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Optimisation and Validation of a Headspace Solid-Phase Micro Extraction GCMS using Central Composite Design for Determination of Polycyclic Aromatic Hydrocarbons in Water Samples

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A headspace solid phase microextraction (HS-SPME) method coupled with gas chromatography-mass spectrometry (GC-MS) was developed for the determination of selected polyaromatic hydrocarbons (PAHs) in water samples. Two extraction parameters i.e. extraction time and temperature were investigated using central composite design (CCD) of response surface methodology. The regression line fitted well with the data with an r^2 value of 0.9048. The lack of fit test gives the highest value of the Sum of Squares of 1.786×10^{13} with a probability F value of 13.31, showing a significant quadratic model. Under the optimal condition, the method provided good linearity, with a concentration range of 2.0-10.0 mgL^{-1} with coefficients of determination, $r^2 \geq 0.9993$ and good limits of detection, which is (0.287-0.999 mgL^{-1}) and limits of quantification (0.958-1.21 mgL^{-1}). The results also showed good relative recoveries ranging from 60 to 103% with acceptable reproducibility (RSDs $\leq$ 0.67%, $n = 3$). The study's results revealed that the HS-SPME-GC-MS method is easy, feasible, and selective for the trace analysis of fluorene and phenanthrene in water samples.

Keywords: Headspace solid phase microextraction; response surface methodology; central composite design; polycyclic aromatic hydrocarbons; water samples

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Polycyclic aromatic hydrocarbons (PAHs) are organic compounds usually formed from incomplete combustion [1]. PAHs is a class of chemicals that occur naturally in coal, crude oil, and gasoline. They result from burning coal, oil, gas, wood, garbage, and tobacco. PAHs can bind to form small particles in the air. Therefore, cooking meat and other foods at high heat will form PAHs. PAHs found in significant concentrations in the marine environment are divided into two categories: pyrogenic and petrogenic. Pyrogenic PAHs are by-products of combustion and are predominantly emitted to the atmosphere via the burning of fossil fuels such as coal, petroleum, wood and biomass, and the natural sources of petrogenic PAHs are from oil seepages and erosion of petroliferous shales [2]. Petrogenic PAHs can be found in oil and some oil products [3].

Access to clean drinking water is a significant public health challenge linked to many health disorders [4]. A study found that treated drinking water, the source of our daily hydration, contains chemicals which significantly can affect our health, such as Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), which was found in very low concentration in samples of wastewater and drinking water [5]. Not only NSAIDs what is most horrifying is that carbon-based compounds, namely PAHs, decently known for their carcinogenic characteristic, are also found in other studies, and the main factor of the accumulation of PAHs in water sources is water pollution [6]. Carcinogenicity of several of these compounds in experimental marine animals was reported causing concern among the community. As two to eight conjugated ring systems, PAHs can have a range of substituents, such as alkyl, nitro, and amino groups, in their structure. In their ring system, nitrogen, sulphur, and oxygen atoms can also be in their ring system. As a pollutant, PAHs are wide-spread and, at high temperatures, can be formed through the combustion of carbonaceous materials. PAHs can also contaminate indoor air through indoor emission sources such as smoking, cooking, and candle and incense emissions. In addition, exposure to high levels of PAHs has been shown to produce immunosuppressive effects and can cause oxidative stress during its metabolism [7].

Due to their mutagenic and carcinogenic character, polycyclic aromatic hydrocarbons (PAHs) are well-known priority pollutants of the water. Different ways involving industrial and municipal

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activities or even natural processes are the sources of PAH's pollution of surface waters. PAHs have been found in many water environments, and maximum contaminant levels for these compounds have been established [8]. Specifically, fluorene is one of the 16 PAHs priority hazardous compounds for human health, according to the USEPA. Fluorene is used as an intermediary in industrial applications to produce dyes, drugs, plastics, resins and pesticides. It is also considered one of the main PAHs foods contaminants, identified at significant concentrations in various food products [9]. Fluorene and phenanthrene are also widely distributed in the aquatic environment and have been identified in surface water, tap water, wastewater, and dried lake sediments. It has also been identified in seafood collected from contaminated waters and smoked and charcoal-broiled foods [10].

