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SIMPLE μ-SOLID PHASE EXTRACTION USING C_{18} FILM FOR THE EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN COFFEE BEVERAGE

(Pengekstrakan μ-Fasa Pepejal yang Mudah Menggunakan Filem C_{18} untuk Pengekstrakan Hidrokarbon Aromatik Polisiklik dalam Minuman Kopi)

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are produced as by-products in the coffee roasting process. A simple and efficient micro-solid phase extraction (μ-SPE) procedure coupled with high performance liquid chromatography and fluorescence detection has been developed for the simultaneous analysis of selected PAHs, namely phenanthrene (PHE) and benzo[a]pyrene (BaP) in coffee beverage. The method offered trace detection limits in the range of 0.003 to 0.1 μg/L and good relative recovery average in the range of 88.3 to 95.7%. The μ-SPE procedure has been simplified by employing C_{18} film as an adsorbent. The method is beneficial in terms of rapidity, simple operation, in-expensive, consumption of micro volumes of solvent and biodegradable agarose film as the adsorbent holder. The method was then applied to analyse five coffee beverage samples and the results indicated the presence of BaP in the range of 1.0 to 1.9 μg/L.

Keywords: agarose, polycyclic aromatic hydrocarbons, coffee, C_{18}, micro-solid phase extraction

Abstrak

Hidrokarbon aromatik polisiklik (PAHs) dihasilkan sebagai produk sampingan dalam proses memanggang kopi. Suatu prosedur pengekstrakan mikro-fasa pepejal (μ-SPE) yang mudah dan cekap bergabung dengan kromatografi cecair berprestasi tinggi dan pengesanan pendarfluor telah dibangunkan untuk analisis serentak PAHs terpilih, iaitu fenanterena (PHE) dan benzo[a]pirena (BaP) dalam minuman kopi. Kaedah ini menawarkan had pengesanan surih dalam julat 0.003 hingga 0.1 μg/L dan purata perolehan semula relatif yang baik dalam julat 88.3 hingga 95.7%. Prosedur μ-SPE ini telah diringkaskan dengan menggunakan filem C_{18} sebagai penjerap. Kaedah ini berfaedah daripada aspek ianya cepat, beroperasi mudah, tidak mahal, penggunaan isipadu pelarut yang mikro dan filem agarosa yang terdegradasi-bio sebagai pemegang penjerap. Kaedah ini kemudian diaplikasikan untuk menganalisa lima sampel minuman kopi dan keputusan menunjukkan kehadiran BaP dalam julat 1.0 hingga 1.9 μg/L.

Kata kunci: agarosa, hidrokarbon aromatik polisiklik, kopi, C_{18}, pengekstrakan mikro-fasa pepejal

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are classified as relatively persistent organic environmental contaminants. Several PAHs, including benzo[a]pyrene (BaP) are classified as carcinogen and mutagen [1]. Human are usually exposed to PAHs via intake of food. Heat processing of food such as roasting, charcoal grilling and smoking are factors which cause the food contaminated with PAHs. PAHs are non-desirable by-products produced by incomplete combustion during the roasting step in coffee production [2]. The 1971 World Health Organisation International Standards has recommended a guideline for six representative PAH compounds namely fluoranthene, 3,4-benzfluoranthene, 11,12-benzfluoranthene, 3,4-benzpyrene, 1,12-benzpyrene and indeno[1,2,3-cd]pyrene as not exceeding 0.0002 mg L^{-1} in drinking water [3]. To the best of our knowledge, there is no specific maximum residue guideline established for beverage.

Classic techniques such as liquid-liquid extraction (LLE) and solid phase extraction (SPE) are frequently used to extract PAHs in various matrices [4-7]. However, these techniques are not eco-friendly as they applied large amounts of organic solvent during extraction. Therefore, micro-scale sample preparation techniques that work towards green chemistry are developed.

