บทความวิจัย

การสกัดระดับจุลภาคด้วยวัฏภาคของเหลวโดยใช้เมมเบรนชนิดเส้นใยกลวง เพื่อวิเคราะห์ปริมาณสารกลุ่มพอลิไซคลิกอะโรมาติกไฮโดรคาร์บอนในตัวอย่างน้ำชา

วิชุดา ทองภูสวรรค์ และ นภา ตั้งเตรียมจิตมั่น*

บทคัดย่อ

งานวิจัยนี้ได้พัฒนาวิธีการวิเคราะห์สารพอลิไซคลิกอะโรมาติกไฮโดรคาร์บอน (PAH) 9 ชนิด ในน้ำชาโดยใช้การสกัดระดับจุลภาคด้วยวัฏภาคของเหลวและใช้เมมเบรนชนิดเส้นใยกลวงเป็นตัวพยุง แล้วนำไปวิเคราะห์ด้วยเครื่องแก๊สโครมาโทกราฟี-แมสสเปกโทรเมทรี โดยเมมเบรนชนิดเส้นใยกลวงที่ใช้ทำจากพอลิพรอพิลีนที่มีรูพรุนภายในบรรจุตัวทำละลายอินทรีย์ 25 ไมโครลิตร ในการสกัดสารละลายตัวอย่าง 15 มิลลิลิตร จากการศึกษาสภาวะที่เหมาะสมพบว่าออกเทนเป็นตัวทำละลายที่เหมาะสมในการสกัดด้วยเมมเบรนชนิดเส้นใยกลวงนี้ โดยใช้อัตราเร็วในการปั่นกวนที่ 700 รอบต่อนาที เป็นเวลา 20 นาที สามารถวิเคราะห์ปริมาณ PAH ทั้ง 9 ชนิด ได้ขีดจำกัดการตรวจวัดในระดับนาโนกรัมต่อลิตร ค่าสัมประสิทธิ์ของกราฟมาตรฐาน (coefficient of determination, R^2) อยู่ในช่วง 0.9852-1 ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 15 เปอร์เซ็นต์ เมื่อนำวิธีสกัดนี้มาหาค่าร้อยละการได้กลับคืนของ PAH ทั้ง 9 ชนิดในตัวอย่างน้ำชาพบว่าอยู่ในช่วง 25-100 เปอร์เซ็นต์ จึงได้ใช้วิธีสแตนดาร์ดแอดดิชันในการวิเคราะห์หาปริมาณสารพอลิไซคลิก อะโรมาติกไฮโดรคาร์บอนในตัวอย่างน้ำชา 11 ตัวอย่าง พบว่ามีปริมาณของ PAH อยู่ในช่วงความเข้มข้น 5.0-199.8 นาโนกรัมต่อลิตร ซึ่งเป็นปริมาณที่ปลอดภัยตามมาตรฐาน USEPA ข้อดีของวิธีการสกัดนี้ คือ ใช้ปริมาณตัวทำละลายอินทรีย์น้อยในระดับไมโครลิตรจัดเป็นเคมีสะอาด (Green Chemistry) เป็นการเตรียมตัวอย่างที่ง่าย รวดเร็ว สามารถสกัดและเพิ่มความเข้มข้นได้ในขั้นตอนเดียว อุปกรณ์ไม่สลับซับซ้อนและราคาไม่สูง

คำสำคัญ: PAH พอลิพรอพิลีนเมมเบรน แก๊สโครมาโทกราฟี-แมสสเปกโทรเมทรี

ศูนย์ความเป็นเลิศด้านนวัตกรรมทางเคมี ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยบูรพา

*ผู้นิพนธ์ประสานงาน, e-mail: napa@buu.ac.th

Hollow Fiber Membrane Liquid-Phase Microextraction for Determination of Polycyclic Aromatic Hydrocarbons in Tea Samples

Wichuda Tongpoosawan and Napa Tangtreamjitmun*

ABSTRACT

The determination of nine polycyclic aromatic hydrocarbons (PAH) in tea samples has been developed using hollow fiber membrane liquid-phase microextraction and detected by gas chromatography with mass spectrometer. PAH were extracted from 15 mL of aqueous samples through the immobilized organic solvent in the pores of polypropylene hollow fiber membrane and finally into 25 μL of the same organic solvent. The optimum conditions were studied and found that octane was a good extraction solvent at the stirring rate of 700 rpm for 20 min. The detection limit of this method can be achieved at nanograms per litre level. The calibration curves with the coefficient of determination (R^2) in the range of 0.9852-1 were obtained. The relative standard deviations were below 15% and recoveries were in the range of 25-100%. Thus, a standard addition method has been applied to determine 9 PAH in 11 tea samples. The determined PAH in all tea samples were in a range of 5.0-199.8 nanograms per litre. All tea samples contained safe levels of PAH when compared to USEPA criteria. The advantage of using micro-litre volume of organic solvent for extraction leads to the extremely low consumption of toxic solvents. The method can thus be considered as a green chemistry initiative. In addition, the extraction and pre-concentration process are combined into a single step. Thus, the technique requires minimal sample preparation time.

