

Research Article

Detection, Risk Assessment, and Survey of Four Polycyclic Aromatic Hydrocarbon Markers in Infant Formula Powder

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Received 15 October 2019; Revised 19 December 2019; Accepted 2 January 2020; Published 21 February 2020

Academic Editor: Antonio J. Signes-Pastor

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A gas chromatography-mass spectroscopy (GC-MS) method was developed to assess the infant exposure assessment from four important polycyclic aromatic hydrocarbon (PAH) markers in infant formula powder: benzo[a]anthracene, chrysene, benzo[b]fluoranthene, and benzo[a]pyrene (collectively referred to as PAH4). The developed method required the addition of an isotopically labeled internal standard, sample extraction under alkali conditions, a saponification step, and a solid-phase extraction purification step. In a controlled spike test, the average recovery rates of PAH4 were 77.3% to 111.8% and the relative standard deviations were 4.8% to 14.2% ($n=6$). The quantitative limit (LOQ) and detection limit (LOD) of the method were 0.5 and 0.1 $\mu\text{g}\cdot\text{kg}^{-1}$, respectively. The PAH4 content was analyzed in 30 commercially available infant formula powders. The PAH4 content was found to be in the range of 0.1 to 0.87 $\mu\text{g}\cdot\text{kg}^{-1}$. Combined with the daily intake of infant milk powder in China, the average and maximum daily exposure of BaP for stage-1 infants in China are 0.45 $\text{ng}/\text{kg}\cdot\text{bw}\cdot\text{d}^{-1}$ and 1.9 $\text{ng}/\text{kg}\cdot\text{bw}\cdot\text{d}^{-1}$ and the PAH4 values are 8.6 $\text{ng}/\text{kg}\cdot\text{bw}\cdot\text{d}^{-1}$ and 18.6 $\text{ng}/\text{kg}\cdot\text{bw}\cdot\text{d}^{-1}$, respectively. The PAH4 content in the tested infant formula powders sold in the China were sufficiently low, and all of the tested products were safe for consumption.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of carcinogens that are widely found in the environment and food products due to incomplete combustion and high-temperature decomposition of organic compounds. Ninety percent of human exposure to PAHs comes from food [1], including food raw materials and environmental sources during food processing, storage, and cooking [2–4].

Infant foods are widely concerned for its potential safety issues [5, 6]. It is reported that PAH levels are highest in fat-enriched foods, including infant formula powder, that contain vegetable oils such as coconut oil, palm oil, and soybean oil [4, 7–9]; the oils have been reported to contain PAH contamination to varying degrees in the order of corn oil, grape seed oil, groundnut oil, olive oil, palm oil, pumpkin seed oil, rapeseed oil, rice bran oil, soybean oil, and sunflower seed oil [10–13]; the PAHs enter infant formula

powders through these vegetable oils. Both natural and industrial oil seed drying processes (air drying, smoke drying, seed roasting, and so on) add PAHs to seeds; the seed PAHs levels are largely exacerbated during the oil production process, i.e., from seed drying to the refining of seed extracts [14].

Because there are variety forms of PAHs, the European Commission (EC) has established safe level requirements of benzo[a]pyrene (BaP), as well as benzo[a]anthracene (BaA), chrysene (CHR), and benzo[b]fluoranthene (BbFA) as four key PAH markers (hereinafter referred to as PAH4) (requirement no. 835/2011). The regulations require the concentrations of BaP and the total PAH4 to be less than 1.0 $\mu\text{g}\cdot\text{kg}^{-1}$ in infant formula powders.

Analysis methods such as GC-MS had been widely utilized for food safety evaluation [15]. Some countries including China had developed PAH detecting method in food, but not including infant formula, and some sample

pretreatment procedures reduced the sensitivity of the methods, which cannot meet the detection limit requirements of the EU regulations for infant formula powders. To improve the sample preparation method, several studies have been conducted to investigate extraction and purification methods for PAH analysis in different foods. Marinez-Lopez et al. [16] improved the determination efficiency of 15 PAHs in olive oil samples through liquid-liquid extraction and preparative gel permeation chromatography (GPC) purification before analysis. Fromberg et al. [17] presented a semi-automatic method for the determination of PAHs in edible oils using a combined gel permeation chromatography/solid-phase extraction (GPC/SPE) clean-up. A magnetic solid-phase extraction (MSPE) method for determination of PAHs in edible oils was reported by Zhao et al. [18]. They investigated the parameters affecting extraction efficiency by magnetic multiwalled carbon nanotubes on eight heavy molecular weight PAHs (CHR, BaA, BbFA, benzo[k]fluoranthene, BaP, indeno [1,2,3-cd]pyrene, dibenzo[a,h]-anthracene, and benzo[g,h,i]perylene); under the optimized conditions, a simple and effective method for PAH determination in edible oils was developed using GC-MS.

To date, no safety limits have been established for benzo[*a*]pyrenes in infant food (including milk powder). Benzo[*a*]pyrenes are also exempt from the total PAH4 limits in China's GB2762 national food safety standard. The aim of this study was to develop a simple, accurate, quick, and easy GC-MS method for PAH4 analysis, to survey the PAH4 contents in stage-1 infant formula powders, and to utilize the method of exposure limits (margin of exposure, MOE) [19] to assess the PAH4 exposure risk in powdered infant formula.

