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HEADSPACE MEMBRANE-PROTECTED LIQUID PHASE MICROEXTRACTION OF PHENANTHRENE IN BEVERAGE AND WATER

(Pengekstrakan Mikro Fasa Cecair Dilindungi Membran Ruang Kepala bagi Fenantrena dalam Air Minuman dan Air)

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Abstract

Contamination of low molecular weights polycyclic aromatic hydrocarbons (PAHs) is common in beverage and water. This could pose a health risk to those beverage lovers when they consume the products. Phenanthrene (PHE) is one of the low molecular weights PAHs that consists of three benzene rings in the molecular structure. In this study, PHE was chosen as the model analyte due to its mid-volatility behavior. A headspace membrane-protected liquid phase microextraction (HS-MP-LPME) combined with high performance liquid chromatography-fluorescence detection (HPLC-FD) has been developed for the analysis of PHE in beverage and water samples. The nylon membrane containing 1-octanol as the extractant was exposed to the headspace of the sample vial containing 25 mL of sample solution. The extraction was performed at its optimal conditions with sample temperature fixed at 60 °C, agitation set at 700 rpm and extraction conducted for 30 minutes. Under these optimal extraction conditions, the HS-MP-LPME-HPLC-FD offered ultra-trace detection of PHE and insignificant matrix effects in beverages (green tea and coffee) and water (river, sea and tap) samples with average of relative recovery in the range of 83.7 to 116.1%. The HS-MP-LPME simplifies the routine analysis and resolves the extractant dissolution problem that commonly occurs in hollow fiber-protected LPME. The proposed technique consumes only minimal amounts of organic solvent (200 µL) and this indirectly supports our National Green Technology Policy: together we create a better tomorrow.

Keywords: beverage, water, headspace membrane-protected liquid phase microextraction, high performance liquid chromatography-fluorescence, phenanthrene

Abstrak

Kontaminasi hidrokarbons aromatik polisiklik (PAHs) berjisim molekul rendah dalam air minuman dan air adalah biasa. Ini akan menghasilkan risiko kesihatan kepada pencinta air minuman apabila mereka minum produk tersebut. Fenantrena (PHE) ialah salah satu PAHs berjisim molekul rendah yang mengandungi tiga cincin benzena dalam struktur molekulnya. Dalam kajian ini, PHE dipilih sebagai sebagai analit model kerana kemeruapannya yang sederhana. Satu pengekstrakan mikro fasa cecair dilindungi membran ruang kepala (HS-MP-LPME) bergabung dengan kromatografi cecair berprestasi tinggi-pengesanan pendarfluor (HPLC-FD) telah dibangunkan untuk menganalisis PHE dalam sampel air minuman dan air. Membran nilon yang mengandungi 1-oktanol sebagai pengekstrak didedahkan pada ruang depan botol sampel yang berisi 25 mL larutan sampel. Pengekstrakan dilaksanakan menggunakan keadaan optimum iaitu menetapkan suhu sampel pada 60 °C, mengacau sampel pada

kelajuan pengocakan 700 rpm dan mengekstrak selama 30 minit. Di bawah keadaan pengekstrakan optimum, HS-MP-LPME-HPLC-FD menawarkan pengesanan PHE pada tahap ultra-surihan dan memberi kesan matriks yang tidak signifikan dalam sampel air minuman (tea hijau dan kopi) dan air (sungai, laut dan paip) dengan perolehan semula secara relatif dalam lingkungan 83.7 hingga 116.1%. HS-MP-LPME memudahkan analisis rutin dan menyelesaikan masalah pelarutan pengekstrak yang biasa berlaku dalam LPME dilindungi fiber berongga. Teknik yang dicadangkan hanya menggunakan kuantiti pelarut organik yang minimum dan ini menyokong Polisi Hijau Nasional kita secara tidak langsung: bersama-sama kita membina keesokan yang lebih baik.

