Ultrasonication Assisted Dispersive Liquid-liquid Micro Extraction (USA-DLLME) for Determination of Pyrene Concentration in Water

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Abstract. Pyrene is one of the chemical compounds of polycyclic aromatic hydrocarbons (PAHs) that has been reported as toxic, carcinogenic, and mutagenic which commonly found in water, food, soil, and air. Therefore, a study of ultrasonic assisted dispersive liquid-liquid microextraction (USA-DLLME) was conducted to extract pyrene from polluted water. The analytical parameters such as volume of extractant solvent, volume of disperser solvent, sonication time, and centrifugation speed were optimized using response surface method (RSM). Gas chromatography-flame ionization detector was used to analyse the pyrene content. The extraction recovery obtained for validation on real water based on optimization are in the range of 14.8%-49.8%. The low value of extraction recovery must be due to unsuitable types of extraction solvent used. However, this study have achieved all the objectives based on the developed method.

1. Introduction

Polycyclic aromatic hydrocarbon (PAHs) is a compound produced from incomplete burning activity of an organic materials. In 1997, US Environmental Protection Agency has discovered 16 PAHs compound which cause pollution to environment because of its characteristics that are toxic, carcinogenic, and mutagenic. Pyrene is one of the PAHs group found. It is used in dyes, plastics manufacturing, and pesticides. Pyrene is yellowish solid crystal which is immiscible in water. Pyrene is not used commercially but it is commonly found because it is formed from incomplete burning. Therefore, human can be exposed to pyrene through inhalation, food, drinking water, and soil [1]. Minnesota Department of Health (MDH) reported that pyrene has been detected in drinking water from their water surface sources but not exceed the guideline level limit set by MDH which is 0.05 ppm. Figure 1 shows an illustration of pyrene structure [2].
Figure 1. Molecular structure of pyrene

According to [3], PAHs contain in three fish species from Pulau Perhentian is in the range of 17.89 – 42.18 ng/g for wet weight and 393.98 – 511.07 ng/g based on lipid weight. This PAHs content include phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)pyrene, benzo(a)anthracene, chrysene, benzo(b)flouranthene, benzo(k)fluoranthene and dibenzo(a,h)anthracene. But, this value is still in the safe limit set up by USEPA [3,1]. However, this value can be increased since fish have the potential to store the pollutant through gill, skin and digestive system [5]. Based on the study, we know that our water also contain PAHs pollutant. Hence, it is important to develop an extraction method to determine PAHs content in water sample.

There are conventional methods used for extraction such as liquid-liquid extraction (LLE) and solid phase extraction (SPE). But, this method required long procedures and consumed large amount of hazardous organic solvent such as xylene and chlorinated solvents. Then, the researcher has improve the conventional technique by developing micro extraction method to reduce the amount of organic solvent used and higher enrichment factor also can be achieved. However, it is needed more time for extraction process and still use hazardous organic chemical as solvent. Therefore, we conduct this study to overcome these weaknesses by adding ultrasonication system in the process to reduce the time needed as well as to increase the efficiency of extraction. The parameter that be measured in this study are volume of extractant solvent, volume of disperser, sonication time and centrifugation speed. The optimum condition of the experimental parameter were optimized using response surface method (RSM) in Design Expert v10.

2. Methodology

2.1. Reagent and Standard
Standard pyrene (99.9%) which was purchased from Sigma Aldrich is used for standard. The stock solution at a concentration of 1000 mg/L was prepared in acetonitrile (ACN) analysis grade purchased from Merck, Jerman. N-phenyl-bis(trifluoromethanesulfonimide) is used as the extraction solvent in this study. Disperser solvent used is dichloromethane (DCM) was purchased from Merck, Jerman. Water samples were collected from river water and lake in Bandar Baru Bangi, Selangor for validating the proposed method. River water was collected from Sungai Langat, Sungai Kuyoh, Sungai Kantan, Sungai Ramal dan Sungai Buah. Water samples at lake was taken from Tasik Seri Serdang, Tasik Fakulti Kejuruteraan dan Alam Bina (UKM), Tasik Bandar Tun Hussien Onn, Tasik Cempaka dan Tasik Idaman.

2.2. Instrumentation
The extraction and quantification of pyrene was carried out using gas chromatography with a flame ionization detector (GC-FID) system. A column type ZB-1MSZB-1MS (30 m x 320 µm x 1 µm) is used. 1µL of sample was injected to the injection pore and each sample was analyzed for about 42 minutes. The temperature was set at 100 oC for the first 5 minutes and then increased by 10 oC/minutes until 200 oC then 5 oC/minutes until 310 oC for 5 minutes. The ultrasonication system model type 19H Ney brand
was used for ultrasonication process. A Sigma 2-16 centrifuge model was purchased from Sartorius from Gottingen, Germany.