Membrane-assisted solvent extraction (MASE) required shorter extraction time (30 min) as compared to stir bar sorptive extraction (SBSE) (3 hours) for the analysis of pesticide and BaP residues in Brazilian sugarcane juice. MASE offered a faster and better analyte recovery technique whereas SBSE has greater sensitivity, repeatability and required lower amounts of sample [8]. Dispersive liquid-liquid microextraction (DLLME) is simple, sensitive and rapid for the extraction and pre-concentration of PAHs from beverage samples because it applies dispersive concept throughout the extraction [9]. Magnetic solid phase extraction simplified the collection of adsorbent disperse in the sample solution and offered ultra-trace analysis of PAHs in both coffee and tea samples [10]. Ishizaki and co-researchers demonstrated online in-tube solid phase microextraction (SPME) that was simple, selective, sensitive and rapid to extract PAHs from food samples [11]. These techniques minimize solvent usage and has gained significant attention.

This study employs agarose as adsorbent holder, which is made up of natural polymer extracted from seaweed. It is a toxic-free and inert material. The proposed approach in this study displays a double "green chemistry" nature due to its micro-extraction format and the biodegradability of the adsorbent holder.

Materials and Methods

Chemicals and materials

Phenanthrene (PHE) and benzo[a]pyrene (BaP) were purchased from Sigma-Aldrich (Missouri, United States). HPLC grade isopropyl alcohol, methanol and acetonitrile were obtained from Merck (New Jersey, United States). Agarose and C_{18} adsorbent were purchased from Sigma-Aldrich (Missouri, United States).

Preparation of standards and samples

Standard stock solutions of PHE and BaP (500 mg/L) were prepared individually by weighing 0.005 g of each analyte in 10 mL of volumetric flask and diluted to volume with acetonitrile, respectively. A series of working mixture standard solutions were prepared by diluting the stock solutions with methanol. The solutions were stored at 0 ˚C in refrigerator when not in use.

Coffee beverage samples were obtained from selected local enterprise shops. Samples selections were more preferred with different brands. Samples were extracted using optimized micro-solid phase extraction (μ-SPE) technique without pre-treatment.

Preparation of C_{18} film

C_{18} (0.1 g) was dispersed and sonicated in 5 mL of ethanol. The C_{18} solution (1 mL) was pipetted into a petri dish (5 cm diameter). Agarose (0.1 g) was added into 10 mL of deionized water and the solution was heated to boil. The resulting hot agarose solution (3.5 mL) was pipetted into the petri dish containing the C_{18} solution. The mixture was allowed to cool and form a C_{18}-impregnated agarose gel. The gel was dried at room temperature for 2 days to form a C_{18} film. The film was cut into a small piece of square sheet (1.5 cm x 1.5 cm) to function as an adsorbent film.

Micro-solid phase extraction procedure

The C_{18} film was dipped into methanol for 2 minutes and followed by deionized water for 30 s to activate and condition the C_{18}, respectively. Sample solution (15 mL) was pipetted into a 25-mL sample vial and the C_{18} film was tumbled into the sample solution for extraction. The sample vial was capped and sonicated for 10 minutes. After sonication, the film was withdrawn and added into a safe-lock screw vial containing 100 µL of acetonitrile. The desorption of target analytes from the C_{18} film was performed by sonicating the screw vial for 10 minutes. The acetonitrile extract was then filtered through 0.2 µm nylon syringe filter prior to quantification using high performance liquid chromatography coupled with fluorescence detection (HPLC-FD). Figure 1 shows the schematic diagram of the proposed µ-SPE.

