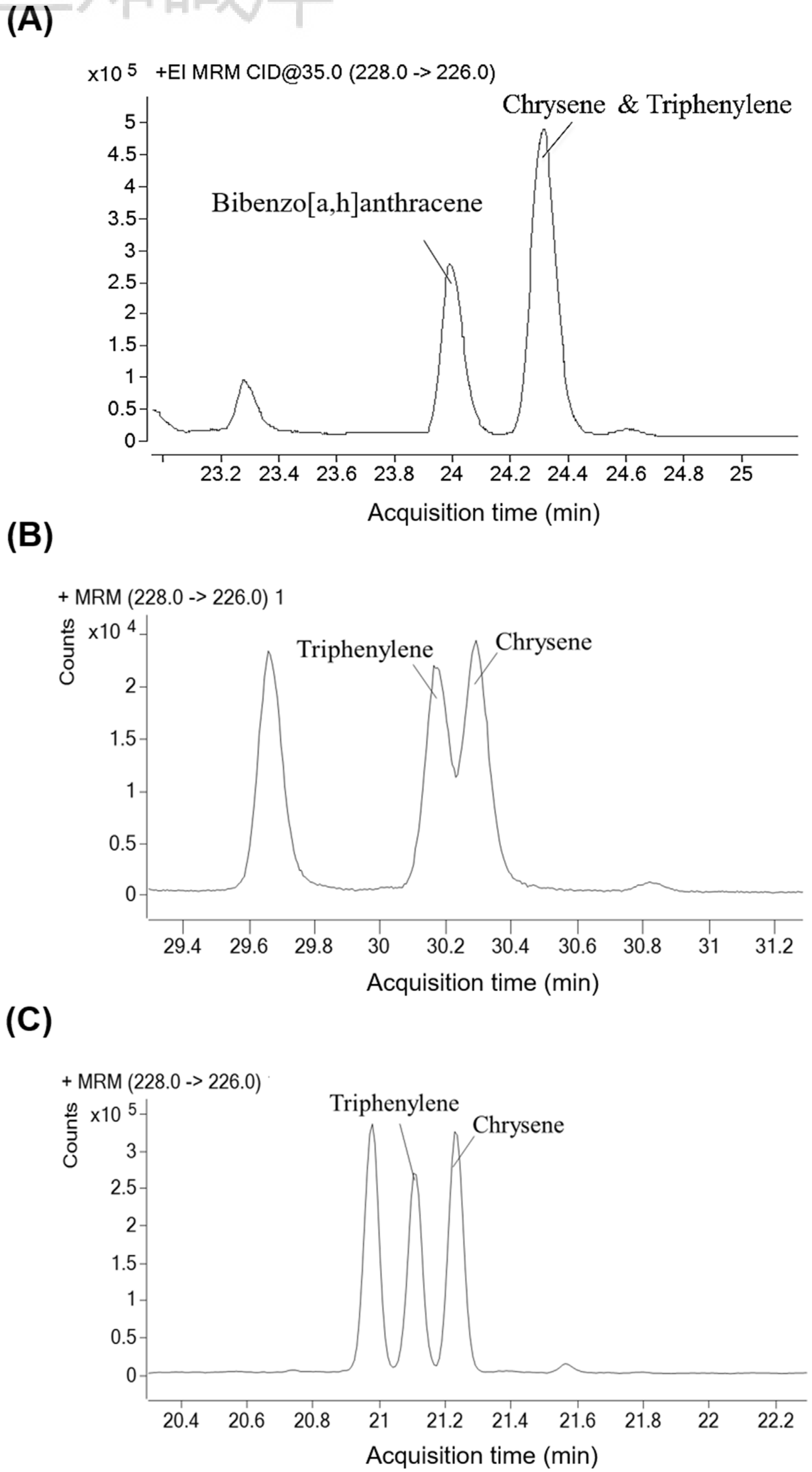

Fig. 2. Chromatographic separation of PAH isomers, including BaA, TRP, and CHR on a (A) DB-EUPAH column, (B) Select PAH column and (C) ZB-PAH-SeleCT column.

