

The Study of Matrix Interference of Vegetable Samples in Dispersive Liquid-Liquid Microextraction (DLLME)

Premla Devi Chander¹, Chai Mee Kin¹ and Wong Ling Shing²

1. University Tenaga Nasional, Malaysia

2. Inti International University, Malaysia

Abstract: The matrix problems of vegetable samples such as Radish (Raphanus sativus), Cabbage (Brassica oleracea) and Cucumber (Cucumis sativus) in Dispersive liquid-liquid microextractio (DLLME) were studied. High yield of extraction efficiency (%) was obtained when the matrix interference in the sample reduced by using optimal dilution. In present study, six different volume of distilled water (50 ml, 100 ml, 150 ml, 200 ml, 250 ml and 300 ml) were added into the cabbage, cucumber and radish samples and the extraction efficiencies at three times injection in GC-FID for each studied volumes were evaluated. The extraction recoveries of PAHs in vegetable samples were improved from the ranged value of 80.07% to 111.19% by using an optimal dilution method. All three types of vegetable have different matrix effect due to different group of complex structure's presence in the matrix. The physicochemical properties of extraction solvent (1-bromo-3-methylbutane) affected the adsorption of PAHs from the complex matrix. The solubility, polarity and hydrophobic interaction of 1-bromo-3-methylbutane with PAHs were discussed.

Key words: PAHs, 1-bromo-3-methylbutane, matrix interference

1. Introduction

Polycyclic Aromatic Hydrocarbon (PAH) is a contaminant that presences in the surrounding environment such as food productions, rivers, soils, crops and also in atmosphere. Besides that, presence of tobacco smokes and implementing the heat devices also increase the PAH concentrations in the environment [1]. Polycyclic Aromatic Hydrocarbons (PAHs) comprise of two or more condensed aromatic rings are the end product of incomplete combustion of organic carbon materials that induce cancer disease [2]. It consists of carbon and hydrogen with more than 2 benzene fused rings. PAHs with more than 3 rings have low solubility in water and low vapor pressure but they have great affinity for soil organic material [3]. In fruits and vegetables, the presences of PAH can

be found due to the location of the growing sites which placed near to the roadways or industrialized area surrounded with air pollution. Thus, the high levels of PAH can be found on the outer layers of plant surfaces such as lettuce, kale and cabbage [4].

These compounds known to be genotoxic, mutagenic pollutants and priority organic pollutants that expose to human in three levels which are ingestion, inhalation and skin contact [5]. Large molecular weight of **PAHs** increased the carcinogenicity level. One of the common exposures for human is food as these contaminants found on the waxy surface of many vegetables and fruits. Sixteen types of PAH namely acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, fluorene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene, fluoranthene, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene and pyrene were monitored by the United States Environmental Protection Agency

Corresponding author: Premla D. Chander, Msc., research area/interests: environment, food safety. E-mail: premla2890@gmail.com.

(US-EPA) and closely regulated by the European Union [4, 5].

Various conventional extraction methods were developed for the determination of polycyclic aromatic hydrocarbons (PAHs) in the food safety and environmental analysis. These extraction methods affect the accuracy and precision of the results [6]. The development of the efficiency, miniaturized and environmental friendly extraction methods were well focused. Dispersive Liquid-Liquid Microextraction (DLLME) was developed in the year of 2006 by Assadi and teammates for PAHs determination in liquid matrices Mostly. DLLME [7]. was implemented in water samples (e.g., river, lake water). In brief, the procedure of DLLME method was comprise of the injection of the mixture of extraction solvent (water immiscible) and dispersive solvent (water miscible) into the samples solution [4, 7]. Upon cloudy formation, fine droplets of extraction solvent formed as it disperses in dispersive solvent. The sediment phase contains target analytes (e.g., PAHs) and the analytes will be further analysed using GC-FID after centrifugation process [4, 7]. DLLME restricts the implications on the vegetables sample due to interference problem. The trace concentration of PAHs and also the presence of interfering complex compounds in the vegetable encounter difficulties to maximize the recovery rate of analyte (PAHs).