Keywords: PAH, polypropylene membrane, gas chromatrography-mass spectrometry

Department of Chemistry and Centre for Innovation in Chemistry, Faculty of Science, Burapha University
*Corresponding author, e-mail: napa@buu.ac.th

Introduction

The history of tea began in China almost five thousand years ago. Nowadays billions of people around the world drink tea as a beverage regularly in everyday life. The health benefits of tea make it the most widely consumed beverage after water. However, some pollutants found in tea leaves may cause a health threat to tea drinkers. The main pollutants in tea leaves include metal ions [1] fluoride ions [2,3] pesticides [4,5] and PAH [6,7]. In a tropical climate country like Thailand, hot tea infusion is not consumed as much as iced tea. The first ready to drink iced tea was a black tea, which came in a can. Five years ago ice green tea in a bottle was produced and became very popular among Thai people. All types of tea have been made and marketed in a bottle or in a box with foil liners and sold throughout the country.

Polycyclic aromatic hydrocarbons (PAH) are chemical species with two to six fused benzene rings. PAH are well known toxic pollutants and are highly potent carcinogens. The analysis of trace PAH in tea samples has been accomplished by two main chromatographic methods; liquid chromatography and gas chromatography. These methods require preliminary extraction for enrichment of the analytes. Traditionally, these processes are performed by solid-phase [8,9] and liquid-liquid extraction [6,7,10,11]. Liquid-liquid extraction is applicable to the determination of certain PAH which are partitioned into an organic solvent and analyzed by gas chromatography. This classical liquid-liquid extraction method was time-consuming and used a large amount of toxic organic solvent. Therefore, a novel method of liquid-phase microextraction (LPME) using a hollow fiber membrane was first introduced by Pederson-Bjergaard and Rasmussen [12]. A short piece of porous hollow fiber membrane was filled with solvent. This fiber was immersed in the aqueous sample and the extraction of PAH occurred by mass transfer of analyte between the aqueous sample and the solvent inside the fiber. The method was adapted to many application as reviewed by Pederson-Bjergaard and Rasmussen [13]. This study will investigate the applicability of hollow fiber liquid-phase microextraction for determination of PAH in tea infusions and bottled tea samples. The selected nine PAH for this work were acenaphthylene (ACY), acenaphthene (ACE), fluorene (FL), phenanthrene (PHEN), anthracene (AN), fluoranthene (FLUR), pyrene (PY), benzo[a]anthracene (BaA) and chrysene (CHRY). These 3-4 aromatic ring PAH were reported to be the predominant PAH found in tea infusions [6].

Materials and methods

1. Tea samples

Two types of tea samples; tea infusion samples and bottled tea beverage samples were analyzed in this work. Both tea samples were obtained from local supermarkets in Thailand. The tea infusions were prepared by weighing 20 grams of tea leaves into 500 mL hot water, covering the container with aluminum foil and heating it at 90-95°C for 60 min. The tea infusion was then decanted into a closed container and cooled to room temperature before analysis. Bottled tea beverage samples were sampled directly from the bottle without any preparation.

2. Standards and reagents

The 9 PAH standards were purchased from Supelco (Bellefonte, PA, USA). HPLC grade acetonitrile and octane were purchased from Merck (Darmstadt, Germany). Individual 200 mg/L PAH stock solutions; acenaphthylene (ACY), acenaphthene (ACE), fluorene (FL), phenanthrene (PHEN), anthracene (AN), fluoranthene (FLUR), pyrene (PY), benzo[a]anthracene (BaA) and chrysene (CHRY) were dissolved separately in acetonitrile. The individual or mixed standard working solutions of each PAH were prepared from these stock solutions by appropriately dilution with acetonitrile. All standards and working solution were stored at 4°C in amber glass bottles.

3. Hollow fiber LPME procedure

Hollow fiber polypropylene membrane (Accurel PP Q3/2 with 0.2 μm pore size, 600 μm internal diameter and 200 μm wall thickness) was purchased from Membrana GmbH, Wuppertal, Germany. It was cut into 5 cm. lengths and sonicated in acetone for 2-3 min to clean the membrane and left to dry before use. The membrane was then soaked in a solvent for 2-3 min to impregnate the solvent into the pores of membrane. Excess solvent in the hollow fiber was blown out with air using a syringe. The impregnated membrane was immediately connected to the extraction unit shown in Figure 1. The hollow fiber was used only once for each extraction and was discarded after use.