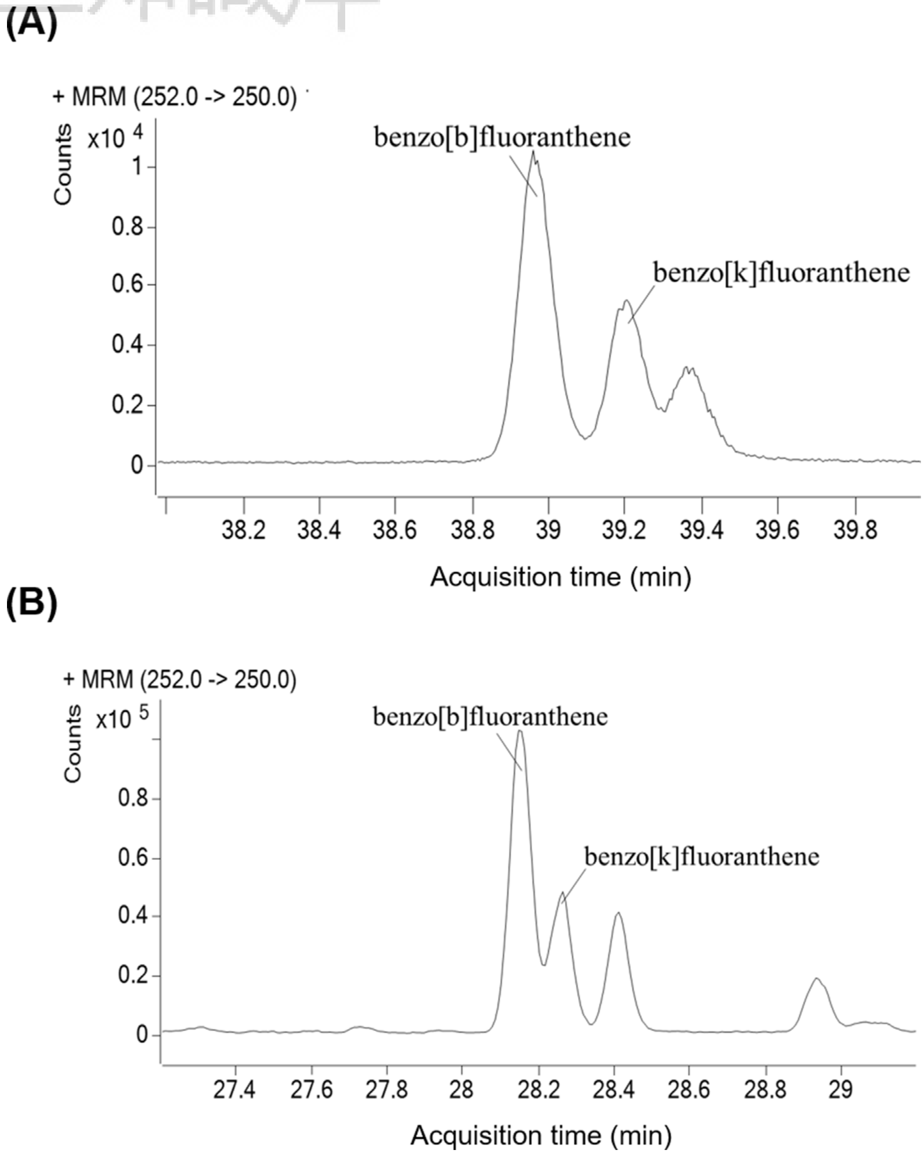

Fig. 3. Chromatographic separation of PAH isomers, including BbF and BkF on a (A) Select PAH column and (B) ZB-PAH-SeleCT column.

the ZB–PAH–SeleCT column achieved superior separation of TRP and CHR (Fig. 2C). In summary, different columns have their limitations in PAH separation; when TRP and CHR are challenging to distinguish, the ZB–PAH–SeleCT column should be used for confirmation.