The need for analysis methods that comply with green analytical goals, including miniaturisation and simplification of the entire analytical technique, has been a significant focus of recent sample preparations [11]. The analysis methods such as solid phase microextraction (SPME), solid phase extraction (SPE), liquid-liquid extraction (LLE), and solid bar sorptive extraction (SBSE) have been widely used in determining the composition of PAHs in water samples as well as other different kinds of samples. According to Dani and co-workers, in the determination of PAHs in water using headspace solid phase microextraction (HS-SPME), most of the PAHs were found in the leachates from contaminated soils showing a maximum global value of 0.0755 mgL^{-1} [12].

In sample preparation, optimisation is crucial to improve the method's efficiency in detecting and determining the concentration of PAHs. There is numerous research to determine PAHs conducted using one separate factor at a time in the optimisation [13]. PAH samples must be protected against oxidation and photoirradiation processes prior to PAH analysis because PAHs are light-sensitive [14]. As a result, minimising light exposure to the samples during matrix pre-treatment is strongly advised. A few aromatic rings of PAHs were found to be easily sublimated during the concentration processes during sample preparation to extract PAHs. As a result, concentration to dryness should be carefully monitored to minimise PAHs evaporation and losses of lower molecular weight PAHs. It is recommended that internal standards or surrogates be added to the samples before extraction to ensure precise and accurate quantification by analytical instruments [15]. However, it does not lead to a real optimum, lacking information on the interaction between the factors and many unnecessary experiments. Therefore, response surface methodology (RSM) based on central composite design (CCD) was applied to minimise the number of experiments, reduce cost and provide information on the interaction between the factors [16].

Due to environmental and economic concerns, miniaturisation has recently been a trend in the development of sample preparation processes. The most crucial aspect of the approaches is the reduction in the use of organic solvents, apart from being a quick, affordable, and simple method to use. Several extraction methods, such as liquid-iquid extraction (LLE), solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE), and solid-phase extraction (SPE), coupled with other instruments such as GC-MS, are widely used for the determination of aroma compounds such as PAHs [17].

Thus, in this study, HS-SPME has been applied using GC-MS to analyse selected PAHs, fluorene and phenanthrene in water samples. An experimental response surface methodology (RSM) design with central composite design (CCD) was applied to optimise and evaluate the interactive effects of parameters of the two most influencing variables in HS-SPME. The present work aims to optimise and validate the implementation of HS-SPME coupled with GC-MS to determine PAHs in water samples using CCD to improve our knowledge of the composition of PAHs in water. The method is expected to be eco-friendly, simple, rapid, accurate, precise, and highly sensitive.

EXPERIMENTAL

Chemical Reagents

Selected PAHs standards, Phenanthrene (PHE) and Fluorene (FLU), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (MeOH) with high-performance liquid chromatography (HPLC) grade, which was employed as the solvent, was purchased from Merck (Darmstadt, Germany).

Preparation of Standard and Sample Collection

Tap water and drinking water were used as samples. The tap water samples were obtained from the laboratory at UiTM Shah Alam. The sample used for validation will be the drinking water obtained from a water purifier. The samples will be stored at ambient temperature and away from light before analysis.

The water samples were collected in pre-cleaned bottles. The tap water and drinking water samples were spiked with 100 ppm phenanthrene and fluorene standards to give each analyte a final concentration of 10 ppm. Each tap water sample (3 mL) was placed in a 5 mL glass vial, tightly capped with poly-tetrafluoroethylene (PTFE) septum, and left for 10 min at 40 °C to allow for the equilibration of the volatiles in the headspace. After the equilibration time, the septum covering each vial was pierced with an SPME needle, and the fibre was exposed to the headspace at varied extraction temperatures and extraction times. Only these two variables were selected to focus on how

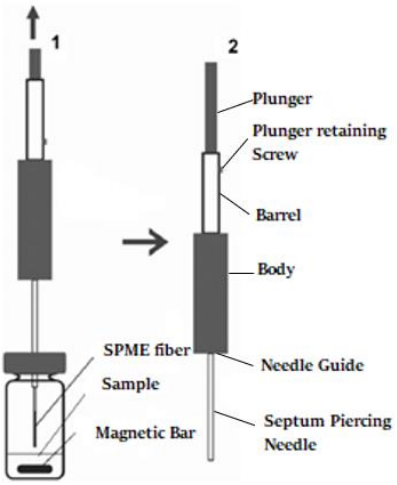

Figure 1. Illustration schematic of HS-SPME [19].

the temperature and the extraction time influence the PAH adsorption. Since SPME is an equilibrium extraction mode, the maximum amount of analyte extracted by the fibre is achieved at equilibrium time. The extraction time was examined to obtain the highest PAH recovery and sensitivity. Thus, the range selected for the extraction time is from 10 to 25 min. Temperature plays a significant role in the sensitivity of SPME, as it affects the distribution coefficient and diffusion coefficient [18]. To obtain the optimal temperature, PAHs were extracted from 50°C-95°C according to the CCD for extraction. SPME fibre (1 cm in length and 100 µm thick film) was obtained from Merck, Sigma-Aldrich, and it was endowed with the Stable Flex stationary phase of Polydimethylsiloxane (PDMS).