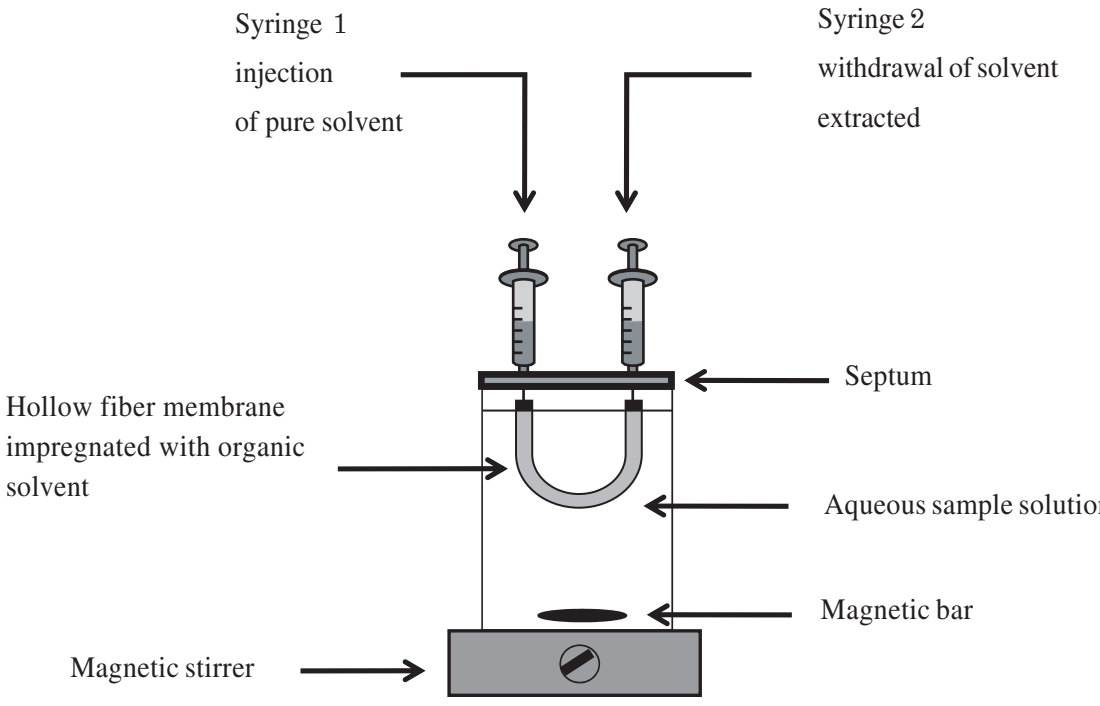

Figure 1 The unit used for PAH extraction by hollow fiber membrane.

The extraction unit was similar to that used by others [12]. The needle of two 100 μL micro syringes (Hamilton Bonaduz AG, Bonaduz, Switzerland) were pushed through a septum. One end of the fiber was attached to syringe 1 containing 25 μL of solvent. The other end of fiber was attached to syringe 2 to complete the fiber loop. The syringe 1 was raised about 1 cm. higher than syringe 2 and the solvent was injected into the fiber. 15 mL of sample was added into a 20 mL vial containing a small magnetic bar (2 × 7 mm). The septum was placed on top of the vial with the fiber loop immersed in the sample solution as in Figure 1. The extraction vial was then placed onto a magnetic stirrer. When the extraction was finished, the septum unit was removed from the vial. One end of the fiber was removed from the syringe 1. The extracted solvent in the membrane was withdrawn from the other end of membrane through the syringe 2 and was delivered into an insert vial for direct GC-MS analysis.

4. GC-MS Analysis

The GC-MS used in this work was a Hewlett-Packard 6890 GC system equipped with HP 5972 A MS and split/splitless autoinjector. PAH were separated on a fused silica HP-5 MS capillary column (30 m length × 0.25 mm internal diameter, 0.25 μm film thickness). Ultra pure helium (99.9995%) was used as carrier gas.

The GC conditions were optimized by a scan mode MS (m/z 40-400) for the simultaneous separation of the selected 9 PAH. The optimized conditions were as follows; 1 mL/min flow rate of helium carrier gas, 1 μL injection volume of a mixed 9 PAH standard with splitless mode at 30 second split delay and the injector temperature was maintained at 250°C. The temperature program was: 80°C (1 min) to 180°C at 20°C/min, to 240°C at 10°C/min, to 243°C (3 min) at 1°C/min. The total run time was 18 min. The identification of each PAH mass spectra was achieved by comparing the mass spectra at each peak to the data base of each standard spectra in a NIST library (National Institute of Standards and Technology). For quantification of the PAH in samples, the selected ion monitoring mode MS (SIM mode) was performed using the quantification ion and confirmation ion as reported by Rodil and coworkers [14].

Results and discussion

1. PAH extraction by hollow fiber LPME

There were four parameters to be optimized for hollow fiber LPME analysis of PAH; type of solvent, stirring rate, stirring time and salt effect. This 5 mL of a mixed standard working solution (1 mg/L of each PAH) was diluted to 50 mL with deionized water in a volumetric flask to make a 100 μg/L per PAH spiked water sample. This spiked water sample was used to perform optimization for all parameters in the extraction except for the salt effect. Three replicate trials were performed in each variation for every parameter.