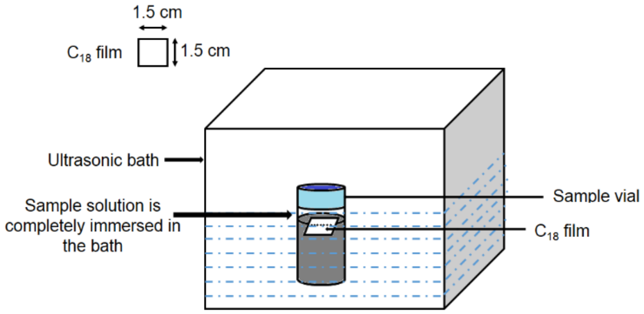

Figure 1. Schematic diagram of µ-SPE

Chromatographic conditions

Analytes quantification was performed using high performance liquid chromatography (Shimadzu, Kyoto, Japan) coupled with fluorescence detection (Shimadzu, Kyoto, Japan). The chromatographic separation of polycyclic aromatic hydrocarbons (PAHs) was carried out on a reversed phase Platinum EPS C_{18} column (4.6 × 250 mm, 5 µm). The separation was performed using isocratic mobile phase acetonitrile-water (80:20) (v/v) at column temperature of 30 ˚C. The flow rate, injection volume and detection wavelengths were fixed at 1.0 mL/min, 10 µL and 250/400 nm of excitation/emission wavelengths, respectively.

Optimization and validation of micro-solid phase extraction

In this study, several extraction parameters were thoroughly investigated to enhance the analytes enrichment. Sample volume, extraction time, types of desorption solvents, desorption time and volume of desorption solvent were optimized before the application of the proposed method for the analysis of coffee beverage samples. Minimal validation was carried out to assess the viability of method and these included linearity, repeatability, relative recovery, limit of detection (LOD) and limit of quantification (LOQ), which were determined based on signal to noise 3:1 and 10:1, respectively.

Results and Discussion

Optimization of micro-solid phase extraction

Five parameters which influenced the extraction efficiency of micro-solid phase extraction (µ-SPE) were optimized and these included sample volume, extraction time, type of desorption solvent, desorption time and desorption solvent volume. Optimization was carried out using deionized water sample spiked with 10 µg/L each of phenanthrene (PHE) and benzo[a]pyrene (BaP), respectively. Triplicate extractions were carried out for each parameter optimized in this study.

Sample volume

Sample volume was examined as it influenced the adsorption capacity of the C_{18} film. Sample volume in the range of 10 to 20 mL was studied. Figure 2a shows that the C_{18} film adsorbed lesser PAHs when smaller sample volume

(10 mL) was extracted. The amount of analytes extracted increased as the sample volume increased and reached optimum at 15 mL and the extraction efficiency did not increase significantly with further increase in sample volume. This was due to the C_{18} film was saturated with the PAHs and further increase in sample volume would not result in the increment of the peak areas [12]. Therefore, 15 mL sample solution was applied for next parameter study.

Extraction time

Extraction time ranging from 5 to 15 minutes was examined to identify the equilibrium time required for the analytes to adsorb onto C_{18} film. PAHs are hydrophobic compound. The octanol-water partition coefficient (K_{ow}) increases from PHE to BaP with the increasing of their molecular weight. The hydrophobic PAHs required some time to diffuse through the static layer of water to be adsorbed onto the C_{18} film [13]. The extraction efficiency increased gradually when the extraction time increased as indicated in Figure 2b. Extraction time of 10 minutes was chosen over 15 minutes even though the extraction efficiency showed an increase at 15 minutes. Both PHE and BaP increased for 10.9 and 8.4%, respectively. However, the increment was not significant according to ANOVA-Tukey test, $P > 0.05$. Therefore 10 minutes extraction time was chosen for the subsequent study as it was sufficient to obtain a better extraction efficiency and save time for the extraction process.