Headspace Solid Phase Microextraction

The 100 µm PDMS SPME fibre was evaluated in this work. The injector and detector temperatures were set up at 250°C and 300°C, respectively. The carrier gas flow rate was set at 30 cm sec^{-1}. The column temperature was set from 60°C to 170°C for 10 min. For the SPME procedure, the PDMS fibre in a GC injection port was conditioned at 250 °C for 10 min. Then, about 2 mL of a sample was placed in the glass vial with the septum. The vial is then placed in a water bath on a hot plate. The sample was heated to 50 °C. The sample was agitated using a magnetic stirrer. The SPME fibre was exposed to the headspace of the vial for 20 min. The fibre was then injected into the GC - MS with a desorption time of 50 s. The major compounds of each sample were identified using the mass spectra in the NIST library. Figure 1 demonstrates the schematic of HS-SPME.

Chromatographic Condition

The analysis of selected PAHs in the water samples was performed using gas chromatography-Mass Spectrometry. A Gas Chromatographer (Agilent Technologies 5890 Series II) equipped with an HP 5971A mass spectrometry detector (MSD) and a 30 m x 250 µm x 0.25 µm HP5-MS capillary column was used.

Experimental Design

HS-SPME extraction conditions were optimised using a central composite design (CCD) with α=2.000. In order to obtain the optimum conditions for the simultaneous extraction of PAHs, RSM and CCD were used to optimise the two independent variables (extraction temperature and extraction time). Finally, the experimental design was generated using the software for regression analysis of the experimental data to fit the equations.

Statistical Data and Analysis

An Analysis of Variance (ANOVA) was applied to the experimental data and the results. These statistical analyses and the CCD were performed using Design-Expert software, version 13. The experimental variables to be tested were randomly designed by CCD.

Validation of Analytical Method

The validation of HS-SPME was assessed to ensure that the analytical procedure was reliable and fit for the intended purpose. Linearity (R^2), precision, accuracy, the limit of detection (LOD) and limit of quantification (LOQ) were calculated from the data obtained. Linear regression of the calibration curve was used to determine LOD and LOQ. The precision of the method was expressed in terms of relative standard deviation (RSD %) and accuracy in terms of relative recovery.

RESULTS AND DISCUSSION

Experimental Design using CCD

Experimental design using RSM with CCD is helpful

in studying the effects of several variables influencing the responses by varying them simultaneously [20]. The CCD is an effective design used to reduce the number of experimental trials needed, maximise efficiencies and investigate the relationship between variables [21]. The equation below shows the number of experiments that should be run:

$$N = 2^n + 2n + n_c, \quad \text{(Eq. 1)}$$

where n is the factor number and (n_c) is the replicate number of the central point.

This study investigated two selected variables (extraction time and extraction temperature). According to Equation 1, 13 experiments were generated with the design matrix consisting of five levels of two factors. The coded level of selected factors (−α, −1, 0, +α, +1). The coded or actual values and the result of experiments are summarised in Table 1. CCD consists of experiments for the study of two experimental factors in coded levels, and experimental results are shown in Table 2.

For an experimental design with two factors, the quadratic model can be expressed by the following equation:

$$Y = a_0 + a_1A_1 + a_2B_1 + a_3A^2 + a_4B^2 + a_5A_1B_1, \quad \text{(Eq. 2)}$$

where Y is the predicted peak area or response value, A is the extraction time, B is the extraction temperature.

Using equation (2), the total predicted area response,

$$Y = 5189540.5399613 - 222887.58357877A + 25357.007924564B + 321190AB - 1033223.3761879A^2 - 1374432.4299517B^2$$

Table 1. Independent Variables and their Coded Level for the CCD Design Parameters.

Parameters (factors)	Code	Code Variables				
		-α	-1	0	+1	+α
A	1	6.89	10	17.50	25	28.11
B	1	40.68	50	72.50	95	100

Table 2. CCD Consists of Experiments for the Study of Two Experimental Factors at the Coded Level and Experimental Results.