1.1 Type of solvent

The PAH were all non-polar compounds, thus non-polar solvents should be used for extraction. Octane and toluene were claimed to be suitable solvents used for PAH extraction using hollow fiber membrane [15-17]. Thus, octane and toluene were compared for the extraction of 9 PAH from the spiked water samples preparing in the laboratory. The stirring rate was fixed at 1000 rpm and the extraction time was 15 min [15]. The extraction efficiency was reported in terms of peak area of each PAH as given in Figure 2. Octane had almost 50% higher peak area than toluene of all extracted PAH. The solubility of toluene in water is 0.47 g/L but octane is much less soluble in water. Therefore some toluene in the hollow fiber was lost into the water sample resulting in a lower peak area of extracted PAH. As a result, Octane was selected as the extraction solvent in this work.

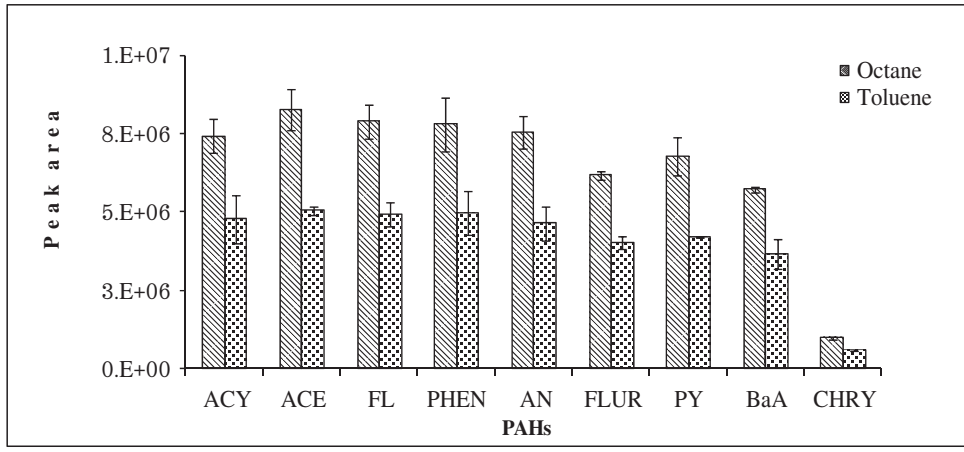

Figure 2 Peak areas of each PAH standard with two different extraction solvents (n=3).

1.2 Stirring rate

The stirring rate was varied from 0, 300, 500, 700, 1000 and 1200 rpm using a 15 min extraction time. Figure 3 showed that a 700 rpm produced the highest peak area. This is due to the fact that initially greater stirring helps enhance mass transfer but after 700 rpm the peak area decreased as the shear was too high resulting in solvent loss through the pores of the membrane into the water sample. Furthermore, a high stirring rate could produce bubbles which reduce the interface area between the membrane and the water sample thus obstructing extraction. On the basis of the results obtained, a stirring rate of 700 rpm was used for the extraction.

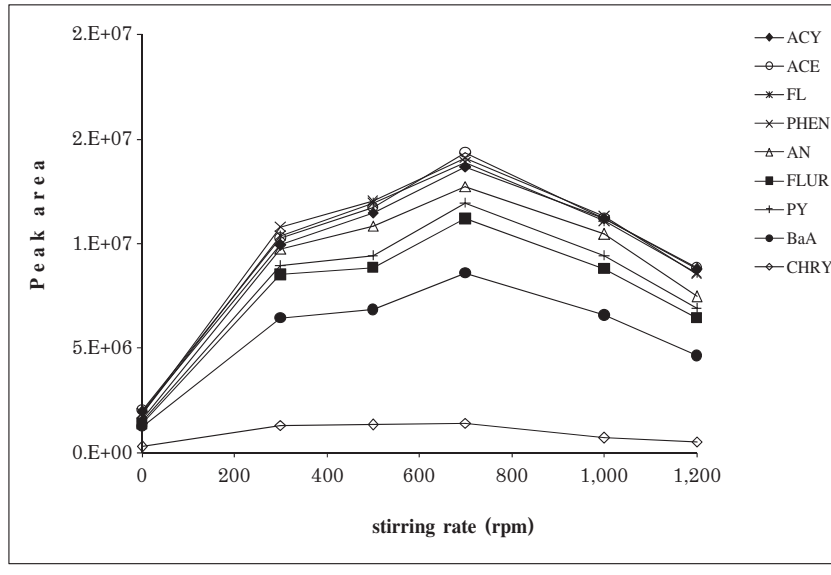

Figure 3 Peak areas of each PAH standard with different stirring rate (n=3).