3.2. Optimization of the clean-up method

In recent years, Chiang et al. have advocated the use of the QuEChERS method to extract PAHs from diverse food matrices such as salmon, seafood, bacon, wheat flour, curry spice powder, and infant formula [29,32]. To test the QuEChERS method for katsuobushi, a 5-g sample was weighed and fortified at 5 μg kg^{-1} of PAH4. Subsequently, 5 mL of water was added and the sample solution was shaken. Thereafter, 10 mL of acetonitrile was incorporated, along with salts comprising 1.5 g of sodium acetate and 6 g of anhydrous $MgSO_4$. Following 1 min of shaking and centrifugation at 5000 ×*g* for 5 min, the upper acetonitrile layer was transferred to a tube containing d-SPE sorbents (400 mg PSA, 400 mg C18 EC, and 1200 mg anhydrous $MgSO_4$) for purification. However, the recoveries of BaA ranged from 229.3% to 291.4% at 5 μg kg^{-1}, indicating that the purification was not satisfactory. In contrast, Lee et al. have reported recoveries between 86.9% and 103.6% for PAH8 when using Sep-Pak silica cartridges for purification in seafood and dairy products [33]. Hence, in our study, we opted for SPE cartridges for purification. The procedure, depicted in Fig. 1, involves the use of cyclohexane to extract katsuobushi via sonication. For purification and clean-up, Sep-Pak Silica cartridges were employed. Compared to the QuEChERS approach, the method we developed reduced matrix interference and yielded excellent recoveries for BaA at 5 μg kg^{-1}, ranging from 108.8% to 116.9%, with a stable CV of 3.1%. This procedure will serve as a reference for the validation of other matrices, as discussed in Section 3.3.

3.3. Method validation

The limits of quantitation (LOQs) for PAH4 were determined through replicate analyses of matrix-matched calibration in conjunction with an IS. In this study, favorable linearity was observed in the matrix-matched calibration curves for katsuobushi, cocoa bean shell, and plant-based food supplements. The coefficients of determination (R^2) for the four analytes were all greater than 0.995. A validation process was conducted via intra-day and inter-day experiments, and the resulting accuracy and precision metrics confirmed the suitability of the optimized method for the intended application. The experiment involved spiking two concentrations—low and high—of the PAH4 mixture standards and their isotopic IS into blank matrices of katsuobushi, cocoa bean shell, and plant-based food supplements. The accuracy tests were conducted in five replicates and analyzed using GC–MS/MS.

For katsuobushi, the processing procedures lead to the formation of PAHs, including BaA, CHR, BbF, and BaP, complicating the acquisition of blank samples [20]. Therefore, in this study, blank katsuobushi samples were prepared using freeze-dried bonito, which was subsequently homogenized into a powdered form. Analytical results indicated that intra-day recoveries for matrices spiked with low (5 μg kg^{-1}) and high (10 μg kg^{-1}) concentrations of PAH4 ranged from 101.5% to 113.4%, with CV values between 2.1% and 4.7%. For the inter-day assay, recoveries for PAH4 ranged from 102.7% to 109.4%, and CV values varied between 2.9% and 5.3%. In the case of plant-based food supplements, intra-day recoveries for matrices spiked with low (5 μg kg^{-1}) and high (10 μg kg^{-1}) concentrations of PAH4 ranged from 101.1% to 109.4%, with CV values between 1.1% and 6.5%. For the inter-day assay, the recoveries for PAH4 were between 105.0% and 110.7%, with CV values ranging from 2.4% to 6.5%. In cocoa bean shell samples, to comply with indicative values in Taiwan, the sample size was increased to 1 g for potentially lower the LOQ. Intra-day recoveries for matrices spiked with low (1 μg kg^{-1}) and high (5 μg kg^{-1}) concentrations of PAH4 ranged from 106.9% to 115.6%, with CV values between 0.8% and 5.9%. In the inter-day assay, the recoveries for PAH4 ranged from 101.2% to 111.7%, with CV values between 1.3% and 8.0% (Table 2). Taken together, the average recoveries of PAH4 for fortified samples in each matrix ranged from 101.1% to 115.6%, with CVs less than 6.5%. These findings show that both the recovery rates and CV (%) for low concentrations of PAH4 were in the acceptable range with Commission Regulation (EU) No 836/2011 for quantification [30]. This demonstrates that the employment of matrix-matched calibration curves combined with isotopic internal

Table 2. Recoveries, limits of quantification and the linear range of PAH4 in food matrices.