Kata kunci: air minuman, air, pengekstrakan mikro fasa cecair dilindungi membran pada ruang kepala, kromatografi cecair berprestasi tinggi-pendarfluor, fenantrena

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds consist of two or more aromatic benzene rings in one structure. Sources of PAHs can be found from natural and anthropogenic activities. For example, forest fires, volcanic eruptions and incomplete combustion. These pollutants tend to migrate through food chain and be transferred over long distances. PAHs are very toxic to human health because they have potential to cause cancer, affect male and female reproduction and cause obesity [1]. Contamination of low molecular weights polycyclic aromatic hydrocarbons (PAHs) is common in beverage and water. This could pose a health risk to those beverage lovers when they consume the products.

Microextraction techniques have been applied as alternatives to conventional sample preparation techniques since 1996, when the single drop microextraction (SDME) was first demonstrated [2, 3]. However, the application of SDME is less popular due to the dissolution of the hanging organic drop into the sample solution during extraction especially when the sample is agitated. Various membranes have been employed to address this limitation, with the aim to protect the organic extractant from losing into sample solution. Polypropylene hollow fiber protected liquid phase microextraction (PPHF-LPME) are the most employed membrane-protected LPME. This technique is reliable and proven to be sensitive and selective for the extraction of various organic and inorganic analytes in aqueous samples [4-8]. Other membrane-protected LPME techniques that utilized agarose film [9], agarose gel [10-12], nylon membrane [13, 14], polypropylene membrane bag [15] and silica monolith [16] were also successfully demonstrated to address the solvent dissolution problem.

This project offered an alternative method for the extraction of phenanthrene in beverage and water samples using headspace membrane-protected LPME coupled with high performance liquid chromatography and fluorescence detection. The extractant was doubled protected using a nylon membrane that was hung in the headspace of sample vial. This geometry design of this technique kept away the interruption the membrane from the agitation and thus freed the extractant from dissolution into sample solution.

Materials and Methods

Chemicals and reagents

Phenanthrene (PHE) reference standard was obtained from Dr. Ehrenstorfer (United Kingdom) and 1-octanol (analytical grade) were purchased from Sigma-Aldrich (Missouri, United States). HPLC grade reagents like acetonitrile (ACN), and methanol (MeOH) were purchased from Merck (New Jersey, United States).

Preparation of standard and samples

Standard stock solutions of PHE (500 mg/L) was prepared by dissolving 0.005 g of PHE in a 10 mL of volumetric flask and diluting to volume with acetonitrile. A series of working standard solutions were prepared by further diluting 500 mg/L of PHE standard stock solution with methanol. All of the standard solutions were stored at 0 ˚C in the refrigerator when not in use.

Different environmental water samples namely, river water, tap water and sea water were collected from area around Universiti Malaysia Terengganu whereas ready packed beverage samples (green tea and coffee) were purchased from local enterprise shops. The samples were kept in the fridge until analysis. The environmental water samples were filtered through Whatman filter paper No. 1 to exclude larger particles prior to extraction, whereas ready packed beverage samples were analyzed without any pre-treatment.

Preparation of nylon membrane

Round-shaped nylon membrane filters (I.D 47 mm, pore 0.2 μm) were purchased from Sartorius (Goettingen, Germany). The membrane filters were cut, folded and sealed according to Loh et al. [14]. The two edges of the membrane top were then poked and threaded through with a thread to enable the membrane hanging in the headspace of samples vial during extraction.

Headspace membrane-protected liquid phase microextraction procedure

The outer layer of the pre-sealed membrane was immersed into 1-octanol to impregnate the pores of the membrane wall. Then, the membrane was exposed to the headspace of a 40 mL of sample vial containing 25 mL of sample solution and a stir bar. The 1-octanol (200 μL) was then added into the membrane, this was followed by screwing the vial cap. Next the sample vial was placed in a 500 mL beaker containing pre-heated water at 60 °C on a hot plate. The sample solution was stirred at 700 rpm at 60 °C for 30 minutes. Later, 100 mL of the extract (1-octanol) was withdrawn with a micropipette and transferred into a 2.5 mL safe-lock tube prior to quantification using high performance liquid chromatography coupled with fluorescence detection (HPLC-FD). Figure 1 shows the schematic of headspace membrane-protected liquid phase microextraction (HS-MP-LPME).