Run order	Code level values		The sum of peak areas
	Extraction time, (A)	Extraction temperature, (B)	
1	0	0	2.36E+06
2	0	+α	3.44E+06
3	0	0	2.83E+06
4	+1	-1	2.53E+06
5	0	0	9.25E+05
6	0	0	1.36E+06
7	0	-α	1.19E+06
8	+α	0	5.00E+06
9	0	0	1.69E+06
10	-1	+1	2.32E+06
11	+1	+1	4.36E+06
12	-α	0	4.22E+06
13	-1	-1	1.22E+06

Table 3. Central composite design (CCD) for the analysis of PAHs.

Run order	Extraction temperature (℃), (A)	Extraction time (min), (B)	Total Peak Area (mAu*min)
1	6.89	72.5	3593450
2	17.5	72.5	5829410
3	25	50	2727560
4	17.5	72.5	5355110
5	17.5	72.5	5093480
6	17.5	40.68	2021470
7	28.11	72.5	2397810
8	10	95	2446270
9	17.5	72.5	4357940
10	17.5	72.5	5267250
11	10	50	3416060
12	17.5	100	3342590
13	25	95	3042530

Table 3 shows the total peak area of predicted response (Y) for both analyte.

Analysis of Variance

Analysis of variance (ANOVA) and regression analysis is used to assess the significance of variables, including the p-value, sum of squares, mean square, F-value and degree of freedom, and DF, which are presented in Table 4.

In the present study, R^2 is 0.9048 for extraction of both analytes (Table 5). The value of R^2 shows that there is an acceptable relationship between the predicted and actual values, therefore the models are significant (Figure 2).

Table 4. Analysis of variance (ANOVA) regression model for response quadratic model.

Source of Variation	Sum of Squares	DF	Mean Square	F Value	P Value	Significance
Regression	5	1.79E+13	3.57E+12	13.31	0.0018	significant
A	1	3.98E+11	3.98E+11	1.48	0.2631	
B	1	4.73E+09	4.73E+09	0.0176	0.8981	
A2	1	4.13E+11	4.13E+11	1.54	0.255	
B2	1	7.50E+12	7.50E+12	27.92	0.0011	
AB	1	1.09E+13	1.09E+13	40.5	0.0004	
Residual	7	1.88E+12	2.69E+11			
Lack of Fit	3	7.36E+11	2.45E+11	0.8582	0.5313	not significant
Pure Error	4	1.14E+12	2.86E+11			

Table 5. ANOVA Analysis of Both Selected PAHs.

Transform	Model	Lack of Fit	DF	R-square	Equation
Square Root	Quadratic Significant	Not Significant	5	0.9048	Total peak area = 5.190E+06 – 2.229E+05·A + 25357.01·B + 3.212E+05·A·B – 1.033E+06 A2 – 1.374E+06·B2

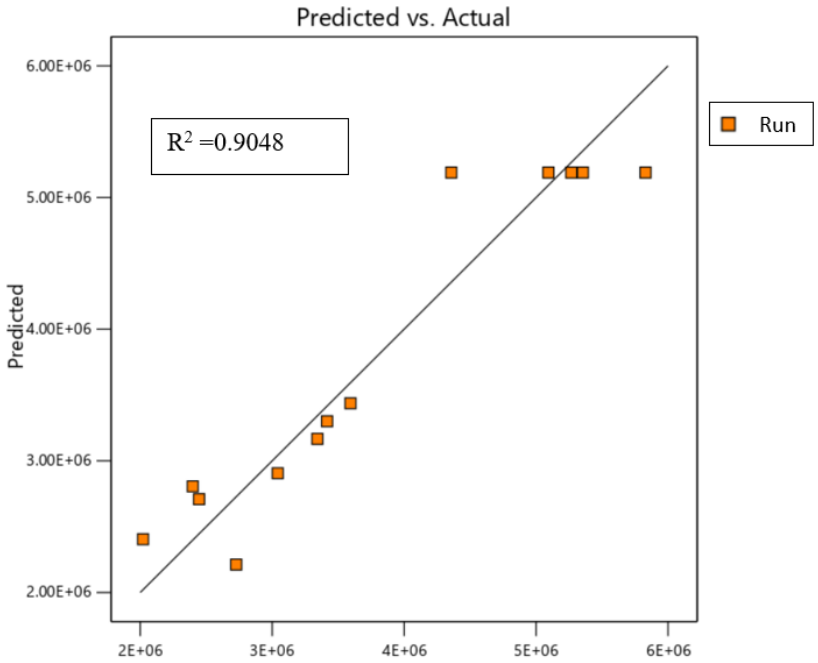

Figure 2. The parity plot between predicted and actual (experimental) values for selected PAHs which is fluorene and phenanthrene.