2. Materials and Methods

2.1. Chemical Reagents. 10.0 µg/mL PAH4 (BaA, CHR, BbF, and BaP) mixed standard solution (LA20950183CY PAH-Mix183) and 100 µg/mL D12-PAH4 (D12-BaA, D12-CHR, D12-BbFA, and D12-BaP) mixed internal standard solution (XA20950902CY PAH—Mix9) were purchased from Dr. Ehrenstorfer GmbH Corp. Olive oil polycyclic aromatic hydrocarbon quality control material (no. T0665QC) was purchased from Fapas (UK). Solid-phase extraction columns (divinylbenzene polymer + *n*-propyl ethylenediamine 250 mg/6 mL) were purchased from Kang Yuan Technology co., Ltd. (Changsha, China). Chromatography grade acetone, isoocane, ethyl acetate, and *n*-hexane were purchased from Merck (USA). Analytical grade anhydrous ethanol, anhydrous ether, petroleum ether, concentrated ammonia water, potassium hydroxide, and anhydrous sodium sulfate were purchased from Sinopharm (China). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, USA). Thirty domestic and imported stage-1 infant formula powders were purchased from a local supermarket in Hangzhou, China.

2.2. Analytical Instrumentation. An Agilent 6890gc-5973MS gas chromatography-mass spectrometer equipped with a DB-PAHEU capillary column, 20 m × 0.18 mm (inner

diameter) × 0.14 µm (film thickness), part number 121-9627, (USA) was used for sample analysis. The injection port temperature was 280°C. The column temperature was initially set at 80°C for 2 min and then increased to 250°C in 10°C/min intervals and maintained for 2 min and finally, to 320°C in 8°C/min intervals and maintained for 5 min. High purity helium (99.99%) was used as the carrier gas. The sample injection (2 µL) was splitless with a solvent delay time of 25 min, and the column was ionized by an electron ionization source at 70 eV and 230°C with a 250 V electron multiplier and a transmission line temperature of 280°C. The monitoring ions are presented in Table 1.

2.3. Preparation of Standard Solutions. The 0.2 µg/mL PAH4 mixed standard stock solution was prepared by diluting 10.0 µg/mL PAH4 mixed standard solution (0.2 mL) with acetone (10 mL). This stock solution was then stored in the dark at 4°C. The 0.1 µg/mL D12-PAH4 mixed internal standard stock solution was prepared by diluting 100.0 µg/mL D12-PAH4 mixed internal standard solution (0.05 mL) with acetone (50.0 mL). This D12-PAH4 stock solution was stored in the dark at 4°C. Five working solutions containing 0.5, 1.0, 2.0, 5.0, or 10.0 ng PAH4 and 5 ng D12-PAH4 were prepared for the GC-MS analysis by portioning 2.5 µL, 5 µL, 10 µL, 25 µL, or 50 µL of the 0.2 µg/mL PAH4 standard solution to five volumetric flasks already containing 50 µL of 0.1 µg/mL D12-PAH4 mixed standard solution, and then fill up the volumetric flasks with additional acetone.

2.4. Sample Collection and Preparation. All the samples of different brands are from Hangzhou supermarket. A flow-chart of the sample preparation procedure is presented in Figure 1. For the first step, sample extraction, PAH4 were extracted according to the reference method GB5009.6-2016: In brief, infant formula powder (2.00 g), 0.1 µg/mL D12-PAH4 mixed internal standard solution (50 µL), and ultrapure water (10 mL) were added to a 50 mL centrifuge tube. The sealed tube was vortexed and incubated at 45°C in a water bath for 10 min, and then ammonia water (2 mL) was thoroughly blended into the mixture. Thereafter, anhydrous ethanol (10 mL) was slowly added and the sealed tube was agitated thoroughly. To this mixture, anhydrous ether (8 mL) was added, and the sealed tube was vortexed for 5 min. Finally, petroleum ether (8 mL) was added and the sealed tube was vortexed for 5 min. The sealed 50 mL tube was centrifuged at 8000 r/min for 5 min and the organic layer (top) was collected and condensed by a flowing stream of N₂ while the sample was heated at 40°C in a water bath. The obtained residue was subjected to the saponification step [8]. The residue was taken up in a 1.5 mol/L potassium hydroxide ethanol solution (5 mL), and this mixture was placed in a 70°C water bath for 3 min. After cooling down using cold tap water, *n*-hexane (5 mL) and ultrapure water (4 mL) were added. The sealed tube was vortexed for 2 min and centrifuged at 10000 r/min for 2 min. Finally, the upper *n*-hexane phase was collected for the third step of purification. Anhydrous sodium sulfate (1 g) was added to the solid-phase extraction (SPE) column, and the column was

TABLE 1: Chemical monitoring ions and linear equations.