1.3 Extraction time

The efficiency of extraction was evaluated using different extraction times (stirring time) of 5, 10, 15, 20, 30 and 50 min. As can be seen in Figure 4, the higher the extraction time, the higher the peak area of extracted PAH. The extraction process involves steady state mass transfer between the aqueous sample phase and the organic solvent phase [13]. The results showed that it would take much more than 50 min to reach the equilibrium for this extraction. In quantitative analysis, equilibrium does not need to be reached as it is enough just to allow sufficient mass transfer into the solvent in the hollow fiber membrane as long as an exact reproducible extraction time is used. Thus, 20 min extraction time was chosen in combination with the GC-MS analysis procedure (18 min for one injection) in order to obtain the required sensitivity. The optimized conditions for extraction, 20 min extraction time at 700 rpm stirring rate were used throughout this analysis with octane as the extraction solvent.

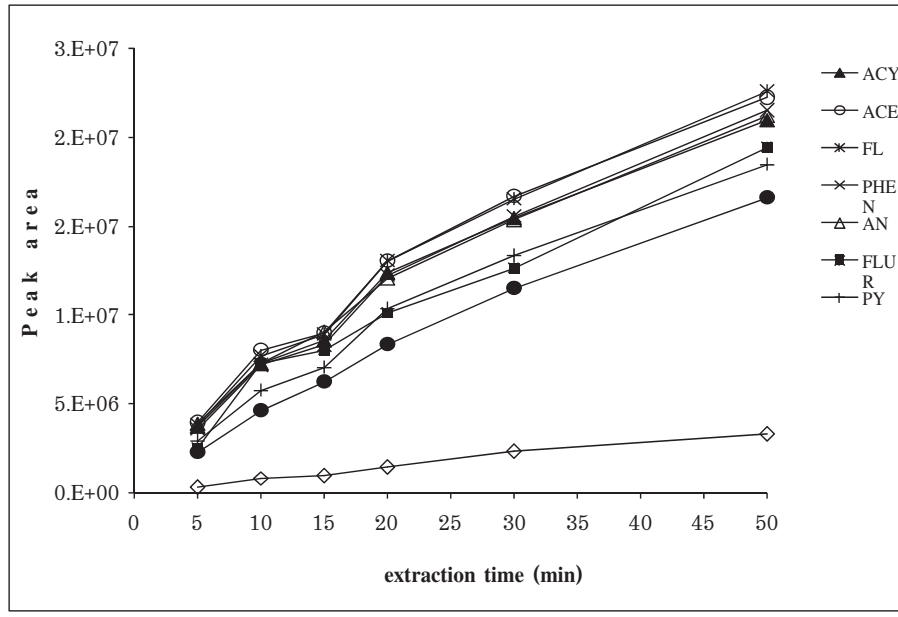

Figure 4 Peak areas of each PAH standard with different extraction time (n=3)

1.4 Salt effect

There have been different trends reported for the effect of addition of salt to aqueous samples for extraction with hollow fiber LPME. The effect seems to depend on the polarity of the analyte. For decreasing polarity, extraction usually increases when salt added. Some polar analytes such as nitroaromatics or organochlorine pesticides (OCPs) were reported to have a negative effect from salt addition [5,18]. However, 20% of salt was needed for the efficient extraction of aromatic amines in water samples [19] and 30% of salt was needed for extraction of OCPs [16]. Nonpolar compounds were also reported to have a negative effect from salt addition [20]. Thus the effect of salt in this extraction was studied using 100 µg/L per PAH spiked water samples with different amounts of sodium chloride added. The concentration of sodium chloride solution was 0, 5, 10, 15, 20 and 30 (%w/v). The effect of sodium chloride addition is shown in Figure 5. It appears that the presence of salt decreases the peak area of extracted PAH often by more than a factor of 2. So, it was decided not to add any salt in extraction of further experiments.

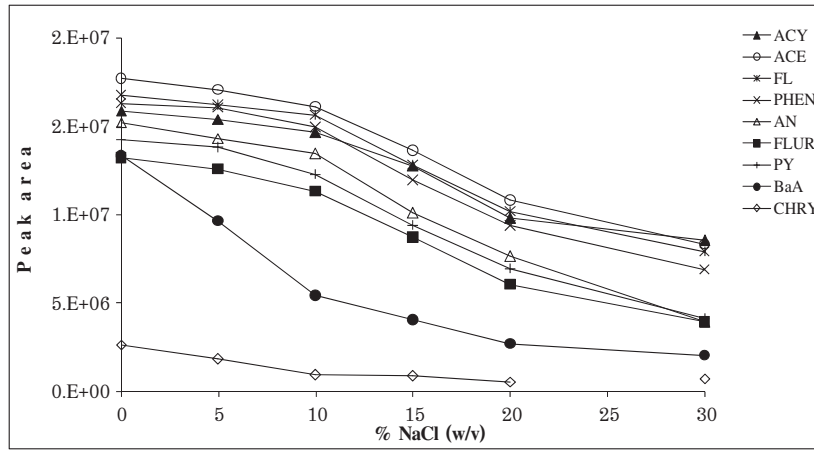

Figure 5 Peak areas of each PAH standard with different sodium chloride concentrations (n=3)

2. Analytical figures of merit

Each 15 mL of mixed PAH standard was extracted using the procedure described in section 3 (in Materials and methods) with the optimized parameters; octane as a solvent, stirring rate of 700 rpm and 20 min extraction time without salt addition. The extracted solvent was followed by GC-MS analysis as in section 4 (in Materials and methods). A calibration curve was obtained from triplicate injections of each extracted PAH standard in the concentration range 10-2000 ng/L. The linear regression equation and coefficient of determination for each PAH calibration curve is shown in Table 1.

