Determination of benzo(a)pyrene in fried and baked foods by HPLC combined with vesicular coacervative supramolecular solvent extraction

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Abstract A simple, rapid and low-cost determination method of benzo(a)pyrene in fried and baked foods was proposed by high performance liquid chromatography combined with vesicular coacervative supramolecular solvent (SUPRAS) extraction. The vesicular coacervate was composed of 1-octanol and tetrabutylammonium bromide. 200 mg of dried samples with 600 μ L SUPRAS could be mixed to extract benzo(a)pyrene. Neither evaporation nor further clean-up steps for the extracts were needed. The overall sample treatment took approximately 30 min, and several samples could be simultaneously treated using conventional lab equipment. Then, benzo(a)pyrene was analyzed via liquid chromatography-fluorescence detection. Parameters affecting the extraction efficiency were investigated and optimized. The results showed good linearity of benzo(a)pyrene with the coefficients of determination (R^2) of more than 0.9999 in the range of 0.1–50.0 μ g/kg. The limit of detection of the method was 0.11 μ g/kg. Recoveries for spiked samples in the range of 1–10 μ g/kg were between 89.86 and 100.01%, with

relative standard deviations from 1.20 to 3.20%. Benzo(a)pyrene was present in food samples (including instant noodles, biscuits, rice crust and fried bread stick) at concentrations in the range of 0.08–0.39 μ g/kg according to the proposed method. The proposed pretreatment method significantly reduces the analysis time. Furthermore, the solventless approach is in accordance with the green chemistry development trend and has significant application prospects.

Keywords Supramolecular solvent (SUPRAS) · Benzo(a)pyrene · Liquid chromatography-fluorescence detection · Green chemistry

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous and persistent organic compounds with multiple aromatic rings, the number of which determines their physico-chemical properties and toxicity (Zachara et al. 2017a; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2010). Several PAHs have been shown to be genotoxic and carcinogenic to animals and humans. Among the large number of PAHs polluting the environment and food, benzo(a)pyrene (BaP), the prototypical PAHs, has attracted significant public attention due to its adverse effects (mutagenesis, carcinogenesis and teratogenesis) and been classified by the International Agency for Research on Cancer (IARC) as a Group 1 human carcinogen (Surma et al. 2014). In fact, the main source of exposure to benzo(a)pyrene for the average person is food (Janoszka et al. 2004). However, in recent years, food safety problems caused by benzo(a)pyrene are increasingly serious. In October 2012, carcinogens-benzo(a)pyrene

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were detected in six types of instant noodles, which were produced by Nongshim Ltd. In 2017, there were 17 notifications about benzo(*a*)pyrene in food in a range from 2.1 to 113.2 µg/kg reported by the Rapid Alert System for Food and Feed (RASFF) in the European Union. The news and notifications have aroused significant concern of people from all lifestyles. In view of the presence of benzo(*a*)pyrene in food and its significant carcinogenicity, monitoring of food is very important.

It is well known that most of the problems associated with the determination of benzo(*a*)pyrene are derived from its low concentration and complex media where it occurs, especially for food matrices. Hence, traditional methods applied for the determination of benzo(*a*)pyrene in food matrices always involve a two-stage methodology. Firstly, samples are extracted with hexane or cyclohexane. Then, the extract is cleaned up using a solid phase extraction column (silica, alumina, florisil, etc.). Available scientific reports have described the analysis via different techniques of extraction, purification and detection (Zachara et al. 2017a; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2010; Conchione et al. 2015; Zachara et al. 2017b; Rey-Salgueiro et al. 2009; Kumari et al. 2013; Liu et al. 2016). Zachara et al. (2017b) adopted a method including the process of saponification, extraction and clean-up using a self-made column packed with aluminum oxide for the determination of PAHs in vegetable oils. The detection limit was 0.18 µg/kg. Then, they used a similar method for determining PAHs in smoked meat and fish (Zachara et al. 2017a). Surma et al. (2014) proposed a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method with ethyl acetate as an extraction solvent, primary–secondary amine and C₁₈ sorbents and evaporation to dryness and dissolving the residues in the hexane for determination of PAHs in food. The recoveries ranged from 72.4 to 110.8%. Rey-Salgueiro et al. (2009) analyzed 11 PAHs in milk formulae and infant cereals. The sample treatment comprised ultrasound-assisted solvent extraction, enzymatic hydrolysis and solid-phase clean-up. Most of the methods described above can remove lipidic material to minimize the maintenance of the chromatographic system and reach lower detection limits (Surma et al. 2014). However, there are some problems, such as high consumption of hazardous organic solvents, experimental steps being too cumbersome and the extraction times being too long. Therefore, it is still required to develop time-saving, easy-to-operate and environmentally friendly methods for the analysis of trace contaminants in food.