Chemicals	Abbr.	Identification ions	Internal standards	Abbr.	Identification ions	Linear equations	R^2
Benzo[a]anthracene	BaA	226, 229, 228*	D ₁₂ -benzo[a]anthracene	D12-BaA	236, 240*	$y = 0.326x - 0.016$	0.999
Chrysene	CHR	226, 229, 228*	D ₁₂ -chrysene	D12-CHR	236, 240*	$y = 0.304x + 0.005$	0.998
benzo[a]fluoranthene	BbFA	250, 253, 252*	D ₁₂ -benzo[a]fluoranthene	D12-BbFA	260, 264*	$y = 0.284x - 0.023$	0.998
benzo[a]pyrene	BaP	250, 253, 252*	D ₁₂ -benzo[a]pyrene	D12-BaP	260, 264*	$y = 0.276x + 0.004$	0.997

Note: "*" represents the quantifier ions.

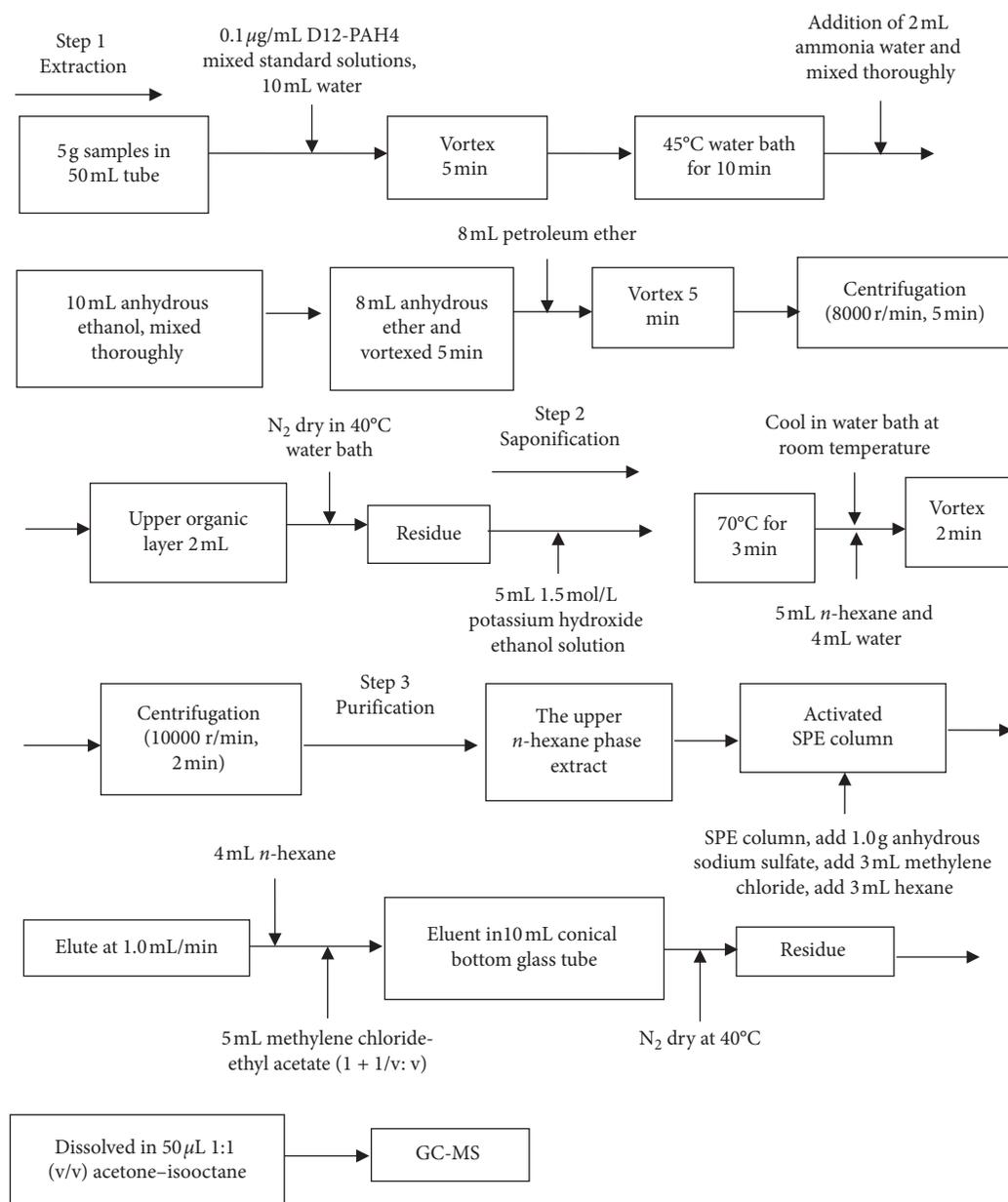


FIGURE 1: Sample preparation flowchart.

activated with sequential washing with methylene chloride (3 mL) and hexane (3 mL). The collected organic phase after the saponification protocol was loaded onto the SPE column and washed with *n*-hexane (4 mL) at 1.0 mL/min to remove any impurities. Thereafter, the PAHs of interest were eluted

with a methylene chloride: ethyl acetate mixture (1:1 v: v, 5 mL). The eluent was collected and dried by flowing N₂ at 40°C. As the final step, this obtained residue was taken up in an acetone: isooctane solution (1:1 v: v, 50 µL) for the GC-MS analysis.

2.5. Quantification and Method Validation

2.5.1. Blank. To ensure the absence of contamination of solvents and cartridges, a blank sample without milk formula was carried out according to the procedures in Figure 1.