2.3. Preparation of Calibration Curve

The preparation of calibration curves by dilution of 1000 ppm stock solution to 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm. The DLLME method was performed in the preparation of calibration curve at different concentration of stock solution. These prepared solution will be filtered into vial before being analyzed using GC-FID.

2.4. Ultrasonication Assisted Dispersive Liquid-liquid Micro Extraction Procedure

1.5mL aqueous sample solution containing pyrene is filled in 15ml centrifuge tube. Dichloromethane and n-phenyl-bis(trifluoromethanesulfonimide) were added to the centrifuge tube. The solution is then sonicated for 5 minutes until the solution turns cloudy. The solution was then centrifuged at a speed of 5000 rpm for 5 minutes. The aqueous solution will be removed while the residue was dissolved in 2 mL ACN. The samples were filtered with 0.45 µm nylon membrane into vial before injected to gas chromatography-flame ionization detector for analysis. Error! Reference source not found. shows the illustration of the dispersive liquid-liquid micro extraction method.

Figure 2. Dispersive liquid-liquid micro extraction method of optimization parameters

Optimization of parameter was carried out before validation on the real water samples. In this study, the optimization was carried out using response surface method (RSM) in Design Expert v10 software based on central composite design (CCD). Table 1 shows the parameters and level used based on micro extraction of pyrene. Percentage of extraction recovery (ER) is set to be the evaluating response for optimization. The formula for extraction recovery is shown as follows:

\[ ER = \left( \frac{C_s}{C_0} \times \frac{V_s}{V_0} \right) \times 100\% = \left( EF \times \frac{V_s}{V_0} \right) \times 100\% \]

Cs and C0 indicates the concentration of the sediment and initial concentration of analyte respectively. Vs express the volume of sediment while V0 express the concentration of aqueous phase.

Table 1. Value of low level and high level set in RSM for each parameters

| Code | Factor               | Unit      | Low level | High level |
|------|----------------------|-----------|-----------|------------|
| A    | Volume of extractant | microliter| 600       | 1000       |
| B    | Volume of disperser  | microliter| 600       | 1000       |
| C    | Sonication time      | minutes   | 1         | 5          |
| D    | Centrifuge speed     | rpm       | 3000      | 5000       |
3. Result and Discussion

3.1. Optimization of Parameter

In order to achieve an optimum condition to be applied during validation on real water samples, the optimization of the most influential parameters such as volume of extractant, volume of disperser, sonication time and centrifuge speed were carried out using response surface method (RSM).

The ANOVA results were analyzed with linear model (suggested) Equation (1) to illustrate the plot function of desirability between two parameters. According to the analysis of variance, it found that parameter volume of disperser is significant because the P-value obtained is 0.0007 which is at 95% confidence level. The other parameters are not significant because the P-value obtained are slightly higher which is below 95% confidence level [6]. Summary of variance analysis shown in Table 3.

In order to display the interaction influence of each pair of design variables on the required responses, 3-D response surfaces contour plots are applied to describe the regression response surface model (RSM) [7,8]. The four response surfaces included in each pair of each variable shown in Figure 3. Volume of extractant and volume of disperser measured at 600 μL – 1000 μL. According to graph in figure 3, it showed that percentage of extractant less than 50% when 600 μL is used and more than 100% when the volume used up to 1000 μL. Figure 3(c) shows when parameter applied at minimum and maximum volume of solvent, volume of extractant is less than 50% and more than 50% respectively. Thus, percentage of extractant increase uniformly with an increase of extractant solvent and shows that this parameter is significant.

Table 2 presents the factorial design and analytical responses predict by RSM for optimization using analysis of variance (ANOVA) to explore the significance of the parameters.

Figure 3. 3D plot for response surface obtained based on the desirability between parameters a) volume of disperser vs volume of extractant, b) sonication time vs volume of extractant, c) sonication time vs volume of disperser, d) centrifuge speed vs sonication time