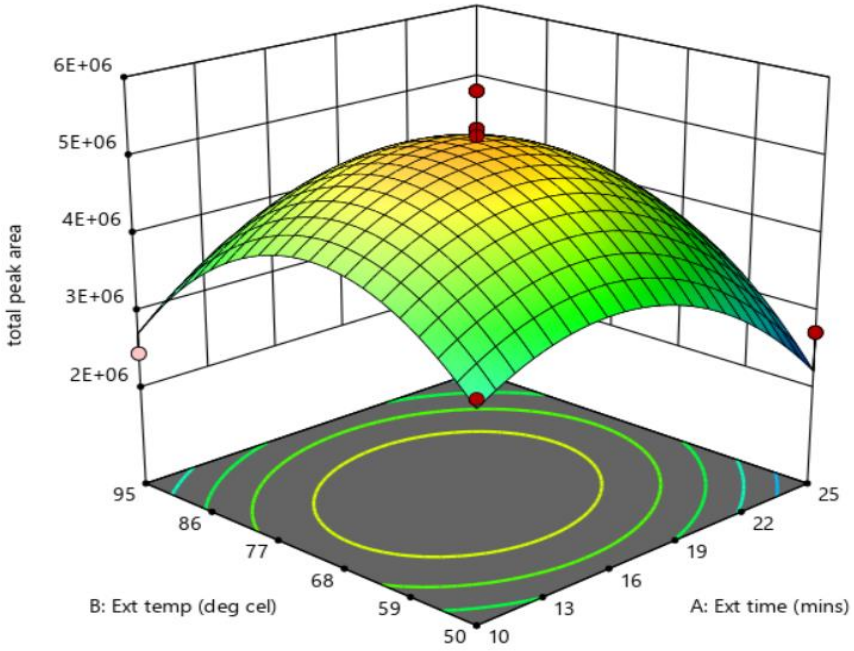

Figure 3. Estimated response surfaces with related contours by plotting factor versus extraction time (A) and extraction temperature (B).

Response Contour Plot

In this work, CCD was used to investigate the effects of extraction time and temperature in the form of three-dimensional (3D) plots. Variables in quadratic and interaction terms with the largest absolute coefficients in the fitted model were chosen for the axes of response surface plots to account for the curvature of the surfaces. This visualises the interaction between each parameter's response and experimental levels. Thus, the response was plotted against two experimental parameters. Figure 3 shows a 3D response surface and contour plot of the model in which the responses were mapped against two experimental parameters for both analytes.

The increment of the extraction temperature and time resulted in the peak area increasing. It is observed that the total peak area of both analytes increased to a point and then decreased after that. The maximum point is located inside the experimental region. Therefore, the optimum extraction time and temperature are around 16.69 min and 72.43℃, respectively. In research of the extraction time and determination of PAHs by Dynamic sonication-assisted solvent extraction (DSASE) GC-MS method, a range of 2 to 20 min of extraction of analytes was implemented, which complemented the optimum extraction time we obtained throughout this study using RSM [22]. Meanwhile, there is also a study that compliments our finding of optimal extraction temperature at 72.43℃ which the researchers stated that an increase in temperature (above 60℃) leads to higher extraction recoveries [23]. Thus, these values were chosen as the optimal extraction condition.

Method Validation of HS-SPME-GCMS

To investigate the applicability of the proposed method for determining PAHs, several factors, including implementing the optimised condition of 16.69 min of extraction time and 72.43℃ extraction temperature, linearity, the limit of detection (LOD), the limit of quantification (LOQ), precision and percentage recovery were evaluated. The linearity test was established by using five (5) different concentrations of both analytes, and the calibration graph of peak area (mAUs) as a function against the concentration (mgL^{-1}) was plotted under the optimal conditions.