Chromatographic conditions

Analyte quantification were performed using high performance liquid chromatography (Shimadzu, Kyoto, Japan) coupled with fluorescence detection (Shimadzu, Kyoto, Japan). The chromatographic separation of PHE was carried out on a reversed phase C_{18} column (4.6 × 150 mm, 5 μm). The separation was performed with isocratic elution utilizing mobile phase acetonitrile-water (70:30) (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 HS-MP-LPME-HPLC-FD

In this study, several extraction parameters were thoroughly investigated to enhance the analytes enrichment. Agitation speed (300-900 rpm), temperature (50-80 °C), and extraction time (20-40 minutes), were optimized before the application of the proposed method for the analysis of beverages and environmental water samples. Minimal validation were carried out to assess the viability of method and these included linearity, repeatability, relative recovery, limit of detection (LOD) and limit of quantification (LOQ). The relative recovery was calculated by comparing the peak area of spiked samples and the peak area of spiked deionized water. The LOD and LOQ were determined using linear regression approaches as the following (equation 1 and 2):

$$LOD = 3.3\ \sigma/S \quad (1)$$

$$LOQ = 10\ \sigma/S \quad (2)$$

where σ = the standard deviation of the y-intercept and S = the slope of the calibration curve.

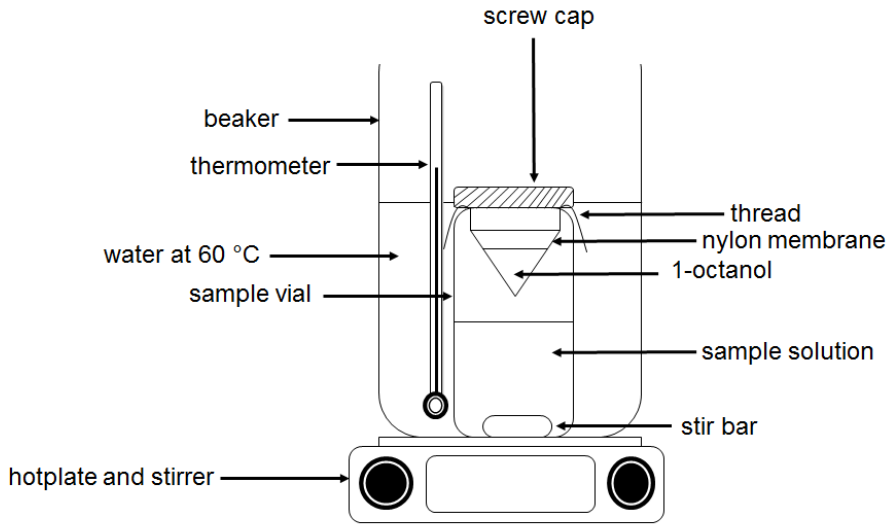

Figure 1. Schematic of headspace membrane-protected liquid phase microextraction

Results and Discussion

Optimization of HS-MP-LPME

There are several types of parameters that need to perform in this investigation to make sure optimum condition for the extraction of PHE in the beverages and environmental water samples are achieve using headspace membrane-protected liquid phase microextraction (HS-MP-LPME) technique. These parameters included extraction time, sample temperature and agitation speed. The condition for the optimization was carried out using deionized water samples spiked level with 200 ppb of PHE. The triplicate injection into HPLC-FD instrument were perform for each parameters to confirm the consistency of the results.

Extraction time is important to determine the time taken to reach equilibrium stage [17] because microextraction is non–exhaustive extraction technique. In this study, the extraction time for the analysis of PHE was carried out in the range 20 to 40 min whereas other parameters were kept constant. For instances, extraction temperature and agitation speed were both maintained at 60 °C and 700 rpm, respectively. Based on Figure 2(a), it was found that the peak area increased for more than 50% when extraction time was prolonged from 20 to 30 minutes. A decline in extraction efficiency was observed when the extraction time was extended to 40 min. The depletion of mid-volatile PHE was observed when the 1-octanol was evaporated due to the long exposing time at elevated temperature. Extraction time was then maintained at 30 minutes for the subsequent experiments.