Table 1 A linear regression equation, coefficient of determination (R^2), LOD (S/N = 3) and LOQ (S/N = 10) of each PAH.

PAH	A linear regression equation	R^2	LOD (ng/L)	LOQ (ng/L)
ACY	y = 57.629x + 178.27	0.9985	6.0	20.0
ACE	y = 69.266x + 675.00	0.9955	5.5	15.5
FL	y = 60.243x + 802.97	0.9852	4.5	14.5
PHEN	y = 34.375x + 1643.00	0.9921	3.0	10.0
AN	y = 29.951x + 215.67	1.0000	4.0	9.0
FLUR	y = 34.728x + 531.27	0.9978	3.0	10.0
PY	y = 40.109x + 570.06	0.9898	4.0	13.5
BaA	y = 133.600x - 774.06	0.9989	4.0	12.0
CHRY	y = 13.590x - 99.63	0.9923	30.0	90.0

The intra-day precision and inter-day precision were determined at three levels of concentrations of each PAH in water. Six replicates were performed for each PAH at each concentration for inter-day precision. One trial at each concentration was run on 6 consecutive days for inter-day precision. The results in Table 2 indicate a good precision of the method for both intra-day precision and inter-day precision according to the Association of Official Analytical Chemists International [21].

Table 2 The relative standard deviation (% RSD) of intra-day and inter-day precision (n=6)

PAH	% RSD					
	25 ng/L		60 ng/L		90 ng/L	
	intra-day	inter-day	intra-day	inter-day	intra-day	inter-day
ACY	6	5	6	9	12	10
ACE	5	5	5	9	12	13
FL	7	7	5	8	7	9
PHEN	10	8	11	13	8	10
AN	3	12	15	10	11	10
FLUR	13	14	13	11	14	10
PY	2	4	5	8	4	8
BaA	6	8	10	7	7	3
CHRY*	14	12	8	11	12	10

Note: *concentrations of CHRY were 125, 250 and 475 ng/L

3. Analysis of PAH in tea samples

Matrix interferences in tea sample analysis with this procedure were studied first by recovery experiments. One leaf tea sample and one bottled tea beverage sample were selected for this recovery experiment. The spiked samples were extracted using the optimized parameters. Three replicate extractions were performed and followed by GC-MS analysis. The recovery for the tea infusion sample and tea beverage sample at each concentration level of each PAH is shown in Table 3.

Table 3 The recovery determined from a tea infusion sample and a tea beverage sample at three concentration levels of each PAH (n=3).

PAH	Recovery from tea samples (%)					
	25 ng/L		60 ng/L		90 ng/L	
	Infusion	Beverage	Infusion	Beverage	Infusion	Beverage
ACY	79	56	83	66	80	69
ACE	53	61	67	71	71	77
FL	75	85	71	71	66	69
PHEN	44	29	49	57	52	64
AN	29	38	47	58	53	61
FLUR	100	37	59	55	50	54
PY	67	47	41	47	35	46
BaA	79	53	50	37	44	35
CHRY*	45	40	31	37	25	33

Note: *Spiked concentrations at 125, 250 and 475 ng/L

The results show a wide range of recoveries from 25-100% indicating a negative interference of the tea sample matrixes in this procedure. The matrixes of the tea samples contain some components with large molecule structure similar in some ways to PAH. Polyphenol such as catechin, theaflavin and other large structures of pectin, flavones and polysaccharide are examples of those components in tea matrix [22,23]. It is possible these natural compounds can form association complexes with PAH molecules which could reduce the extraction from water by various amounts. This is analogous to the known solubilisation of nonpolar molecules by humic substances in organismic natural waters [24,25]. Therefore, a method of standard addition was used for PAH analysis from the tea samples.

Table 4 reports the PAH concentrations (ng/L) obtained in two tea infusion samples and nine bottled tea beverage samples. Acenaphthylene (ACY) and acenaphthene (ACE) were not detected in any of the tea samples, which included several brands. There were not any PAH detected in two of the green tea beverage samples (2 and 5). The 2011 edition of the Drinking water standards and health advisories by USEPA stated the maximum contaminated level (MCL) of benzo(a)pyrene (the most toxic PAH) as 200 ng/L. The MCL of nine PAH in this work can be calculated from the ratio of 200 ng/L to toxic equivalent factors of each

PAH purposed by others [26]. The calculated MCL of benzo[a]anthracene (BaA) and anthracene (AN) is 2 and 20 μg/L, respectively. The calculated MCL of the other seven PAH is 200 μg/L each. Therefore, the amount of PAH found in all tea infusion and tea beverage samples were under the maximum contaminated level.