2.5.2. Method Validation. Because PAHs are vulnerable to environmental pollution, the internal standards were used to track and evaluate the extraction, saponification and purification of milk powder, the internal standard average recovery rate during the steps, and the whole processes. In order to verify the efficiency of the developed method, three concentrations of PAH4 ($0.5 \mu\text{g}\cdot\text{kg}^{-1}$, $1.0 \mu\text{g}\cdot\text{kg}^{-1}$, and $5.0 \mu\text{g}\cdot\text{kg}^{-1}$) were spiked into the infant formula powders ($n = 6$) for a recovery test. For quality control, Fapas olive oil (0.5 g), as a quality control material, was added to $0.1 \mu\text{g}/\text{mL}$ D12-PAH4 mixed internal standard solution ($50 \mu\text{L}$) and this sample was subjected to the above sample preparation protocol. Because there is no quality control substance for PAHs in the milk powder, this Fapas olive oil is selected as a quality control substance to track the accuracy of the evaluation method.

2.6. Risk Assessment for Dietary Exposure to PAHs. The MOE value is the ratio between the reference dose calculated by the dose-response relation curve and the human dietary exposure estimated by the mathematical model. In order to estimate MOE, the chronic daily intake (CDI) of PAHs using the BaP equivalent concentration should be calculated according to EPA [20]. The ratio between benchmark dose lower confidence limit (BMDL) and chronic daily intake (CDI) can be considered as MOE; the CDI value can be calculated by the estimated daily intake (EDI) value calculated by using U.S. EPA's benchmark dose software [21].

The MOE calculation formula is as follows:

$$\text{MOE} = \frac{\text{BMDL}_{10}}{\text{EDI}}, \quad (1)$$

where MOE is the margin of exposure for BaP or PAH4 (dimensionless) and BMDL_{10} is benchmark dose level₁₀, which means the lowest dose that causes adverse reactions in 10% of the population; the BMDL_{10} value of BaP and PAH4 were $0.07 \text{ mg}/\text{kg bw}\cdot\text{d}^{-1}$ and $0.34 \text{ mg}/\text{kg bw}\cdot\text{d}^{-1}$, respectively [6].

3. Results and Discussion

3.1. Optimization of Conditions for GC-MS Detection of PAH4 in Infant Formula. Several methods including HPLC with fluorescence detection [1, 4, 6, 22] and GC-MS [23–26] have been used for PAHs analysis in food and environmental samples. Some of these methods do not meet the necessary sensitivity and accuracy limits. At low analyte concentrations, the GC-MS method for antiinterference is better than that of liquid chromatography and the internal standard quantitative method is better than that of an external standard method. Therefore, a GC-MS method using

isotopic internal standards was developed to determine PAH4 in powdered infant formula.

Commonly used chromatographic columns in GC-MS determination of PAHs include DB-5 MS, DB-17 MS, and DB-EUPAH chromatographic columns. The separation of three isomers, benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene, is a challenging task, and the high amounts of benzo[b]fluoranthene further complicate separation efforts; therefore, the DB-5 MS and DB-17 MS columns were not selected in this study. The DB-EUPAH column was selected because it can effectively separate benzo [b]fluoranthene, benzo[k]fluoranthene, and benzo[j]fluoranthene. A sample chromatogram containing PAH4 and the internal standard ions is presented in Figure 2.

The corresponding peak area of PAH4 and internal standard D12-PAH4 were measured. In the 0.5 to 10.0 ng analyte range, the mass ratio of PAH4 and 5.0 ng internal standard D12-PAH4 was plotted on the X-axis and the peak area ratio of PAH4 and the internal standard D12-PAH4 (response ratio) was plotted on the Y-axis to obtain a linear relationship. The monitoring ions and linear equations of each compound are shown presented in Table 1. Some other methods such as LC-MS/MS have occasionally been used for PAHs analysis and can be further applied to detect PAHs.

3.2. Blank Test. PAHs are widely present pollutants that can be found nearly everywhere. The reagents, experimental setup, and other devices could be potential sources of PAH4 contamination; it is necessary to strictly control the analysis process. In order to reduce the PAH4 blank value, glass vessels were pretreated by baking at 300°C or washed with n-hexane. All plastic tools were used only once because PAHs could stick to plastic tools and transfer to other samples. The blank value can also be effectively controlled by reducing reagent dosage and experimental steps; therefore, N_2 air drying was selected during the concentration steps. The blank test values of PAH4 were less than the limit of detection and guaranteed the reliability of the developed method. The total ion flow chromatogram of the blank test is presented in Figure 3, the peak after D12-BbFA not labeled is D12-BkFA in the internal standard solution of PAH-Mix 9, which was not detected.