Supramolecular solvent (SUPRAS) is a complex, organized and nanostructured liquid, generated from the amphiphiles through a sequential and spontaneous self-assembly process via intermolecular interactions. SUPRAS

is a water-immiscible liquid that is made up of surfactant aggregate dispersed in a continuous phase (usually water) (Ballesteros-Gómez et al. 2009, 2010). Supramolecular solvent extraction is a novel extraction method, which was put forward by Ballesteros-Gómez and Rubio (2012). It is a promising and powerful alternative to organic solvents in analytic extractions because it offers rapid extraction, safe to operate and has high efficiency. Thus, SUPRAS has been widely used for the extraction of different compounds. In the last decades, accessible research has been reported for the application of SUPRAS for the extraction of contaminants from food, involving chili-containing foodstuffs (López-Jiménez et al. 2010), fish (Costi et al. 2010; Caballo et al. 2012; López-Jiménez et al. 2014), raw wheat and cereals (García-Fonseca et al. 2010, 2016; García-Fonseca and Rubio 2016), snacks (Cardeñosa et al. 2011; Caballero-Casero et al. 2012; Alabi et al. 2014), garlic, mint and tea (Moradi et al. 2017). It can be seen that people are gradually recognizing the advantages and great utility of SUPRAS. Feizi et al. (2016) used SUPRAS prepared via gemini surfactant to extract methyl paraben, ethyl paraben and propyl paraben in beverages and water samples with high recoveries. The presentation of gemini surfactants has extended the scope of SUPRAS and provided them the ability to be used for the extraction of a wider range of analytes.

In this paper, the suitability of a novel 1-octanol-based SUPRAS for extracting benzo(*a*)pyrene in foods with the aim of simplifying sample pretreatment was explored. The research included the study of parameters (including mass concentration of 1-octanol, molar quantity of tetrabutylammonium bromide, solvent amount and ultrasonic time), which affect the extraction efficiency and quantitative performance of the method and its application for the determination of benzo(*a*)pyrene in various foods purchased at local supermarkets.

Materials and methods

Reagents and standards

Tetrabutylammonium bromide (TBAB), 1-octanol and benzo(*a*)pyrene standards were purchased from J&K Scientific (Shanghai, China). HPLC-grade acetonitrile was supplied by Sigma-Aldrich (St. Louis, Missouri, USA). Ultra-pure grade water was obtained using a Milli-Q water purification system (Millipore, MA, USA). Stock solutions of benzo(*a*)pyrene (200 mg/L) were prepared in acetonitrile. Working standard solutions were prepared via dilution of the stock solution with acetonitrile. All solutions were stored under dark conditions at 4 °C and were brought to room temperature before use.

Apparatus

The liquid chromatographic system used consisted of a P1201 binary pump, an S3100 auto sampler, a ZW1201 column oven (Dalian Elite Analytical Instruments Co., Ltd., Liaoning, China) and an RF-20AXs fluorescence detector (SHIMADZU, Kyoto, Japan). The stationary-phase column was a SinoPak C18 column (100 mm × 4.6 mm id, 3 μ m). AS3120A Ultrasonic cleaner (120 W, 40 kHz. Tianjin Automatic Science Instrument Co., Ltd., Tianjin, China) and multifunctional food grinder (Shanghai Hailing electric appliance Co., Ltd., Shanghai, China) were applied for sample pretreatment. A 3K15 centrifuge (Sigma, Osterode am Harz, Germany) was used for SUPRAS production. Electronic balance (Mettler Toledo Instrument Co., Ltd., Shanghai, China) was employed for sample weighing. Milli-Q ultra-pure water purification system was used for supplying ultra-pure water (Millipore, Billerica, MA, USA).

Sample pretreatment

Practical and spiked samples

Different food samples were randomly purchased at local markets, including different brands of instant noodles, biscuits, rice crust and fried dough sticks. The samples were chopped and homogenized using a homogenizer–dispenser after drying. All of the samples were sealed dry for preservation until analysis. The concentrations of benzo(a)pyrene in spiked samples were 10 μ g/kg.