In this study, the interfering matrix effects in vegetable samples (radish, cabbage and cucumber) were reduced by using optimal dilution which can enhanced the recovery rate of PAHs. The physicochemical properties of 1-bromo-3-methyl butane were also focused as these properties give important impact on the adsorption of PAHs from complex matrix.

2. Experimental

2.1 Reagents and Materials

Twelve types of PAHs (acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene,

fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b) fluoranthene, benzo(k)fluoranthene and benzo(a) pyrene) were purchased from Supelco. 1-bromo-3-methylbutane was purchased from Sigma-Aldrich. Acetone and Acetonitrile (HPLC Grade) were obtained from Merck. Deionised water (D.I.) was purified on a Milli-Q water system. Stock solution of PAH standards were prepared by dissolving PAHs standard at 10 ppm in acetonitrile. Working standard solutions of 12 types of PAHs were prepared in acetonitrile at concentration of 0.1 ppm to 5 ppm. The solutions were stored at 4°C in a refrigerator. Common vegetables namely radish (Raphanus sativus), cucumber (Cucumis sativus) and cabbage (Brassica oleracea) were selected in this study because they are consumed by locals residents wisely. Vegetables were purchased from the local organic market. The vortex agitator model no. VELP Scientifica ZX³ (Usmate, Italy) and the centrifuger with a model no. Hettich ROTOFIX 32 Benchtop centrifuge (Tuttlingen, Germany) were used in this study.

2.2 Instrumentation

The chromatographic analysis was performed on model Agilent 7890A Gas Chromatography equipped with a split/splitless injection inlet, flame ionization detector (FID) and a 7683 automatic liquid sampler. The optimization of gas chromatography (GC-FID) condition was carried out and set up in which one run of a sample requires 25 minutes. The 12 PAH compounds were separated by using HP-5 5% Phenyl Methyl Siloxan capillary column (30 m x 320 µm x 0.25 µm). The optimum condition of the GC-FID for separation of 12 PAHs was program temperature commenced at 100°C for 1 min and was raised by 15°C min⁻¹ to 280°C for 10 min. Other GC terms were as follows: helium as carrier gas in a constant flow of 1 mL min⁻¹, injector temperature of 290°C and in splitless ratio.

2.3 Sample Preparation

The vegetable sample was weighed, homogenized, centrifuged, filtered and spiked at 50 μ L of 10 ppm of PAHs standard. The homogenized sample (5 mL) was transferred into conical centrifuge tube.

2.4 Modified DLLME Procedure

An appropriate amount of extraction solvent was added into the sample and gently shaken. A cloudy solution formed due to tiny droplets of extraction solvent dispersing in the sample solution. After homogenization (vortex) and centrifugation process at rate of 3000 rpm for 10 mins, the sediment layer formed at bottom of the conical tube. The sediment layer further transferred into the glass insert in a 2 mL vial using microsyringe. 1 μ L of the sediment solution was injected into GC-FID using autosampler for the analysis. Three replicates were done for each trial.

3. Results and Discussion

3.1 The Study of Interferences Matrix Problem in Modified DLLME

Vegetables sample contains mixture of complex structure compounds. The undesired compounds will be classified as interferences. The matrix effects were encountered due to the physicochemical properties of the matrix. They are water content, acidity, colour and pH [3]. The interaction between the target analyte and the matrix may also affect the extraction efficiency. When the extraction solvent is added into the sample solution, the surface area of analyte contacts with the surface area of extraction solvent will be minimized if the amount of interference presence is high [3, 5].

One of the implemented techniques is diluting of the matrix by distilled water to achieve the optimal dilution ratios that yields higher extraction efficiency. Six different volumes of distilled water were added into the cabbage, cucumber and radish samples and the extraction efficiency for each studied volume was determined. The extraction efficiency was calculated by the obtained peak area of analyte divided by the peak area of standard PAHs in distilled water.