The linearity of the method was evaluated using water samples spiked with the two PAHs. Good linearity of response (peak area) for each analyte was observed (Table 6) in the concentration range of 2.0-10 mgL^{-1} with coefficients of determination, $r^2 \geq 0.9993$. The proposed method showed good LODs and LOQs for the targeted analytes in the range of 0.10-0.29 mgL^{-1} and 0.96 -1.05 mgL^{-1}, respectively. The LOD was calculated using a linear regression equation, and the result obtained revealed the sensitivity of the method, which is quite decent at the obtained range. The tabulated validation data obtained is considered a good result; having r^2 more or equal to 0.9993 shows that the method implemented is suitable for the analysis [5].

Relative standard deviation (RSD) was used to determine the precision of the method by analysing one spiked drinking water sample (n=1) at two different concentrations (5 and 7 mgL^{-1}) for each analyte, FLU and PHE. The percentage recovery study was obtained by spiking the drinking water samples to give final concentrations of 5 mgL^{-1} and 7 mgL^{-1}. The results (Table 7) showed good percentage recoveries in the range for FLU and PHE. At concentrations of 5 mgL^{-1} and 7 mgL^{-1}, the average relative recovery for FLU was 60% and 103%, respectively. Random errors or systematic errors somehow caused the low recovery of fluorene at 5 mgL^{-1}. For PHE, the average relative recovery was 101% for 5 mgL^{-1} and 99% for 7 mgL^{-1}. The RSD values are also excellent as the RSD for FLU and PHE at concentrations of 5 mgL^{-1} and 7 mgL^{-1} were 0.31% and 0.33% for FLU and 0.67% and 0.36% for PHE, respectively. Hence, the HS-SPME-GC-MS method proved to be simple, sensitive, and highly selective and can be considered a green extraction method that can potentially be used in a laboratory for water sample analysis.

Table 6. Validation data of HS-SPME-GC-MS for Selected PAHs in Drinking Water Samples.

Sample	Analyte	Linear range (mgL^{-1})	Coefficient of determination (R^2)	LOD, (mgL^{-1})	LOQ (mgL^{-1})
Drinking water	FLU	2.0-10	0.9994	0.29	0.96
	PHE	2.0-10	0.9993	0.1	1.05

Table 7. Relative Recoveries (%) and Method Precisions (RSD %, n = 3) at Two Different Concentrations for HS-SPME-GC-MS in Drinking Water.

Analyte	Average relative recovery, % (RSD, %), Spiking level (n = 3)	
	5 mgL^{-1}	7 mgL^{-1}
FLU	60 (0.31)	103 (0.33)
PHE	101 (0.67)	99 (0.36)

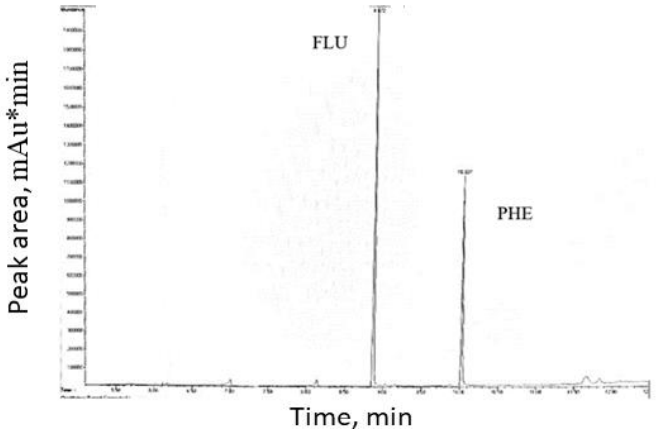

Figure 4. Chromatograms of FLU and PHE at 10 mgL^{-1} of spiked tap water sample under optimum conditions at 16.69 min extraction time and 72.43℃ extraction temperature.

Figure 4 shows the chromatograms of spiked tap water samples in 10 mgL^{-1} of mixed selected PAHs. No significant peak was observed in the plain tap water sample analysis, but this chromatogram revealed that all analytes were successfully extracted and separated from the sample. This shows that HS-SPME-GC-MS is suitable for the determination of PAHs in water samples. Despite that, FLU displayed a higher peak than PHE, which could be due to the degradation rate of the PHE, which is greater than FLU which may occur during extraction [19].

Comparison of HS-SPME-GC-MS with other Reported Methods

The comparison of the analytical method between HS-SPME-GC-MS and other reported methods is tabulated in Table 8. A few methods, such as microextraction in packed syringes (MEPS), Solid Phase Extraction (SPE), have been applied in the analysis of PAHs. Generally, each method has its advantages and disadvantages. The proposed HS-SPME revealed good performance in sensitivity and recoveries compared to other methods. In addition, the proposed method utilises RSM for the optimisation studies of the most affected parameters to achieve better performance results.