Table 4 PAH concentration (ng/L) found in tea infusion and bottled tea beverage samples (n=3)

Tea samples	PAH (ng/L)								
	ACY	**ACE**	**FL**	**PHEN**	**AN**	**FLUR**	**PY**	**BaA**	**CHRY**
Green tea infusion	ND	ND	30.1	177.0	ND	99.7	104.2	18.0	154.7
Oolong tea infusion	ND	ND	30.8	187.9	26.7	129.8	124.0	5.0	51.5
Green tea beverage 1	ND	ND	23.1	ND	ND	70.1	42.7	17.3	199.8
Green tea beverage 2	ND	ND	ND	ND	ND	ND	ND	ND	ND
Green tea beverage 3	ND	ND	59.6	72.0	ND	20.1	24.2	110.0	38.2
Green tea beverage 4	ND	ND	29.2	38.7	ND	5.0	10.7	ND	ND
Green tea beverage 5	ND	ND	ND	ND	ND	ND	ND	ND	ND
Black tea beverage 1	ND	ND	33.6	49.9	ND	16.8	31.5	ND	ND
Black tea beverage 2	ND	ND	31.7	38.1	ND	6.2	13.3	ND	ND
Oolong tea beverage 1	ND	ND	21.9	36.1	ND	4.3	9.1	ND	ND
Oolong tea beverage 2	ND	ND	51.0	60.1	ND	14.9	19.6	ND	ND

ND = not detected

Conclusions

Hollow fiber LPME can be used to extract PAH from tea infusion and bottled tea beverage samples. Even though, the extraction is interfered with some tea matrixes, a standard addition can be performed for quantitation of PAH in tea samples to overcome problem of matrix interferences. The method is rapid and low cost compared to solid-phase extraction or liquid-liquid extraction procedures. The method performs a combination of extraction and pre-concentration in one step. The total PAH contained in bottled tea beverage is less than the tea infusion samples. However, both types of tea samples are safe to drink.

Acknowledgements

Financial support from The National Research Council of Thailand. A Grant from the Faculty of Science, Burapha University and Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, are gratefully acknowledged.

References

1. Mierzwa, J., Sun, Y. C., Chung, Y. T., and Yang, M. H. 1998. Comparative Determination of Ba, Cu, Fe, Pb and Zn in Tea Leaves by Slurry Sampling Electrothermal Atomic Absorption and Liquid Sampling Inductively Coupled Plasma Atomic Emission Spectrometry. *Talanta* 47: 1263-1270.
2. Wong, M. H., Fung, K. F., and Carr, H. P. 2003. Aluminium and Fluoride Contents of Tea, with Emphasis on Brick Tea and Their Health Implications. *Toxicology Letter* 137: 111-120.
3. Cao, J., Zhao, Y., Liu, J. W., Xirao, R., Danzeng, S., Daji, D., and Yan, Y. 2003. Brick Tea Fluoride as a Main Source of Adult Fluorosis. *Food Chemistry Toxicology* 41: 535-542.
4. Cai, L. S., Xing, J., Dong, L., and Wu, C. 2003. Application of Polyphenylmethylsiloxane Coated fiber for Solid-Phase Microextraction Combined with Microwave-Assisted Extraction for the Determination of Organochlorine Pesticides in Chinese Teas. *Journal of Chromatography A* 1015: 11-21.
5. Huang, S., -P. and Huang, S., -D. 2006. Dynamic Hollow Fiber Protected LiquidPhase Microextraction and Quantification Using Gas Chromatography Combined with Electron Capture Detection of Organochlorine Pesticides in Green Tea Leaves and Ready-to-Drink Tea. *Journal of Chromatography A* 1135(1): 6-11.
6. Lin, D., Tu, Y., and Zhu, L. 2005. Concentrations and Health Risk of Polycyclic Aromatic Hydrocarbons in Tea. *Food and Chemical Toxicology* 43: 41-48.
7. Fiedler, H., Cheung, C. K., and Wong, M. H. 2002. PCDD/PCDF, Chlorinated Pesticides and PAH in Chinese Teas. *Chemosphere* 46: 1429-1433.
8. Wei, M. C. and Jen, J. F. 2007. Determination of Polycyclic Aromatic Hydrocarbons in Aqueous Samples by Microwave Assisted Headspace Solid-Phase Microextraction and Gas Chromatography Flame Ionization Detection. *Talanta* 72: 1269-1274.
9. Vinas, P., Campillo, N., Aguinaga, N., Perez-Canovas, E., and Hermandez-Cordoba, M. 2007. Use of Headspace Solid-Phase Microextraction Coupled to Liquid Chromatography for the Analysis of Polycyclic Aromatic Hydrocarbons in Tea Infusions. *Journal of Chromatography A* 1164: 10-17.