3.3. Extraction and Purification. The extraction of PAHs in infant formula powders includes direct saponification [1, 2, 23], solvent extraction [22, 25, 26], and dispersive solid phase extraction, and a simplified version of conventional method named the quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method has been used for the multiresidue analysis. The analytical method for the determination of the US EPA priority pollutant of 16 PAHs in edible oils was developed by an isotope dilution GC-MS method [27], which is a reference for this study, and all parameters are listed in Figure 1. In brief, samples were extracted by ultrasonication in acetonitrile followed by purification using a narrow gel permeation chromatographic column [27]. Due to the necessary processes for extraction

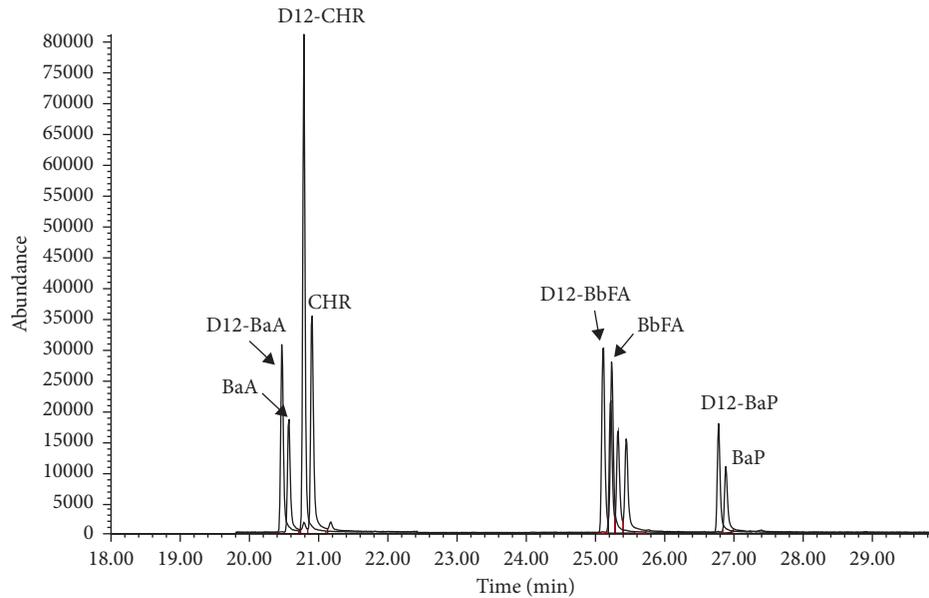


FIGURE 2: Ion chromatograph of PAH4 and the D12-PAH internal standards.

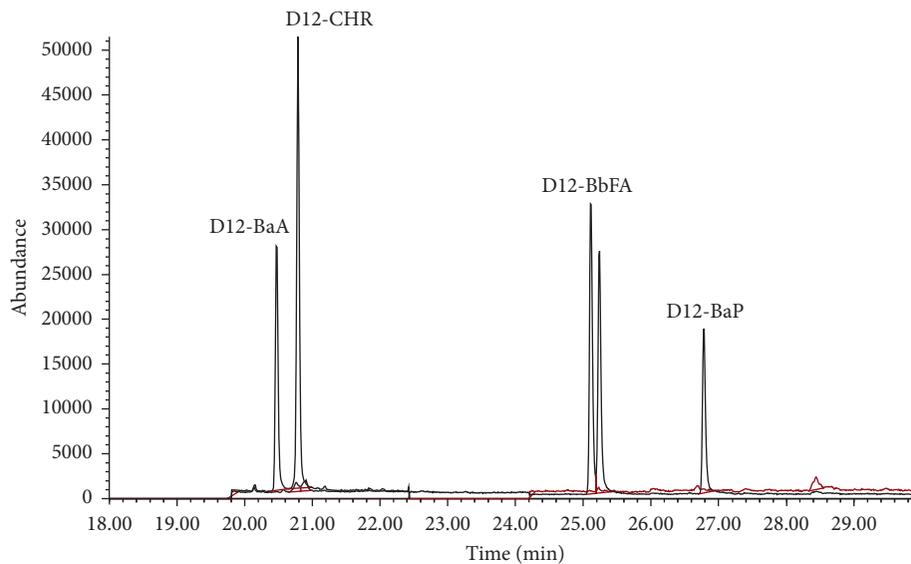


FIGURE 3: Total ion flow chromatogram of the blank test.

utilized, such as SPE, and the ingredients in infant formula powder, there were several undesired drawbacks during our sample preparation procedure: it is difficult to extract all PAHs using the direct solvent extraction method; saponification directly, without a first purification step, would consume large amounts of reagent and might jeopardize the quality of the blank control; for purification, the SPE method alone does not deliver satisfactory results, combination utilization of QuEChERS with SPE had been used for PAHs analysis, and the rapid, quick, easy, cheap, effective, rugged, and safe procedures will be more efficient; the procedures utilized in this study shows the same level efficiency compared with the QuEChERS method. PAHs are stable chemicals and soluble in lipid; therefore, it is necessary to extract the fat in milk powders under alkali conditions

(GB5009.6) and then to remove the fat by a saponification process. After a further SPE purification step, the PAHs will be sufficiently purified for the GC-MS analysis.

3.4. Accuracy, Precision, and Quality Control of the Developed Method. Three concentrations of PAH4 ($0.5 \mu\text{g}\cdot\text{kg}^{-1}$, $1.0 \mu\text{g}\cdot\text{kg}^{-1}$, and $5.0 \mu\text{g}\cdot\text{kg}^{-1}$) were spiked into the infant formula powders ($n = 6$) for a recovery test. Because a known PAH quality control substance in powdered milk existed, Fapas olive oil containing low concentrations of PAHs was selected as a quality control substance for internal control. There is no milk powder material of polycyclic aromatic hydrocarbons in quality control, thus in the FAPAS olive oil (low), to track the accuracy of the evaluation method.