CONCLUSION

It can be concluded that the head space solid phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) can be optimised by setting up the extraction time and extraction temperature by using the response surface method (RSM). Optimum conditions of 16.69 min extraction time and 70.43 ℃ extraction temperature have achieved the highest peak area. This method was successfully applied for extracting PAHs compounds with good relative recovery and an insignificant lack of fit model used.

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Table 8. Comparison study of the determination of PAHs in Water Samples.

Analysis method	*Linear range (µg/L)*	*LOD (ng/L)*	*Recoveries (%)*	*Ref.*
MEPS-GC-MS	(0.05–2.0)	0.8 - 8.2	70-117	[24]
HS-SPME-GC-MS	(>10)	95 -742	Not Available	[25]
HS-SPME-GC-MS	(2.0 – 10) mg/L	(0.1 – 0.29) mg/L	60 - 103	Present work

REFERENCES

1. Abdel-Shafy, H. I. & Mansour, M. S. M. (2016) A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, **25(1)**, 107–123.

2. Boehm, P. D. (1964) Polycyclic Aromatic Hydrocarbons (PAHs). *Environmental Forensics: Contaminant Specific Guide*, 313–337.

3. Pampanin, D. M. & Sydnes, M. O. (2013) Polycyclic Aromatic Hydrocarbons a Constituent of Petroleum: Presence and Influence in the Aquatic Environment. *Hydrocarbon.*

4. Karyab, H., Yunesian, M., Nasseri, S., Mahvi, A. H., Ahmadkhaniha, R., Rastkari, N. & Nabizadeh, R. (2013) Polycyclic aromatic hydrocarbons in drinking water of Tehran, Iran. *Journal of Environmental Health Science and Engineering*, **11(1)**, 1–7.

5. Mohamad Hanapi, N. S., Sanagi, M. M., Ismail, A. K., Saim, N., Wan Ibrahim, W. N., Wan Ibrahim, W. A., & Mohd Marsin, F. (2017) Rapid Determination of Non-steroidal Anti-inflammatory Drugs in Aquatic Matrices by Two-phase Microelectro-driven Membrane Extraction Combined with Liquid Chromatography. Journal of Chromatographic Science, **56(2)**, 166–176. October 23, 2017, https://doi.org/10.1093/chromsci/bmx092.

6. Honda, M. & Suzuki, N. (2020) Toxicities of polycyclic aromatic hydrocarbons for aquatic animals. *International Journal of Environmental Research and Public Health*, **17(4)**.

7. Singh, V. K., Patel, D., Jyoti, Ram, S., Mathur, N. & Siddiqui, M. (2008) Blood levels of polycyclic aromatic hydrocarbons in children and their association with oxidative stress indices: An Indian perspective. Clinical Biochemistry, 41(3), 152–161. February, 2008, https://doi.org/10.1016/j.clinbiochem.2007.11.017.

8. Marwani, H. M., Danish, E. Y., Alhazmi, M. A., Khan, S. B., Bakhsh, E. M. & Asiri, A. M. (2017) Cellulose acetate-iron oxide nanocomposites for trace detection of fluorene from water samples by solid-phase extraction technique. *Separation Science and Technology*, **53(6)**, 887–895. https://doi.org/10.1080/01496395.2017.1405982.

9. Beltrán, F. J., Rivas, F. J., Gimeno, O. & Carbajo, M. (2005) Photocatalytic enhanced oxidation of fluorene in water with ozone. Comparison with other chemical oxidation methods. *Industrial & engineering chemistry research*, **44(10)**, 3419-3425.

10. Gad, S. E. (2014) Phenanthrene. Encyclopedia of Toxicology, 865–867. https://doi.org/10.1016/b978-0-12-386454-3.00901-5.

11. Herrera-Herrera, A. V., Hernández-Borges, J., Borges-Miquel, T. M. & Rodríguez-Delgado, M. A. (2010) Dispersive liquid-liquid microextraction combined with nonaqueous capillary electrophoresis for the determination of fluoroquinolone antibiotics in waters. *Electrophoresis*, **31(20)**, 3457–3465.

12. Zuazagoitia, D., Millán, E. & Garcia, R. (2007) A screening method for polycyclic aromatic hydrocarbons determination in water by headspace SPME with GC-FID. *Chromatographia*, **66(9–10)**, 773–777.