Supramolecular solvent production

1-Octanol (113 mg, amounting to a mass concentration of 1.2%) was mixed with TBAB (322 mg) and ultra-pure water (9 mL) in a 10-mL centrifuge tube. The mixture was centrifuged for 10 min at 3000 rpm. The SUPRAS formed was less dense than water and was separated as a new liquid phase at the top of the solution. Then, the aqueous phase was removed with a syringe, and the supernatant was stored at 4 °C in a closed glass vial until use.

Microextraction of benzo(a)pyrene

200 mg of food sample and 600 μ L of SUPRAS were mixed in a 1.5 mL centrifuge tube. Then, the mixture was sonicated for 10 min to accelerate the dissolution of the target analyte from food samples into the solvent phase at room temperature. Next, the SUPRAS extract was withdrawn using a 1-mL syringe, filtered through 0.45- μ m filters, and transferred to an auto-sampler vial for chromatographic analysis. A general workflow for

benzo(a)pyrene determination in foods using SUPRAS extraction prior to liquid chromatography-fluorescence detection (LC-FD) is shown in Fig. S1 in the Supplementary Material.

Optimization of SUPRAS extraction

Optimization of SUPRAS extraction was carried out using blank instant noodles (200 mg) spiked with the target benzo(a)pyrene at a concentration of 10 μ g/kg per sample. Recovery experiments were performed in quintuplicate. The investigated variables were as follows: composition (including mass fraction of 1-octanol, molar quantity of TBAB in the SUPRAS), volume of the extractant and ultrasonication time required for effective extraction. When one variable was being investigated, other variables were as same as the conditions in the section of “[Supramolecular solvent production](#)”.

Liquid chromatography-fluorescence detection

The column used in LC-FD was a SinoPak C18 (100 mm × 4.6 mm id, 3 μ m) with the column temperature of 35 °C. Benzo(a)pyrene was monitored at E_x = 290 nm and E_m = 410 nm. The mobile phase consisted of acetonitrile and water (80:20, V/V) at a flow rate of 1.0 mL/min. The volume of injection of samples was 10 μ L. The limit of detection (LOD) was defined as the lowest level of the analyte that can be detected and could be calculated when the ratio of signal to noise was 3 ($S/N = 3$).

Results and discussion

Optimization of the supramolecular solvent-based extraction

Supramolecular solvent description

There are three different structures of SUPRAS (e.g., reverse micelles, aqueous micelles and vesicles) according to the type of amphiphiles and dispersants (Ballesteros-Gómez et al. 2010). The vesicles SUPRAS are usually used for the extraction of solid samples. The typical physico-chemical properties of the supramolecular solvents make them very attractive for replacing organic solvents in analytical extractions. The main intrinsic properties of these solvents are as follows. Amphiphiles are ubiquitous in both nature and synthetic chemistry. Thus, SUPRAS can be easily prepared. The synthetic process is based on self-assembly and can be performed by anyone. Solvent properties can be tuned by varying the hydrophobic or polar

group of the amphiphile. The aggregates that make up SUPRAS have different polarity regions, which provide excellent extraction performance to a large number of organic compounds. The aggregates that make up SUPRAS also have a large number of binding sites that increase the extraction efficiency, which is of great importance for microextraction. Most importantly, high molecular mass components, such as lipids and carbohydrates, cannot be extracted using SUPRAS (Ballesteros-Gómez and Rubio 2012).

In our research, synthesis of vesicular SUPRAS was a spontaneous and fast process involving the mixture of 1-octanol and TBAB in an aqueous solution. Tetrabutyl ammonium (Bu_4N^+) induces coacervation during the solvent preparation (Ballesteros-Gómez et al. 2010). 1-Octanol and TBAB are subjected to a continuous and ordered self-assembly process in an aqueous solution. Hydrophilic moieties both inside and outside the vesicles were formed based on the hydrophobic interaction between the hydrocarbon chains. Then, these nanostructures self-assemble into bigger aggregates that are immiscible and have a lower density than the bulk solution. Figure 1 shows the self-assembly processes of supramolecular solvent formation. In fact, the liquid provided an effective enrichment for the low molecular mass polar and most organic compounds while excluding high molecular mass components such as proteins and carbohydrates in solid matrices (Ballesteros-Gómez et al. 2010).