Analyte	Intra-day precision[a]				Inter-day precision[b]				LOQ (μg kg^{-1})	Linear range (ng mL^{-1})	R^2
	Low conc.		High conc.		Low conc.		High conc.				
	Recovery (%)	CV (%)	Recovery (%)	CV (%)	Recovery (%)	CV (%)	Recovery (%)	CV (%)			
Katsuobushi (Spiked 5 and 10 μg kg^{-1})											
BaA	112.0	3.1	103.8	2.1	108.6	4.9	103.7	3.1	5	2.5–50	0.9993
CHR	113.4	2.7	103.0	2.4	109.4	5.2	102.9	3.0	5	2.5–50	0.9993
BbF	106.5	4.7	101.5	2.6	109.2	5.1	102.7	3.2	5	2.5–50	0.9995
BaP	108.6	2.4	103.1	2.8	104.6	5.3	102.7	2.9	5	2.5–50	0.9992
Cocoa bean shell (Spiked 1 and 5 μg kg^{-1})											
BaA	112.8	5.1	109.3	0.8	110.6	2.7	110.0	1.3	1	1–50	0.9996
CHR	106.9	1.2	110.0	2.3	107.5	2.4	111.7	3.4	1	1–50	0.9993
BbF	107.7	4.2	112.8	1.0	111.2	8.0	111.0	2.9	1	1–50	0.9998
BaP	115.6	5.9	111.0	3.2	101.2	2.5	110.7	2.4	1	1–50	0.9999
Plant-based food supplements (Spiked 5 and 10 μg kg^{-1})											
BaA	106.9	2.7	102.6	1.7	107.9	2.4	106.1	3.8	5	2.5–50	0.9967
CHR	105.7	3.7	101.1	1.1	106.4	3.7	105.0	4.1	5	2.5–50	0.9968
BbF	107.9	3.8	105.0	3.2	110.7	5.6	107.7	3.4	5	2.5–50	0.9966
BaP	109.4	6.5	102.7	3.1	107.0	6.5	105.7	4.0	5	2.5–50	0.9977

Conc. = concentration, LOQ = limit of quantitation. CV = coefficients of variation.

[a] n = 5.

[b] n = 10.

Table 3. Analytical results of PAH4 in commercial katsuobushi, plant-based food supplement and cocoa bean shell products.

Sample	Sample No.	Analyte ($\mu g\ kg^{-1}$)				
		BaP	BaA	CHR	BbF	∑ PAH4
Katsuobushi	K1	9.7	35.7	34.5	18.7	98.6
	K2	9.7	42.6	42.8	18.9	114.0
	K3	9.4	42.6	45.0	20.4	117.4
	K4	13.8	48.3	50.3	23.9	136.3
Plant-based food supplement	S1	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]
	S2	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]
	S3	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]
	S4	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]
Cocoa bean shell	C1	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]	N.D.[a]

[a] N.D. = Not detected.

standards for quantification in this study, effectively overcoming matrix effects.

The outcomes of the accuracy and precision assessments were employed to determine the LOQs for difference foodstuffs. At the lowest spike levels, the signal-to-noise ratios for the quantitative ions exceeded 10, while those for the qualitative ions were greater than 3. These conditions demonstrated satisfactory accuracy and precision, both in terms of repeatability and reproducibility, fulfilling with the "Validation guideline for food chemical testing method" established by the Taiwan Food and Drug Administration [34]. Therefore, the LOQs for the four PAHs in katsuobushi, cocoa bean shells, and plant-based food supplements were established at 5, 1, and 5 $\mu g\ kg^{-1}$, respectively. These findings suggest that the method is suitable for routine laboratory analysis.