13. Ibrahim, M., Makky, E. A., Azmi, N. S. & Ismail, J. (2018) Optimization parameters for Mycobacteria confluentis biodegradation of PAHs. *MATEC Web of Conferences*, **150**, 1–5

14. Purcaro Giorgia, Moret Sabrina, Conte Lanfranco, S. (2013) Overview on polycyclic aromatic hydrocarbons: Occurrence, legislation and innovative determination in foods. *Talanta*, **105()**, 292–305. doi:10.1016/j.talanta.2012.10.041.

15. Peng, P. L. & Lim, L. H. (2022, January 1). Polycyclic Aromatic Hydrocarbons (PAHs) Sample Preparation and Analysis in Beverages: A Review. Food Analytical Methods, **15(4)**, 1042–1061. January 1, 2022, https://doi.org/10.1007/s12161-021-02178-y.

16. Mohammadi, A., Malek-Mohammadi Jahani, S., Kamankesh, M., Jazaeri, S., Eivani, M., Esmaeili, S. & Abdi, S. (2020) Determination of Polycyclic Aromatic Hydrocarbons in Edible Oil Using Fast and Sensitive Microwave-assisted Extraction and Dispersive Liquid–Liquid Microextraction Followed by Gas Chromatography-Mass Spectrometry. *Polycyclic Aromatic Compounds*, **40(3)**, 705–713.

17. Zhang, X., Wang, C., Wang, L., Chen, S. & Xu, Y. (2020) Optimization and validation of a head space solid-phase microextraction-arrow gas chromatography-mass spectrometry method using central composite design for determination of aroma compounds in Chinese liquor (Baijiu). *Journal of Chromatography A*, **1610**, 460584.

18. Lei, F. -F., Huang, J. -Y., Zhang, X. -N., Liu, X. -J. & Li, X. -J. (2011) Determination of Polycyclic Aromatic Hydrocarbons in Vegetables by Headspace SPME-GC. *Chromatographia*, **74(1-2)**, 99–107. doi:10.1007/s10337-011-2024-4.

19. Camarasu, C., Madichie, C. & Williams, R. (2006) Recent progress in the determination of volatile impurities in pharmaceuticals. *TrAC* -

Trends in Analytical Chemistry, **25(8)**, 768–777. https://doi.org/10.1016/j.trac.2006.05.013.

20. Ebrahimi-Najafabadi, H., Leardi, R. & Jalali-Heravi, M. (2014) Experimental Design in Analytical Chemistry—Part I: Theory. Journal of AOAC International, **97(1)**, 3–11. January 1, 2014, https://doi.org/10.5740/jaoacint.sgeebrahimi.

21. Camposeco-Negrete, C. (2015) Optimization of cutting parameters using Response Surface Method for minimizing energy consumption and maximizing cutting quality in turning of AISI 6061 T6 aluminum. Journal of Cleaner Production, 91, 109–117. March, 2015, https://doi.org/10.1016/j.jclepro.2014.12.017.

22. Domeño, C., Blasco, M., Sánchez, C., Nerín, C. (2006) A fast extraction technique for extracting polycyclic aromatic hydrocarbons (PAHs) from lichens samples used as biomonitors of air pollution: Dynamic sonication versus other methods. **569(1-2)**, 103–112. doi:10.1016/j.aca.2006.03.053.

23. Rabodonirina, S., Rasolomampianina, R., Krier, F., Drider, D., Merhaby, D., Net, S. & Ouddane, B. (2019) Degradation of fluorene and phenanthrene in PAHs-contaminated soil using Pseudomonas and Bacillus strains isolated from oil spill sites. *Journal of Environmental Management*, **232**, 1–7. February, 2019, https://doi.org/10.1016/j.jenvman.2018.11.005.

24. Fu, S., Fan, J., Hashi, Y. & Chen, Z. (2012) Determination of polycyclic aromatic hydrocarbons in water samples using online micro-extraction by packed sorbent coupled with gas chromatography–mass spectrometry. *Talanta*, **94**, 152–157. https://doi.org/10.1016/j.talanta.2012.03.010.

25. King, A. J., Readman, J. W. & Zhou, J. L. (2004) Determination of polycyclic aromatic hydrocarbons in water by solid-phase microextraction–gas chromatography–mass spectrometry. *Analytica Chimica Acta*, **523(2)**, 259–267.