From the results in Table 1, the matrix effect of radish was solved by addition of 50 g of sample with 150 mL of distilled water (1: 3). The extraction recovery was enhanced from 80.07% to 111.19%. Reduction of matrix effect increases the surface area contact of target analytes with the extraction solvent and yielded better efficiency. Table 2 shows the matrix effect of cabbage was solved by addition of 50 g of sample with 250 mL of distilled water (1: 5). The extraction recovery obtained was increased from 83.18% to 101.57%.

 Table 1
 Recovery Percentage (%) of Modified DLLME with Different Dilution Volumes in Radish Samples

Radish (Recovery Percentage % at $n = 3$)						
РАН	50g: 50 ml	50 g:100 ml	50 g:150 ml	50 g: 200 ml	50 g: 250 ml	
Acenaphthylene	70.09	83.05	110.02 89.39		85.65	
Acenaphthene	74.22	87.88	111.19 88.77		82.03	
Fluorene	75.32	88.07	111.14 88.66		84.6	
Anthracene	72.78	82.14	105.49	88.82	82.16	
Phenanthrene	71.05	81.81	102.81	89.11	84.96	
Fluoranthene	70.77	78.02	93.84	88.34	82.99	
Pyrene	73.49	78.68	101.04	88.78	81.44	
Benzo(a)anthracene	72.21	77.18	84.77	80.09	78.21	
Chrysene	73.04	75.79	84.06	80.01	79.75	
Benzo(b)fluoranthene	72.15	80.94	82.52	79.14	75.69	
Benzo(k)fluoranthene	70.9	71.34	84.14	80.55	74.03	
Benzo(a)pyrene	70.8	72.72	80.07	70.01	71	
			Optimum			

The Study of Matrix Interference of Vegetable Samples in Dispersive Liquid-Liquid Microextraction (DLLME)

Cabbage (Recovery Percentage % at $n = 3$)						
РАН	50 g: 50 ml	50 g: 100 ml	50 g: 150 ml	50 g: 200 ml	50 g: 250 ml	50 g: 300 ml
Acenaphthylene	75.63	80.47	81.52	84.4	101.57	80.06
Acenaphthene	73.11	78.18	83.76	88.6	97.79	80.07
Fluorene	74.38	76.67	82.48	85.23	95.98	84.76
Anthracene	70.03	77.96	81.6	87.07	93.85	80.86
Phenanthrene	71.64	76.29	78.05	84.64	96.42	80.53
Fluoranthene	70.71	77.86	79.38	82.33	97.96	80.59
Pyrene	70.44	75.08	77.73	82.53	89.51	78.77
Benzo(a)anthracene	71.78	74.23	76.5	81.88	87.72	77.22
Chrysene	71.09	74.61	79.2	80.35	86.03	74.87
Benzo(b)fluoranthene	70.28	75.56	79. 72	81.26	85.07	79.22
Benzo(k)fluoranthene	71.17	74.25	77.61	80.4	84.45	79.06
Benzo(a)pyrene	70.19	75.62	78.67	80.38	83.18	80.28
					optimum	

 Table 2
 Recovery Percentage (%) of Modified DLLME with Different Dilution Volumes in Cabbage Samples

In Table 3, the matrix effect of cucumber was reduced by addition of 50 g of sample with 200 mL of distilled water. The extraction recovery obtained was improved from 83.21% to 107.38%. However, addition of 50 g of sample with 300 mL of distilled water reduced the extraction efficiency. This may due to high dilution rate flushes out the target analytes from the sample. The concentration of target analytes will become low and will lose the surface area contact

between the extraction solvent with target analytes. In total, all three types of vegetable have different matrix effect due to different group of complex structure's presence in the matrix. For instance, presence of flavonoids in cucumber enhanced the pigments in cucumber which acquired more dilution compared to the radish. The matrix effects were reduced at optimum dilution and produced higher extraction efficiency.