According to the study of SUPRAS by Ballesteros-Gómez and Rubio (2012), alkanol-based SUPRAS provided a mixed-mode mechanism for dissolving medium polar and nonpolar solutes. As for the prepared SUPRAS above, two types of interactions were expected to be the driving forces for extraction, namely, dispersion forces between the hydrocarbon chains of the amphiphile (1-octanol) and the analytes, and π -cation interactions between the aromatic rings of analytes and the tetrabutyl ammonium (Bu_4N^+). Both types of interactions should provide good solubilization of benzo(a)pyrene in the SUPRAS that

facilitate their efficient extraction, isolate and concentrate benzo(a)pyrene from solid matrices.

Optimization of SUPRAS composition and amount

Optimization of SUPRAS extraction was carried out according to recovery experiments. The selection of the optimal conditions was based on the recoveries and standard deviations obtained. A higher recovery with a lower standard deviation would be the best result. Meanwhile, it was also needed to balance the reagent consumption and the recoveries.

Figure 2a shows the effect of varying the mass fraction of 1-octanol (0.3–1.8%) on the extraction efficiency for benzo(a)pyrene in spiked samples under the conditions of 1.0 mmol TBAB and 600 μL amount of SUPRAS. The results showed that the recoveries gradually increased and tended to be stable with increasing 1-octanol mass fraction. When the mass fraction of 1-octanol was higher than 1.2%, the recoveries were almost constant. Considering the standard deviation of recoveries, 1.2% 1-octanol was chosen as the best dosage in the subsequent study for its lower standard deviation.

Figure 2b shows the effect of varying the molar quantity of TBAB (0.2–1.4 mmol) on the extraction efficiency for benzo(a)pyrene in spiked samples under the conditions of 1.2% 1-octanol and 600 μL amount of SUPRAS. The results show that the recoveries gradually increased and tended to be stable with increasing the molar quantity of TBAB. Although under the condition of 1.2 mmol TBAB the recoveries were the highest, the standard deviation was rather high that made the extraction process unstable. When the molar quantity of TBAB was 1.0 mmol (322 mg), the recoveries were still high but more stable, which met the detection requirement. Therefore, 1.0 mmol TBAB was regarded as the optimum dosage in the subsequent study.

The volume of supramolecular solvent required for the optimal extraction was determined by extracting a constant

Fig. 1 Self-assembly processes in supramolecular solvent formation: **a** 1-octanol (red folding lines) and TBAB (blue circles) were dispersed in the solution; **b** after molecular self-assembly, vesicles were constructed from 1-octanol and TBAB based on their hydrophobic interaction and **c** vesicles gathered and self-assembled at the nanoscale to form the SUPRAS

Fig. 2 Mean recoveries and standard deviations ($R \pm SD$) obtained during the extraction of benzo(a)pyrene with SUPRAS under different conditions. **a** 1-Octanol at different levels; **b** TBAB at different levels; **c** different volumes of SUPRAS and **d** different ultrasonic time

amount of sample (200 mg of blank instant noodles spiked with benzo(a)pyrene at 10 $\mu\text{g/kg}$ each) with variable volumes (300–1000 μL) of the selected SUPRAS under the conditions of 1.2% 1-octanol and 1 mmol TBAB. Figure 2c shows the recoveries obtained, along with their standard deviations, as a function of the SUPRAS volume used for extraction. In the aspect of average recoveries, there was little difference among the conditions of 600 μL , 800 μL and 1000 μL . However, the standard deviation under the condition of 1000 μL and 800 μL were the smallest and the largest respectively. Besides, in order to increasing the concentration of benzo(a)pyrene extracted in SUPRAS and saving the consumption of SUPRAS, a volume of 600 μL was selected as the optimal amount.

Effect of ultrasonic time

Ultrasound baths always provide a simple and cheap way to accelerate the extraction of analyte from samples. In this

research, extraction was performed using ultrasound to favor the dispersion of sample mass in SUPRAS. The effect of ultrasonication time (0–20 min) on the extraction efficiency of benzo(a)pyrene in the spiked samples were studied at room temperature and other conditions of extraction were as same as the conditions in the section of “Supramolecular solvent production”. The results showed that the recoveries gradually increased and tended to be stable with an increasing ultrasonication time (Fig. 2d). When the ultrasonication time was 10 min, the recoveries were high and stable, which met the requirement of detection. Therefore, the samples were ultrasonicated for 10 min in the subsequent study.