PHE is a low molecular weight polycyclic aromatic hydrocarbon and it is classified as semi-volatile compound due to its low volatility [18]. Therefore, it was conveniently determined using headspace extraction, where the extractant was not contacted directly with the sample solution. This would prevent the extractant from dissolution into sample solution during extraction especially at high agitation speed. In this study, sample temperature ranging from 50 to 80 °C. The extraction was prolonged for 30 minutes as determined from previous experiments. Figure 2(b) shows, average peak area for the PHE gradually increased from 50 to 60 °C. No significant change in extraction efficiency indicated by peak area when the extraction temperature was fixed beyond 60 °C. Increased of sample temperature promoted an increase in PHE vapors in the headspace capacity [19]. The

recovery of PHE increased with temperature up to a certain level where the system achieved equilibrium and no more gain in peak area was observed. Therefore, the optimal sample temperature was fixed at 60 °C for the following experiments.

In this study, agitation speed was investigated in the range of 300 to 900 rpm. Figure 2(c) indicates that increase of agitation speed enhanced the convection in sample. The mass transfer of PHE was promoted from sample solution to headspace and finally reaching the extractant when the sample was agitated from 300 to 700 rpm. Above agitation at 700 rpm, the vortex formed in the sample solution disrupted the mass transfer rate and thus causing a drop in PHE recovery [11].

Validation of HS-MP-LPME-HPLC-FD method

Validation was performed to proof the HS-MP-LPME was acceptable for its application to extract PHE from water and beverage samples. The validation variables namely linearity range, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) were carried out to ensure the HS-MP-LPME was viable in its intended application. Linearity regression was established using five deionized water spiked with PHE in the range of 25 to 500 ppb. A good linearity regression was obtained with correlation of determination (R^2), 0.9960. LOD and LOQ are normally used in an analytical analysis as a measurement to detect the limitation of the analytical method. LOD is defined as the lowest concentration of analyte that can be detected in a sample but not necessarily quantitated, whereas LOQ is defined as the lowest concentration of analyte that can be quantified with acceptable precision and accuracy [20]. The LOD and LOQ were calculated at concentration levels, 18 ppb and 25 ppb respectively. The technique was capable to offer ultra-trace detection and quantification of PHE in water. Both LOD and LOQ were lower than the maximum permissible concentration (MPC) of PHE proposed by National Institute for Public Health and the Environment, Netherlands for drinking water which is 0.14 ppm [21].

Accuracy of the HS-MP-LPME was investigated using relative recovery study. Relative recovery is defined as the initial concentration of the analyte after the extraction process acquired. The relative recovery was conducted by spiking each of the coffee, green tea, tap water, river water, and seawater samples with 25 and 100 ppb of PHE, respectively. Both sample blank and spiked samples were extracted using the optimized HS-MP-LPME. PHE was not detected in all of the sample blanks. Table 1 shows that the average of relative recovery of all samples exhibited acceptable recovery of PHE range from 83.7 to 116.1% with good precision indicated by relative standard deviations (RSDs) of three replicates $\leq$ 8.5%. Indeed, the HS-MP-LPME coupled with HPLC-FD was able to offer ultra-trace detection of PHE and provide negligible matrix effect for the analysis of PHE in beverage and environmental water samples.

The pros and cons of using HS-MP-LPME for the extraction of PHE

The comparison between HS-MP-LPME combined with HPLC-FD and previous methods was tabulated in Table 2. Overall, all of the previous reported methods offered much better sensitivity and wider linearity range as compared to HS-MP-LPME-HPLC-FD. However, all these methods involved several steps in extraction, whereas HS-MP-LPME combined extraction and pre-concentration in one step. Low density solvent based-dispersive liquid-liquid microextraction (LDSB-DLLME) coupled with HPLC-FD involved several steps which were, injection of solvents into sample solution, centrifugation for phase separation and withdrawal of extraction solvent prior to HPLC-FD analysis [22]. The extraction was completed in 6 minutes even though with multi-steps. This was because the extraction applied dispersive concept that accelerated the analytes mass transfer from the sample solution into the extraction solvent.