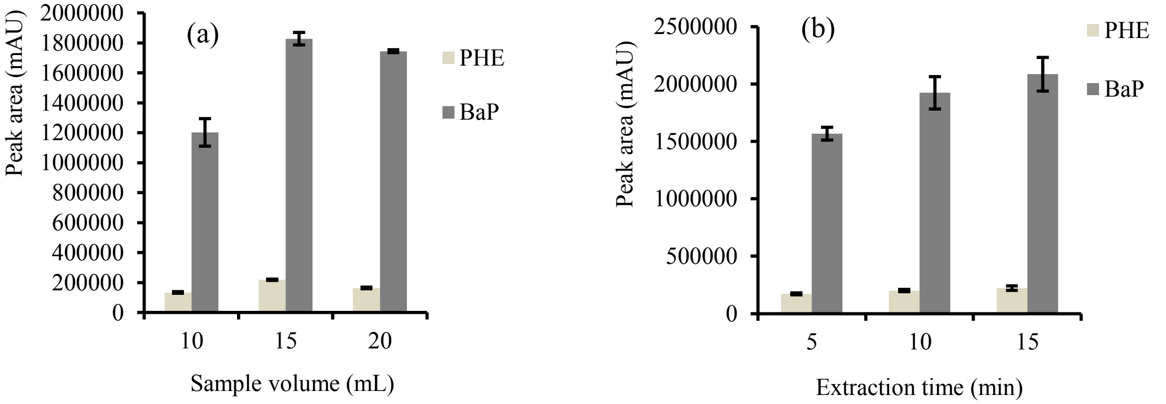

Figure 2. Effect of sample volume (a) and extraction time (b) on the μ-SPE for the extraction of PHE and BaP in the spiked deionized water samples

Type of desorption solvent

Desorption solvent was used to desorb the PAHs from the C_{18} film. The desorption solvents investigated were acetonitrile (ACN), methanol (MEOH) and isopropanol (IPA). PAHs are lipophilic and non-polar compounds, thus it will adsorb strongly on C_{18} film. Therefore, a stronger solvent which is relatively non-polar is required to desorb the PAHs from the film. Among the solvents, the elution strength of IPA is the strongest, it was then followed by ACN and MEOH. The results showed that ACN was a better choice as a desorption solvent for PHE, a PAH with lower molecular weight, as compared with MEOH and IPA. On the other hand, the IPA showed greater affinity to the BaP, a PAH with higher molecular weight. The desorption was less efficient when MEOH was applied. The desorption strengths of both ACN and IPA were comparable and no significant different. The ACN was chosen in subsequent studies as it was also applied as the mobile phase.

Desorption Time

Desorption time in the range of 5 to 15 minutes was investigated. Figure 3a shows the peak area of PHE increased as the desorption time increased and became stagnant at 10 minutes. However, the peak area of BaP was the highest at 5 minutes and decreased gradually when the desorption time was extended. Prolonged desorption time might lead to re-adsorption of the BaP onto the C_{18} film and caused the drop in peak area [14]. The desorption time at 10 minutes was chosen because greater peak area for PHE (increased 30%) was obtained while extraction efficiency for BaP decreased only slightly (declined for 20%).

Volume of desorption solvent

A range of 100 µL to 200 µL of acetonitrile was investigated in this study. Figure 3b indicates that when a lower volume of desorption solvent was used, the greater the peak areas. This is due to the lower volume enhanced the analyte enrichment [15]. In addition, the amount of solvent used should be as minimal as possible in order to obtain a greater sensitivity and the volume of the solvent must be sufficient to completely immerse the entire film [16]. A volume of 100 µL desorption solvent was chosen because it gave higher extraction efficiency for all of the analytes.