The prepared SUPRAS might provide a good solvation property for benzo(a)pyrene as other kinds of SUPRAS (Ballesteros-Gómez et al. 2010), which could facilitate the rapid transfer of analytes from the food sample to the solvent. Only 10 min of ultrasonication-assisted extraction was required to reach equilibrium conditions. At this

duration, the target compound was quantitatively extracted from food samples.

Method validation

Linearity and the lower limit of quantification

Eight different concentrations (0.1 µg/kg, 0.5 µg/kg, 2.0 µg/kg, 5.0 µg/kg, 10.0 µg/kg, 20.0 µg/kg, 25.0 µg/kg and 50.0 µg/kg BaP) of spiked samples were used for the calibration curve construction. The linear regression equation is $Y = 8.5237X + 0.5709$ with a coefficient of determination R^2 of more than 0.9999 and a standard error of estimates SE(Y) of 1.7352. The limit of detection (LOD) of benzo(a)pyrene was 0.11 µg/kg, which was much lower than the value allowed in processed cereal-based foods according to Chinese standard GB 2762-2017 (2017) (5 µg/kg) and European Union Commission Regulation (EC) 2015/1125 (2015) (1 µg/kg).

Precision and accuracy

Optimized approach has been successfully applied for the determination of benzo(a)pyrene. Recovery experiments were made in quintuplicate. The results showed that recoveries for spiked instant noodles with a concentration of 1.0, 5.0 and 10.0 µg/kg BaP were between 89.86 and 100.01% with relative standard deviations from 1.20 to 3.20%, as shown in Table 1, which indicated that the method had high precision and accuracy.

Chromatograms of blank and spiked samples are shown in Fig. 3.

Fig. 3 Chromatograms of **a** blank and **b** spiked samples

Results for food samples

Compared with the traditional pretreatment method based on the Chinese standard GB 5009.27-2016 (2016), the established method was simpler and quicker that could save at least 60% of the pretreatment time. Besides, the established method needed smaller sample amounts and much fewer volume of organic solvent but achieved lower LODs and higher recoveries as shown in Table 2. The proposed method was then successfully applied for the determination of benzo(a)pyrene in 3 brands of instant noodles, 2 brands of biscuits, 2 brands of rice crust and 2 kinds of fried bread stick. As a result, the contents of benzo(a)pyrene were 0.08–0.39 µg/kg in instant noodles ($n = 3$), 0.13–0.34 µg/kg in biscuits ($n = 2$), 0.18 µg/kg in rice crust ($n = 2$) and 0.16–0.17 µg/kg in fried bread stick ($n = 2$). The content of the test substance is less than

Table 1 Recoveries and relative standard deviation (RSD) of benzo(a)pyrene in instant noodles

Sample	Added (µg/kg)	Found (µg/kg)	Recovery (%)	RSD ($n = 5$) (%)
Instant noodles	1.0	0.959	95.92	3.20
		0.959	95.87	
		0.914	91.38	
		0.952	95.21	
		1.000	100.01	
	5.0	4.667	93.34	2.16
		4.625	92.50	
		4.756	95.11	
		4.566	91.32	
		4.493	89.86	
	10.0	9.597	95.97	1.20
		9.631	96.31	
		9.355	93.55	
		9.435	94.35	
		9.498	94.98	

Table 2 Comparison of pretreatment processing parameters between the traditional method and the method established herein

Pretreatment method	Sample amount (g)	Volume of organic solvent (mL)	Processing time (h)	LOD ^a (µg/kg)	Recovery ± SD (%)
Traditional method ^b	1	92	1.5	0.2	80.9 ± 0.6
Established method	0.2	0.2	0.5	0.11	94.1 ± 2.5

^aLOD means limit of detection^bTraditional method was in accordance with Chinese standard GB 5009.27-2016 (2016)

Chinese national standards GB 2762-2017 (2017) (5 µg/kg for processed cereal-based foods).

Conclusions

Supramolecular solvent have a unique array of physico-chemical properties making them very attractive for replacing organic solvents in analytical extractions. In this paper, SUPRAS composed of 1-octanol and tetrabutylammonium bromide were proposed as valuable tools for the extraction of benzo(a)pyrene from food, which offered a simple, low-cost and rapid alternative to conventional sample preparation methods. The overall sample treatment required approximately 30 min with extraction and purification being completed in one step, and several samples could be simultaneously treated. The proposed pretreatment method can be used for the routine control of benzo(a)pyrene in fried and baked food according to Chinese national standards.

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