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| Table 2. Factorial design and analytical responses predict by RSM |
|-----------------------------------------------|
| Run | Volume of extractant (microliter) | Volume of disperser (microliter) | Sonication time (minutes) | Centrifuge speed (rpm) | Extraction recovery (%) |
|-----|-----------------------------------|-----------------------------------|--------------------------|------------------------|------------------------|
| 1   | 600                               | 1000                              | 5                        | 5000                   | 106.28                 |
| 2   | 600                               | 1000                              | 1                        | 5000                   | 123.67                 |
| 3   | 800                               | 800                               | 3                        | 3000                   | 145.47                 |
| 4   | 800                               | 800                               | 3                        | 4000                   | 124.76                 |
| 5   | 600                               | 600                               | 5                        | 5000                   | 48.52                  |
| 6   | 1000                              | 600                               | 5                        | 3000                   | 58.17                  |
| 7   | 800                               | 800                               | 3                        | 4000                   | 59.98                  |
| 8   | 600                               | 600                               | 1                        | 3000                   | 33.16                  |
| 9   | 1000                              | 1000                              | 1                        | 3000                   | 87.64                  |
| 10  | 800                               | 800                               | 3                        | 5000                   | 52.65                  |
| 11  | 800                               | 800                               | 3                        | 4000                   | 47.18                  |
| 12  | 800                               | 800                               | 1                        | 4000                   | 70.97                  |
| 13  | 800                               | 800                               | 3                        | 4000                   | 59.00                  |
| 14  | 1000                              | 1000                              | 1                        | 5000                   | 129.39                 |
| 15  | 600                               | 800                               | 3                        | 4000                   | 37.68                  |
| 16  | 800                               | 1000                              | 3                        | 4000                   | 79.84                  |
| 17  | 1000                              | 600                               | 1                        | 3000                   | 38.83                  |
| 18  | 1000                              | 800                               | 3                        | 4000                   | 100.87                 |
| 19  | 600                               | 1000                              | 1                        | 3000                   | 33.76                  |
| 20  | 600                               | 600                               | 5                        | 3000                   | 40.97                  |
| 21  | 1000                              | 1000                              | 5                        | 3000                   | 132.33                 |
| 22  | 1000                              | 600                               | 5                        | 5000                   | 85.25                  |
| 23  | 1000                              | 1000                              | 5                        | 5000                   | 90.19                  |
| 24  | 800                               | 600                               | 3                        | 4000                   | 43.19                  |
| 25  | 800                               | 800                               | 3                        | 4000                   | 56.25                  |
| 26  | 600                               | 1000                              | 5                        | 3000                   | 88.70                  |
| 27  | 600                               | 600                               | 1                        | 5000                   | 29.14                  |
| 28  | 800                               | 800                               | 5                        | 4000                   | 51.87                  |
| 29  | 800                               | 800                               | 3                        | 4000                   | 57.18                  |
| 30  | 1000                              | 600                               | 1                        | 5000                   | 45.93                  |
Table 3. Summary of variance analysis

| Source     | Degree of freedom | Sum of squares | Mean square (variance) | F value | P-value (α=0.05) | P-value |
|------------|-------------------|----------------|------------------------|---------|-----------------|---------|
| Model      | 14857.71          | 4              | 3714.43                | 5.02    | 0.0041          | Significant |
| A          | 2855.61           | 1              | 2855.61                | 3.86    | 0.0607          |         |
| B          | 11182.23          | 1              | 11182.23               | 15.10   | 0.0007          |         |
| C          | 669.68            | 1              | 669.68                 | 0.90    | 0.3507          |         |
| D          | 150.19            | 1              | 150.19                 | 0.20    | 0.6563          |         |
| Residual   | 18507.55          | 25             | 740.30                 |         |                 |         |
| lack of fit| 14453.62          | 20             | 722.68                 | 0.89    | 0.6194          | Unsignificant |
| Pure error | 4053.93           | 5              | 810.79                 |         |                 |         |

ER = 71.96 + 12.60A + 24.92B + 6.10C + 2.89D  \hspace{1cm} (1)

4. Conclusion
In this study, the USA-DLLME method has been successfully performed to extract pyrene from water. However, further improvement need to be considered to increase the efficiency of this approach in order to increase the value of extraction recovery.

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References
[1] Siddens L K, Larkin A, Krueger S K, Bradfield C A, Waters K M, Tilton S C, Pereira C B, Löffler C V, Arlt V M, Phillips D H, Williams D E and Baird W M 2012 Appl. Pharmacol. 264(3) 377–386
[2] Goeden H 2018 Int. J. Environ. Res. Public Health 15(3)
[3] Tien S K, Heng L Y, Ghaffar M A, Zakaria M P and Surif S 2010 Sains Malaysiana 39(2) 219–226
[4] Nasher E, Heng L Y, Zakaria Z and Surif S 2016 Environ. Forensics 17(1) 97–106
[5] Ramachandran S D, Sweezy M J, Hodson P V, Boudreau M, Courtenay S C, Lee K, King T and Dixon J A 2006 Mar. Pollut. Bull. 52(10) 1182–1189
[6] Sanusi S N A, Halmi M I E, Abdullah S R S, Hassan H A, Hamzah F M and Idris M 2016 Ecol. Eng. 97 524–534
[7] Chen J S, Lee C M and Crap C 1993 58(3) 535–538
[8] Han H, Yu R, Li B and Zhang Y 2019 Appl. Therm. Eng. 159 p. 113731