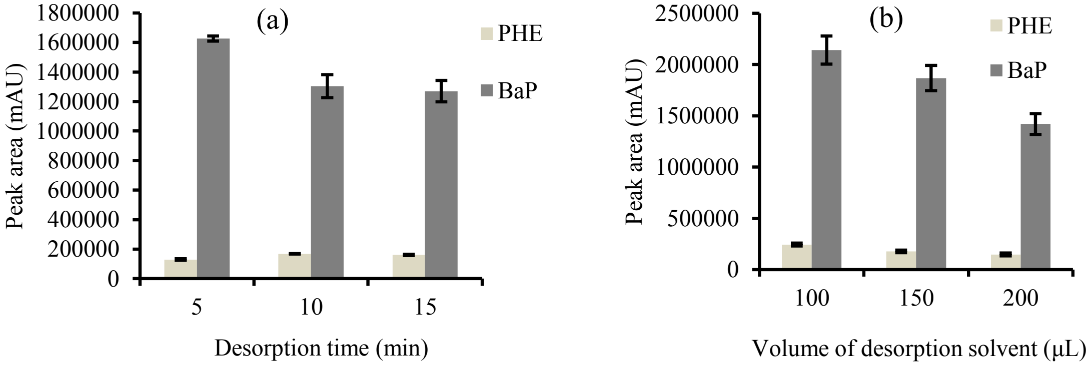

Figure 3. Effect of desorption time (a) and volume of desorption solvent (b) on the µ-SPE for the extraction of PHE and BaP in the spiked deionized water samples

Validation of micro-solid phase extraction

Linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample [17]. Relative calibration for the two analytes of interest, namely PHE and BaP were performed because µ-SPE technique is a non-exhaustive extraction. Five deionized water samples spiked in a specified range were extracted and quantitated using optimized µ-SPE-HPLC-FD. The results indicated that a good linearity was obtained for both PHE and BaP in the range of 5 to 200 µg/L and 1 to 25 µg/L, respectively with correlation of coefficients, $r \geq 0.996$.

Limit of detection (LOD) is defined as the lowest amount of analyte in the sample which can be detected but not necessarily quantitated under stated experimental conditions. Whereas limit of quantification (LOQ) is defined as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy [18]. LOD and LOQ can be determined by visual inspection, signal-to-noise ratio or the standard deviation of the response and the slope [19]. In this study, a signal-to-noise ratio of 3:1 and 10:1 were used to establish the LOD and LOQ, respectively. The results showed that the proposed µ-SPE-HPLC-FD was able to offer LODs in the range of 0.003 to 0.1 µg/L and LOQs in the range of 0.01 to 0.4 µg/L. This revealed that µ-SPE-HPLC-FD method using C_{18} film as the adsorbent film was sensitive to analyse the ultra-trace PAHs in coffee beverage samples.

Relative recovery study was performed by spiking the coffee beverage samples to give a final concentration of 30 µg/L of PHE and 25 µg/L of BaP. Table 1 shows that the µ-SPE was capable to recover PAHs from the spiked coffee beverage samples with relative recovery average ranging from 88.3 to 95.7% coupled with good repeatability with relative standard deviations (RSDs) of ≤ 7.6%. This revealed that matrix effect was not significant and thus the µ-SPE combined with C_{18} film can be employed in the analysis of PAHs residues in the coffee beverage samples.

Table 1. Validation data of the µ-SPE-HPLC-FD for the determination of PAHs in coffee beverage samples

PAH	Linearity range, µg/L	r	LOD, µg/L	LOQ, µg/L	Average relative recovery ± RSD, % (n=3)
PHE	5-200	0.9961	0.1	0.4	88.3±7.6
BaP	1-25	0.9985	0.003	0.01	95.7±0.8

Analysis of selected PAHs in coffee beverage samples

The optimized and validated µ-SPE technique was applied to extract the PAHs from five coffee beverage samples that were obtained from retail shops. The results show that BaP was detected in four out of five coffee beverage samples. The BaP concentration was detected in the range of 1.0 to 1.9 µg/L while PHE was not detected. Currently, there is no regulation that set a maximum limit for BaP level in coffee beverage.

Conclusion

The micro-solid phase extraction using C_{18} film as the adsorbent is a sensitive, simple and green technique. The agarose used is a biodegradable material that makes it environmental friendly coupled with the reduction in the consumption of solvent due its micro-scale format. This technique fulfils the green chemistry concept that is widely approached these days. The technique shall then be applied for the extraction of 16 PAHs to examine the competition among the PAHs for the minute amounts of adsorption sites.

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