The micro solid phase extraction (μ-SPE) using C_{18} film as the adsorbents and combined with HPLC-FD incorporated simpler steps in the extraction of selected polycyclic aromatic hydrocarbons (PAHs) in coffee beverage, where analysts only needed to desorb the analytes from the film using a solvent and ultra-sonic

bath after the extraction [23]. The method offered slight lower and narrower of low concentration limit and linearity range, respectively as compared to HS-MP-LPME-HPLD-FD.

The magnetic solid phase extraction (MSPE) coupled with HPLC-FD were also reported for the analysis of PHE in coffee beverage [24]. The method simplified the collection of adsorbents after the extraction, where an external magnet was positioned outside of the sample vial to collect the adsorbent. This method has similar advantages as HS-MP-LPME, where both are environmentally friendly, inexpensive and easy to perform. Lastly, it is concluded that every method has its own pros and cons, where analysts can be based on their capability and convenience to choose their ideal method to achieve their analysis objectives.

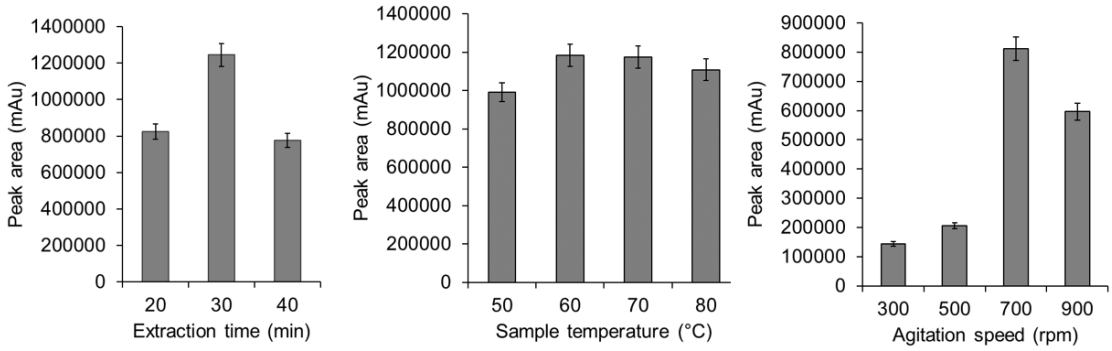

Figure 2. Effect of extraction time (a), sample temperature (b) and agitation speed (c) on the extraction efficiency of HS-MP-LPME for the extraction of PHE in spiked deionized water

Table 1. Relative recovery study using HS-MP-LPME-HPLC-FD for the analysis of PHE in beverage and water samples

Samples	**Average of Relative Recovery ± Relative Standard Deviation, % (n=3)**	
	Spiked at 25 ppb	**Spiked at 100 ppb**
Coffee	95.2 ± 3.2	97.7 ± 5.9
Green tea	112.4 ± 7.4	83.7 ± 8.4
Tap water	96.8 ± 3.8	97.0 ± 3.6
River water	107.9 ± 8.5	86.1 ± 5.0
Sea water	116.1 ± 6.8	98.0 ± 4.4

Table 2. Comparison between HS-MP-LPME and other published methods for the analysis of PHE in beverage and environmental water samples

Analysis Method	Matrix	Linear Range (ppb)	LOD (ppb)	Extraction Time (minutes)	Reference
LDSB-DLLME-HPLC-FD	Beverage	0.005-50 and 0.01-50	0.001 and 0.008	6	[22]
μ-SPE-HPLC-FD	Beverage	5-200	0.1	20	[23]
MSPE-HPLC-FD	Beverage	0.1-200	0.005	12	[24]
HS-MP-LPME-HPLC-FD	Beverage	25-500	18	30	This work

Conclusion

The HS-MP-LPME is proven as a green analysis method because only a small amount of organic solvent is consumed, and no harmful by-product is generated after extraction. This method is easy to perform for the extraction of PHE and the cost is inexpensive as compared to other extraction methods. For future research, the size and shape of the membrane can be further minimized to maximize the nylon membrane usage. Increase of sample temperature causes an increase of analytes in headspace capacity which then increases the extraction rate and shortens the extraction time. Therefore, we suggest that the sample temperature can be decreased to lower than 50 °C to increase the analyte recovery with prolong extraction time.

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