TABLE 2: Recovery of internal standard during the extraction, saponification, purification, and the whole processes.

Chemicals	Extraction (%)	Saponification (%)	Purification (%)	The whole process (%)
D12-BaA	93.5	95.2	95.7	85.2
D12-CHR	92.5	93.4	94.6	81.7
D12-BbFA	91.6	92.7	89.3	75.8
D12-BaP	91.4	92.3	88.9	74.6

Because PAHs are vulnerable to environmental pollution, the internal standards were used to track and evaluate the extraction, saponification, and purification of milk powder; the internal standard average recovery rate during the steps and the whole processes are listed in Table 2 ($n = 3$). As presented in Table 2, the recovery rate of the four compounds in extraction, saponification, and purification steps were over 85% and the developed method of internal standard recovery in the whole process were over 70%, which meets the requirements for the analysis of the compounds.

The results of the accuracy, precision, and quality control tests are presented in Table 3. As presented in Table 3, the average recovery rate of PAH4 in infant formula was 77.3% to 111.8% and the precision was 4.8% to 14.2%. Li et al. analyzed 31 milk powder samples using HPLC with silica cartridges for PAH clean-up; the recoveries were greater than 79% for all PAHs analyzed [23].

The average signal-to-noise ratio (S/N) of PAH4 was 47.5 when the formula was spiked with $0.5 \mu\text{g}/\text{kg}$ ($n = 6$). The S/N value of BaP was 32.4 due its relatively low detection sensitivity. These results demonstrate that the developed method detects BaP to the levels required by international regulatory bodies, such as the EU analysis standard of $1.0 \mu\text{g}\cdot\text{kg}^{-1}$. Therefore, the quantitative limit of PAH4 in this method was determined to be $0.5 \mu\text{g}\cdot\text{kg}^{-1}$, and the detection limit was $0.1 \mu\text{g}\cdot\text{kg}^{-1}$. Figure 4 presents the chromatogram of one infant formula powder sample spiked with $1.0 \mu\text{g}\cdot\text{kg}^{-1}$ PAH4 standard. Several studies had reported the LODs, LOQs, and recoveries of PAHs from different food material including meat products, tea, fish, and crab [28]; the recoveries ranged from 66% to 138% when detecting 16 PAHs in fresh oysters, shrimp, and Atlantic croaker products, with the LODs and LOQs of 0.02 and $0.06 \mu\text{g}\cdot\text{kg}^{-1}$, respectively [29].

3.5. Sample Analysis. The PAH4 content of 30 commercially available domestic and imported stage-1 infant formula powders was analyzed (Figure 5). The total amount of BaP and PAH4 in the 30 domestic and imported stage-1 infant formula powders tested was all lower than the requirement of $1.0 \mu\text{g}\cdot\text{kg}^{-1}$ (Table 4). Cho and Shin reported that the average concentrations of 7 PAHs were 0.435 and $0.457 \mu\text{g}/\text{kg}$ in infant formulas and mixed milk powders, respectively, and BaP contents were much lower than $1 \mu\text{g}\cdot\text{kg}^{-1}$ [1]; Santonicola et al. reported that the average of the total PAHs was of $52.25 \mu\text{g}\cdot\text{kg}^{-1}$ and $11.82 \mu\text{g}\cdot\text{kg}^{-1}$ in milk and meat/fish based baby foods, milk-based samples showed significant higher values ($P < 0.05$) of carcinogenic and possible carcinogenic hydrocarbons than meat/fish-based products [6].

In the South America market, the highest concentration of BaP detected was $0.57 \mu\text{g}\cdot\text{kg}^{-1}$ in milk powder; the sum of 15 analysed PAHs varied between 11.8 and $78.4 \mu\text{g}\cdot\text{kg}^{-1}$, and the PAH4 was between 0.02 and $10.16 \mu\text{g}\cdot\text{kg}^{-1}$. All the samples were below the regulatory limit for BaP, but 65% of commercial milk powders do not comply with the European Union limit for PAH4 [23]; the results indicated the contamination level of PAHs in the baby food. As for this study, PAH4 showed a relatively low level compared with products in other markets [6, 22]. In addition, the positive detection rates of the PAH4 markers in infant formula powders, CHR, BbFA, BaA, and BaP, were 70.0%, 63.3%, 50.0%, and 23.3%, respectively. The average concentration from highest to lowest of the samples was in the following order: CHR, BaA, BbFA, and BaP.

3.6. Exposure Assessment. For substances with genetic toxicity and carcinogenicity, the MOE method is used to assess the risk characteristics. In toxicology, the MOE of a substance is the ratio of its no-observed-adverse-effect level to its theoretical, predicted, or estimated dose or concentration of human intake [30]. This approach is published by U.S. Environmental Protection Agency (U.S. EPA) and preferred by both the World Health Organization (WHO) and the European Food Safety Authority (EFSA) for the risk evaluation of carcinogens [31].