10. Dugay, A., Herrenknecht, C., Czok, M., Guyon, F., and Pages, N. 2002. New Procedure for Selective Extraction of Polycyclic Aromatic Hydrocarbons in Plants for Gas Chromagraphic-Mass Spectrometric Analysis. *Journal of Chromatography A* 958: 1-7.
11. Ratola, N., Lacorte, S., Alves, A., and Barcelo, D. 2006. Analysis of Polycyclic Aromatic Hydrocarbons in Pine Needles by Gas Chromatography-Mass Spectrometry Comparison of Different Extraction and Clean-up Procedures. *Journal of Chromatography A* 1114: 198-204.
12. Pedersen-Bjergaard, S., and Rasmussen, K. E. 1999. Liquid-Liquid-Liquid Microextraction for Sample Preparation of Biological Fluids Prior to Capillary Electrophoresis. *Analytical Chemistry* 71(14): 2650-2656.
13. Pedersen-Bjergaard, S. and Rasmussen, K. E. 2008. Liquid-Phase Microextraction with Porous Hollow Fibers, a Miniaturized and Highly Flexible Format for Liquid-Liquid Extraction. *Journal of Chromatography A* 1184: 132-142.
14. Rodil, R., Schellin, M., and Popp, P. 2007. Analysis of Polycyclic Aromatic Hydrocarbons in Water and Beverages Using Membrane-Assisted Solvent Extraction in Combination with Large Volume Injection-Gas Chromatography-Mass Spectrometric Detection. *Journal of Chromatography A* 1163(1-2): 288-297.
15. Charalabaki, M., Psillakis, E., Mantzavinos, D., and Kalogerakis, N. 2005. Analysis of Polycyclic Aromatic Hydrocarbons in Waste Water Treatment Plant Effluents Using Hollow Fibre Liquid-Phase Microextraction. *Chemosphere* 60: 690-698.
16. Basheer, C., Balasubramanian, R., and Lee, H. K. 2003. Determination of Organic Micropollutants in Rainwater Using Hollow Fiber Membrane/Liquid-Phase Microextraction Combined with Gas Chromatography-Mass Spectrometry. *Journal of Chromatography A* 1016(1): 11-20.
17. King, S., Meyer, J. S., and Andrews, A. R. J. 2002. Screening Method for Polycyclic Aromatic Hydrocarbons in Soil Using Hollow Fiber Membrane Solvent Microextraction. *Journal of Chromatography A* 982(2): 201-208.
18. Psillakis, E., Mantzavinos, D., and Kalogerakis, N. 2004. Development of a Hollow Fibre Liquid Phase Microextraction Method to Monitor the Sonochemical Degradation of Explosives in Water. *Analytica Chimica Acta* 501(1): 3-10.
19. Zhao, L., Zhu, L., and Lee, H. K. 2002. Analysis of Aromatic Amines in Water Samples by Liquid-Liquid-Liquid Microextraction with Hollow Fibers and High-Performance Liquid Chromatography. *Journal of Chromatography A* 963(1-2): 239-248.

20. Sarafraz-Yazdi, A., Amiri, A. H., and Es'haghi, Z. 2008. BTEX Determination in Water Matrices Using HF-LPME with Gas Chromatography-Flame Ionization Detector. *Chemosphere* 71(4): 671-676.
21. AOAC. 1993. AOAC Peer-Verfied Methods Program. *Association Official Analytical Chemists International:* 7-10.
22. Owuor, P. O. and Obanda, M. 2007. The Use of Green Tea *(Camellia sinensis)* Leaf Flavan-3-ol Composition in Predicting Plain Black Tea Quality Potential. *Food Chemistry* 100(3): 873-884.
23. Yin, J.,-F., Xu, Y.,-Q., Yuan, H.,-B., Luo, L.,-X., and Qian, X.,-J. 2009. Cream Formation and Main Chemical Components of Green Tea Infusions Processed from Different Parts of New Shoots. *Food Chemistry* 114(2): 665-670.
24. Beckett, R. 1990. The Surface Chemistry of Humic Substances in Aquatic Systems. In: Surface and Colloid Chemistry of Natural Waters and Water Treatment. Beckett, R., Editor. p. 3-20. New York. Plenum Press.
25. Chiou, C. T., Malcolm, R. L., Brinton, T. I., and Kile, D. E. 1986. Water Solubility Enhancement of Some Organic Pollutants and Pesticides by Dissolved Humic and Fulvic Acids. *Environment Science and Technology* 20: 502-508.
26. Nisbet, I. C. T. and LaGoy, P. K. 1992. Toxic Equivalency Factors (TEFs) for Polycyclic Aromatic Hydrocarbons (PAHs). *Regulatory Toxicology and Pharmacology* 16(3): 290-300.

ได้รับบทความวันที่ 11 มกราคม 2555
ยอมรับตีพิมพ์วันที่ 20 กุมภาพันธ์ 2555