3.4. Application of the method to the commercial products

As previously outlined, the method utilizing Sep-Pak Silica cartridges for purification and GC–MS/MS for chromatographic separation successfully isolated the interfering PAHs from the target analytes. In this study, nine samples, including four katsuobushi (K1–K4), one cocoa bean shell (C1), and four plant-based food supplements (S1–S4), were procured from Taiwan. All samples were analyzed using the analytical method established in the current study, and the levels of PAH4 in these nine samples are detailed in Table 3. The findings indicated that BaP levels ranged from 9.4 to 13.8 $\mu g\ kg^{-1}$, and the sum of PAH4 levels varied between 98.6 and 136.3 $\mu g\ kg^{-1}$ in the katsuobushi samples (K1–K4). The elevated concentration of PAH4 is likely attributable to the processing techniques used for katsuobushi, which involve repeated cycles of smoking and drying, potentially leading to the accumulation of higher concentrations of PAHs [20]. Moreover, a study by Tsutsumi et al. (2020) in Japan reported mean concentrations of 21 $\mu g\ kg^{-1}$ for BaP and 287 $\mu g\ kg^{-1}$ for the sum of PAH4 in dried bonito flakes ($n = 10$), with all samples having BaP levels above the LOQ [20]. In this study, the levels of BaP (9.4–13.8 $\mu g\ kg^{-1}$) and PAH4 (98.6–136.3 $\mu g\ kg^{-1}$) were detected in the katsuobushi samples from the market. These concentrations did not exceed the maximum limits of 30 $\mu g\ kg^{-1}$ for BaP and 150 $\mu g\ kg^{-1}$ for PAH4, respectively, as specified by Taiwan's "Guidelines for Reducing Polycyclic Aromatic Hydrocarbons Content in Foods" [11]. Apart from the four commercial katsuobushi samples, BaA, CHR, BbF, and BaP were not detected in the cocoa bean shell (C1) and plant-based food supplement samples (S1–S4). Therefore, based on the findings of this study, commercially available katsuobushi, cocoa bean shells, and plant-based food supplements comply with the existing regulations in Taiwan.

4. Conclusion

In this study, for the first time, the same sample preparation procedure was employed for katsuobushi, cocoa bean shells, and plant-based food supplements to determine PAH4 levels using GC–MS/MS. The use of cyclohexane for the extraction, followed by clean-up with Sep-Pak Silica cartridges, resulted in excellent accuracy, with recovery rates ranging from 101.1% to 115.6%, and high precision, characterized by CVs less than 6.5%. These results comply with the recommendation of Commission Regulations (EU) 2015/1125 and (EU) 2015/1933. Upon application of this validated method to commercial products, it was confirmed that none had exceeded the indicative thresholds defined by Taiwanese regulations. Additionally, this analytical method offers a viable tool for manufacturers engaged in the regulatory surveillance of agricultural and processed food, thereby contributing to the assurance of food safety.

Conflict of interest

The authors have disclosed no conflicts of interests.

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References

[1] Abdel-Shafy HI, Mansour MSM. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt J Pet 2016;25:107–23. https://doi.org/10.1016/j.ejpe.2015.03.011.

[2] Ravindra K, Sokhi R, Van Grieken R. Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation. Atmos Environ 2008;42:2895–921. https://doi.org/10.1016/j.atmosenv.2007.12.010.

[3] Scientific Committee on Food. Opinion of the Scientific Committee on Food on the risks to human health of polycyclic aromatic hydrocarbons in food. 2002. Available at: https://ec.europa.eu/food/sites/food/files/safety/docs/scicom_scf_out153_en.pdf. [Accessed 18 September 2023].

[4] International Agency for Research on Cancer. Agents classified by the IARC monographs, volumes 1–133. 2018. Available at: https://monographs.iarc.who.int/agents-classified-by-the-iarc/. [Accessed 18 September 2023].

[5] European Food Safety Authority. Polycyclic aromatic hydrocarbons in food scientific opinion of the Panel on Contaminants in the Food Chain. EFSA J 2008;724:1–114. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/724. [Accessed 18 September 2023].