The scientific opinion of the EFSA on a harmonized approach for risk assessment of substances that are both genotoxic and carcinogenic was used to characterize the risk related to consumption of vegetables. In general, it was assumed that a MOE of 10000 or higher would be of low concern for public health and might be considered of low priority for risk management actions [32]. The benchmark dose lower confidence limit 10% (BMDL₁₀), an estimate of the lowest dose (95% confidence level) that causes no more than a 10% cancer incidence in rodents, was used to obtain the MOE. The MOE for each population group was calculated as follows. Taking primary hepatocellular carcinoma as the toxicity effect endpoint to assess the risk of PAHs intake in the population, the BMDL₁₀ of BaP and PAH4 were $0.07 \text{ mg}/\text{kg bw}\cdot\text{d}^{-1}$ and $0.34 \text{ mg}/\text{kg bw}\cdot\text{d}^{-1}$, respectively [6]. According to the report of Jia et al. [33], the recommended daily intake of formula for infants (average body weight 7 kg) at the first stage (6 months old) in China is 150 g. According to Table 4, the average and maximum daily exposure of BaP for stage-1 infants in China are $0.45 \text{ ng}/\text{kg}\cdot\text{bw}\cdot\text{d}^{-1}$ and $1.9 \text{ ng}/\text{kg}\cdot\text{bw}\cdot\text{d}^{-1}$, respectively. For PAH4, the values are $8.6 \text{ ng}/\text{kg}\cdot\text{bw}\cdot\text{d}^{-1}$ and $18.6 \text{ ng}/\text{kg}\cdot\text{bw}\cdot\text{d}^{-1}$, respectively. According to the MOE calculation, the average values and highest values of BaP and PAH4 are 155555, 36842 and 18279, 39534,

TABLE 3: Recovery rate of PAH4 in the spiked infant formula test.

Chemicals	0.5 ($\mu\text{g}/\text{kg}$) ARR	0.5 ($\mu\text{g}/\text{kg}$) RSD%	1.0 ($\mu\text{g}/\text{kg}$) ARR	1.0 ($\mu\text{g}/\text{kg}$) RSD%	5.0 ($\mu\text{g}/\text{kg}$) ARR	5.0 ($\mu\text{g}/\text{kg}$) RSD%	Fapas oil ($\mu\text{g}/\text{kg}$)	Amount recovered ($\mu\text{g}/\text{kg}$)
BaP	97.8	8.3	90.1	10.4	98.4	5.2	2.04–5.24	3.93
CHR	89.2	14.2	94.3	12.4	97.5	6.7	2.83–7.28	6.41
BaFA	77.3	9.6	79.4	8.4	84.7	4.8	1.16–2.98	2.24
BaP	111.8	8.7	86.1	4.2	96.4	4.4	0.90–2.32	1.56

*ARR, average recovery rate (%); RSD, relative standard deviation.

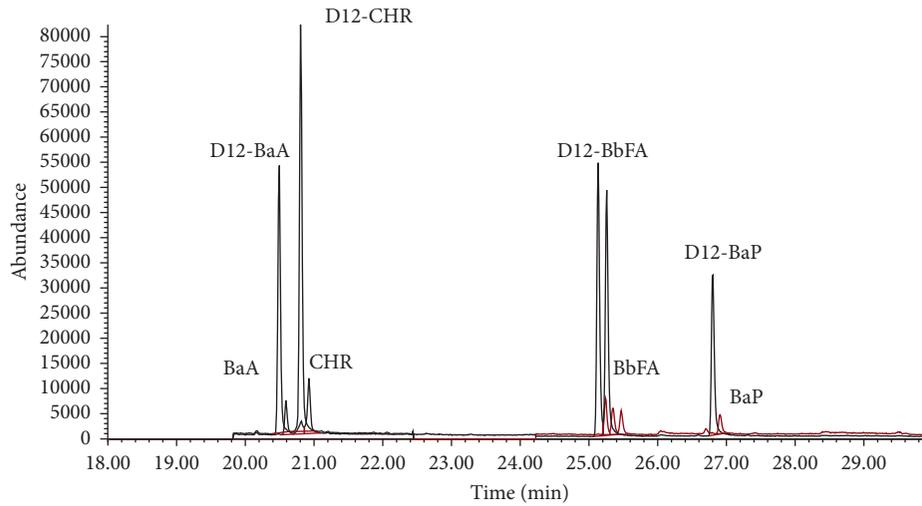


FIGURE 4: Chromatograph of PAH4 in an infant formula powder sample spiked with 1.0 $\mu\text{g}/\text{kg}$ PAH4 standard.