nples
ľ

Cucumber (Percentage Recovery % at $n = 3$)							
РАН	50 g: 50 ml	50 g:100 ml	50 g:150 ml	50 g: 200 ml	50 g: 250 ml	50 g: 300 ml	
Acenaphthylene	72.22	85.29	88.3	103.11	88.34	84.31	
Acenaphthene	70.7	81.94	82.99	107.38	107.38 84.06		
Fluorene	72.24	80.26	85.23	100 84.99		86.69	
Anthracene	70	76.4	87.29	100.11	89.54	83.95	
Phenanthrene	71.27	75.64	83.41	97.05	83.86	82.38	
Fluoranthene	70.68	78.53	83.52	95.22	85.3	86.21	
Pyrene	70.33	77.29	80.15	93.11	77.24	84.81	
Benzo(a)anthracene	71.03	74.37	77.24	88.92	72.13	80.06	
Chrysene	70.28	78.52	79.49	87.17	73.56	81.05	
Benzo(b)fluoranthene	70.2	74.68	77.37	85.18	79.6	76.63	
Benzo(k)fluoranthene	70.26	73.05	76.41	84.77 77.68		75.19	
Benzo(a)pyrene	70.5	72.45	74.37	83.21	75.11	73.91	
				optimum			

3.2 The Physicochemical Relationship of Extraction Solvent in Modified DLLME

The study of physicochemical properties of 1-bromo-3-methylbutane gives the better understanding of the interaction of 12 PAHs with 1-bromo-3-methylbutane as the extraction solvent in modified DLLME. From the results obtained, 12 types of PAHs were extracted at different range of extraction efficiency. Though, all the extractions of PAHs are in the acceptable range.

Chlorobenzene is the common extraction solvent implemented in conventional DLLME, which is more soluble (466.3 mg/L) in water (Table 4) compare to 1-bromo-3-methylbutane. Modified DLLME implies 1-bromo-3-methylbutane as extraction solvent that is denser than water and less soluble in water. Thus, 1-bromo-3-methylbutane enables for layer separation and miscible in PAHs. The high interaction of extraction solvent between PAHs leads better

adsorption and enhances the extraction efficiency. 1-bromo-3-methylbutane comprise of C₅H₁₁Br which is a non-polar solvent. The electronegativity between C-Br is 2.8 which have lower electronegativity compared to other halogen and falls under non-polar group [8]. Thus, it does not interact with water molecules leads on low solubility in water which is 196 mg/L (Table 4). Low solubility in water prevents the interaction of interferences with water or other complex molecule which presents in vegetables. Low polarity in water due to the less solubility of PAHs in water creates high adsorption between the PAHs in the extraction solvent. PAHs have large affinity on the implied extraction solvent because it is comprised of non-polar compounds due to the alkyl aromatic ring substitution. Thus, PAHs lead low solubility and polarity towards water molecule. For instances, acenaphthene and fluorene are total insoluble in water [9].

Table 4 The Physicochemical Properties of Extraction Solvent and PAHs

Compounds	Molecular weight (g/mol)	Density (g mL ⁻¹)	Boiling Point (°C)	Melting Point (°C)	Vapor pressure (mm Hg at 25°C)	Solubility in water (mg/L)
Chlorobenzene	112.56	1.1075	132	-45	11.8	466.3
1-Bromo-3-methylbutane	151.05	1.2	120-121	112	34.5	196 at 16.5°C
Acenaphthylene	152.19	0.8988	265-275	92-93	9.12	3.93 at 25°C
Acenaphthene	154.21	1.0242	279	95	10.0	insoluble
Fluorene	166.22	1.203	295	116-117	3.2	insoluble
Anthracene	178.2	1.25	342	218	6.56	1.29at 25°C
Phenanthrene	178.2	1.179	340	101	1.21	1.15 at 25°C
Fluoranthene	202.3	1.252	384	111	9.22	0.20-0.26
Pyrene	202.1	1.271	404	150.62	4.5	0.135 at 25°C
Benzo(a)anthracene	228.3	1.19	437.6	160	5.0	0.0094 at 25°C
Chrysene	228.3	1.274	448	258.2	6.23	0.00189 at 25°C
Benzo(b)fluoranthene	252.3	1.286	481	168	5.0	0.0015
Benzo(k)fluoranthene	252.3	1.286	480	217	9.7	0.00076 at 25°C.
Benzo(a)pyrene	252.3	1.351	310-312	179	5.5	0.00162 at 25°C