[6] Agency for Toxic Substances and Disease Registry (ATSDR). Toxicity of polycyclic aromatic hydrocarbons (PAHs). 2009. Available at: https://www.atsdr.cdc.gov/csem/polycyclic-aromatic-hydrocarbons/health_effects.html. [Accessed 18 September 2023].

[7] European Commission. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union 2006;L364:5–24. Available at: https://leap.unep.org/countries/eu/national-legislation/commission-regulation-ec-no-18812006-setting-maximum-levels. [Accessed 18 September 2023].

[8] European Commission. Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. Off J Eur Union 2011;L215:4–8. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32011R0835. [Accessed 18 September 2023].

[9] European Commission. Commission Regulation (EU) 2015/1125 of 10 July 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in Katsuobushi (dried bonito) and certain smoked Baltic herring. Off J Eur Union 2015;L184:7–10. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32015R1125&qid=1695027228948. [Accessed 18 September 2023].

[10] European Commission. Commission Regulation (EU) 2015/1933 of 27 October 2015 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in cocoa fibre, banana chips, food supplements, dried herbs and dried spices. Off J Eur Union 2015;L282:11–3. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32015R1933&qid=1695027363044. [Accessed 18 September 2023].

[11] Ministry of Health and welfare (MOHW). Guidelines for reducing polycyclic aromatic hydrocarbons content in foods. 2021 (second revision) (in Chinese) Available at: https://www.fda.gov.tw/tc/includes/GetFile.ashx?id=f637460439088132140&type=3&iid=3395. [Accessed 18 September 2023].

[12] Marzooghi S, Di Toro DM. A critical review of polycyclic aromatic hydrocarbon phototoxicity models. Environ Toxicol Chem 2017;36:1138–48. https://doi.org/10.1002/etc.3722.

[13] Zhang Y, Tao S. Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004. Atmos Environ 2009;43:812–9. https://doi.org/10.1016/j.atmosenv.2008.10.050.

[14] Paris A, Ledauphin J, Poinot P, Gaillard JL. Polycyclic aromatic hydrocarbons in fruits and vegetables: origin, analysis, and occurrence. Environ Pollut 2018;234:96–106. https://doi.org/10.1016/j.envpol.2017.11.028.

[15] Romanova O, Lovell S. Food safety considerations of urban agroforestry systems grown in contaminated environments. Urban Agric Region Food Syst 2021;6:e20008. https://doi.org/10.1002/uar2.20008.

[16] Rojo-Poveda O, Barbosa-Pereira L, Zeppa G, Stévigny C. Cocoa bean shell-A by-product with nutritional properties and biofunctional potential. Nutrients 2020;12:1123. https://doi.org/10.3390/nu12041123.

[17] Abballe C, Gomes FML, Lopes BD, Oliveira APF, Berto MI, Efraim P, et al. Cocoa beans and derived products: effect of processing on polycyclic aromatic hydrocarbons levels. LWT–Food Sci Technol 2021;135:110019. https://doi.org/10.1016/j.fufo.2021.100061.

[18] Sampaio GR, Guizellini GM, da Silva SA, de Almeida AP, Pinaffi-Langley ACC, Rogero MM, et al. Polycyclic aromatic hydrocarbons in foods: biological effects, legislation, occurrence, analytical methods, and strategies to reduce their formation. Int J Mol Sci 2021;22:6010. https://doi.org/10.3390/ijms22116010.

[19] Rose M, Holland J, Dowding A, Petch SRG, White S, Fernandes A, et al. Investigation into the formation of PAHs in foods prepared in the home to determine the effects of frying, grilling, barbecuing, toasting and roasting. Food Chem Toxicol 2015;78:1–9. https://doi.org/10.1016/j.fct.2014.12.018.

[20] Tsutsumi T, Adachi R, Matsuda R, Watanabe T, Teshima R, Akiyama H. Concentrations of polycyclic aromatic hydrocarbons in smoked foods in Japan. J Food Protect 2020;83:692–701. https://doi.org/10.4315/JFP-19-486.