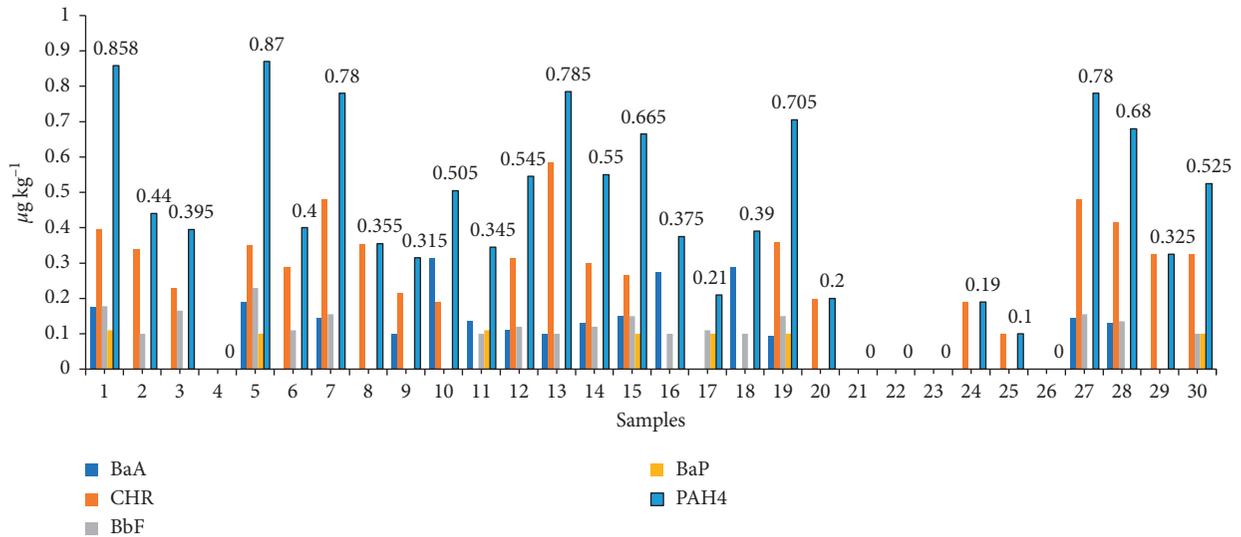


FIGURE 5: PAH4 content in 30 stage-1 infant formulas.

TABLE 4: Detected PAH4 content in 30 stage-1 infant formulas.

	BaA	CHR	BbFA	BaP	PAH4
Positive numbers	15	21	19	7	25
Concentration range ($\mu\text{g}/\text{kg}$)	<0.1–0.32	<0.1–0.59	<0.1–0.23	<0.1–0.09	<0.1–0.87
Average content ($\mu\text{g}/\text{kg}$)	0.085	0.23	0.078	0.021	0.4

respectively. Compared with BaP in infant formula powders, PAH4 should be given more attention. The higher the MOE value is, the lower the attention need to be paid. The MOE value of BaP in infant formula was from 1,370,000 to 2,700,000, which is of negligible concern according to Cho and Shin [1]. As world widely concerned the risk of PAHs contamination, nowadays, the four PAH4 markers in all of the commercially available infant formula powders were tested; they were at low risk, and are all quite safe for consumption.

4. Conclusion

A GC-MS method was developed to analyze the PAH4 content in commercially available infant formula powders. This simple method consisted of the addition of an isotopic internal standard, an extraction under alkali conditions, a saponification step, and a SPE purification step. The developed method was quite accurate and had a quantitative detection limit of $0.5 \mu\text{g}\cdot\text{kg}^{-1}$, which exceeded the EU guideline limit of $1.0 \mu\text{g}\cdot\text{kg}^{-1}$ of PAH4 specified for infant formula. The PAH4 content in 30 domestic and foreign infant formula powders were tested, and all 30 powders were found to contain $<0.1\text{--}0.87 \mu\text{g}\cdot\text{kg}^{-1}$ PAH4. When considering the intake of infant formula recommended for stage-1 infants in China, the PAH4 concentrations in the analyzed samples present low risk levels for consumption. Because PHAs are widely present in the environment and are easy to contaminate lipid-containing foods and oil in apparatus, it is necessary to monitor the food material, processing facilities, and other factors to decrease the possibilities of PHA contamination. Also, rapid and accurate analysis method, PHA internal standard for special foods, and survey of other foods should be further carried out.

Abbreviations

PAH4:	BaP, BaA, CHR, and BbFA
BMDL ₁₀ :	Benchmark dose level ₁₀
BaP:	Benzo[a]pyrene
BaA:	Benzo[a]anthracene
BbFA:	Benzo[b]fluoranthene
BW:	Body weight
CDI:	Chronic daily intake
CHR:	Chrysene
ELISA:	Enzyme-linked immunosorbent assay
EU:	European Union
EC:	European Commission
EFSA:	European Food Safety Authority
GC-MS:	Gas chromatography-mass spectroscopy
GPC:	Gel permeation chromatography
GC-MS/MS:	Gas chromatography/tandem mass spectrometry
GPC/SPE:	Gel permeation chromatography/solid-phase extraction
MOE:	Margin of exposure
PAHs:	Polycyclic aromatic hydrocarbons
QuEChERS:	Quick, easy, cheap, effective, rugged, and safe
WHO:	World Health Organization.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

This research was funded by the Key Research and Development Program of Guangdong Province (no. 2019B020212001), Zhejiang Provincial Natural Science Foundation of China (nos. LGN19C200003 and LGF19C200002), Zhejiang Province and National Health Commission Co-Construction Project (no. WKJ-ZJ-1917), the National Key Research and Development Program of China (2018YFC1603600), and National Natural Science Foundation of China (no. 31871884).

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