[21] Duedahl-Olesen L, Ionas AC. Formation and mitigation of PAHs in barbecued meat - a review. Crit Rev Food Sci Nutr 2022;62:3553–68. https://doi.org/10.1080/10408398.2020.1867056.

[22] Wilde AS, Duedahl-Olesen L. Analysis of four polycyclic aromatic hydrocarbons in plant-based food supplements-results of a collaborative study. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2022;39:1380–9. https://doi.org/10.1080/19440049.2022.2082539.

[23] Vistnes H, Sossalla NA, Røsvik A, Gonzalez SV, Zhang J, Meyn T, et al. The determination of polycyclic aromatic hydrocarbons (PAHs) with HPLC-DAD-FLD and GC-MS techniques in the dissolved and particulate phase of road-tunnel wash water: a case study for cross-array comparisons and applications. Toxics 2022;10:399. https://doi.org/10.3390/toxics10070399.

[24] Poster DL, Schantz MM, Sander LC, Wise SA. Analysis of polycyclic aromatic hydrocarbons (PAHs) in environmental samples: a critical review of gas chromatographic (GC) methods. Anal Bioanal Chem 2006;386:859–81. https://doi.org/10.1007/s00216-006-0771-0.

[25] Badibostan H, Feizy J, Daraei B, Shoeibi S, Rajabnejad SH, Asili J, et al. Polycyclic aromatic hydrocarbons in infant formulae, follow-on formulae, and baby foods in Iran: an

assessment of risk. Food Chem Toxicol 2019;131:110640. https://doi.org/10.1016/j.fct.2019.110640.

[26] Rascón AJ, Azzouz A, Ballesteros E. Assessing polycyclic aromatic hydrocarbons in cereal-based foodstuffs by using a continuous solid-phase extraction system and gas chromatography–mass spectrometry. Food Control 2018;92: 92–100. https://doi.org/10.3390/ijms22116010.

[27] Jira W. A GC/MS method for the determination of carcinogenic polycyclic aromatic hydrocarbons (PAH) in smoked meat products and liquid smokes. Eur Food Res Tech 2014; 218:208–12. https://doi.org/10.1007/s00217-003-0827-8.

[28] Peng PL, Lim LH. Polycyclic aromatic hydrocarbons (PAHs) sample preparation and analysis in beverages. A Review Food Anal Methods 2022;15:1042–61. https://doi.org/10.1007/s12161-021-02178-y.

[29] Al-Thaiban H, Al-Tamimi N, Helaleh M. Development of QuEChERS method for the determination of polycyclic aromatic hydrocarbons in smoked meat products using GC-MS from Qatar. J Anal Methods Chem 2018;2018:9206237. https://doi.org/10.1155/2018/9206237.

[30] European Commission. Commission Regulation (EU) No 836/2011 of 19 August–amending Regulataion (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels lead, cadnium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. Off J Eur Union 2011;L215:9–16. Available at: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32011R0836. [Accessed 18 September 2023].

[31] Šimko P. Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. J Chromatogr B 2002;770:3–18. https://doi.org/10.1016/S0378-4347(01)00438-8.

[32] Urban M, Lesueur C. Comparing d-SPE sorbents of the QuEChERS extraction method and EMR-lipid for the determination of polycyclic aromatic hydrocarbons (PAH4) in food of animal and plant origin. Food Anal Methods 2017; 10:2111–24. https://doi.org/10.1007/s12161-016-0750-9.

[33] Lee SY, Lee JY, Shin HS. Evaluation of chemical analysis method and determination of polycyclic aromatic hydrocarbons content from seafood and dairy products. Toxicol Res 2015;31:265–71. https://doi.org/10.5487/TR.2015.31.3.265.

[34] Taiwan Food and Drug Administration (TFDA). Validation guideline for food chemical testing method. (second revision) (in Chinese). 2013. Available at: http://www.fda.gov.tw/tc/siteList.aspx?sid=4115. [Accessed 18 September 2023].