In terms of density, 1-bromo-3-methylbutane comprise of haloalkanes. It is denser (1.2 g/mL) than water (1.0 g/mL). Since the implied extraction solvent denser than the water, it enables the formation of separation layer at the bottom of the tube and easy to

transfer into vials as well as minimize the losses of analytes. Among the extracted PAHs, most of the PAHs are denser than water except for acenaphthylene. Low density of PAHs may loss adsorption with implied extraction solvent as it floats on the upper

The Study of Matrix Interference of Vegetable Samples in Dispersive Liquid-Liquid Microextraction (DLLME)

layer where presence of impurities in the sample. This causes low ratio of PAHs concentration transfer from the sample solution to sediment phase and yield low extraction efficiency.

All the PAHs 12 of types and 1-bromo-3-methylbutane have respective boiling and melting point. When the analytes were injected into GC-FID, those compounds with higher boiling point than the column temperature such as anthracene, phenanthrene, fluoranthene, pyrene, benzo(a) anthracene, chrysene, benzo(b)fluoranthene, benzo (k)fluoranthene and benzo(a)pyrene had longer retention time compare to other PAHs (Table 4). Meanwhile, 1-bromo-3-methylbutane has lower boiling point where it vaporizes into gas phase faster

with short retention time taken which is 8.443 minute shown in Fig. 1. Extraction solvent as (1-bromo-3-methylbutane) has highest peak concentration compare to other analytes on the chromatogram. The PAHs peaks begin from 7.838 minutes (Acenaphthylene), 8.110 minutes (Acenaphthene), 8.870 minutes (Fluorene), 10.327 minutes (Anthracene), 10.395 (Phenanthrene), 12.167 minutes (Fluoranthene), 12.510 minutes (Pyrene), 14.574 minutes (Benzo[a]anthracene), 14.659 minutes (Chrysene), 17.324 minutes (Benzo (b)fluoranthene), 17.402 minutes (Benzo(k) fluoranthene), 18.396 minutes (Benzo(a)pyrene). The temperature of the column was optimized in order to obtain well separated peaks [10].



Fig. 1 GC-FID Chromatogram of Standard Mixture of 12 PAHs

The interaction of extraction solvent and target analyte was strong due to the hydrophobic interaction. Implied extraction solvent is nonpolar group also known as hydrophobic. As it was injected into the aqueous solution, it causes the breakdown of hydrogen bonding in water molecules and creates the cavity for hydrophobic molecule [11]. The nonpolar molecule does not form hydrogen bonding with water molecules, it forces the water molecules to form rigid cage of hydrogen bonding molecules around them. The breakdown of hydrogen bonding will cause decrease in enthalpy in hydrophobic (nonpolar molecule). The enthalpy increases as it trigger the binding of Van der Waals between nonpolar molecules and form hydrophobic interaction. Van der Waals interaction helps to bind all the nonpolar molecules together and separates the nonpolar molecules from the polar molecules and forms layer formation [11]. Thus, the implied extraction solvent (1-bromo-3-methylbutane) and target analyte (PAHs) forms hydrophobic interactions and a separation layer through Van der Waals interaction as both are nonpolar groups. Therefore, good extraction recovery yielded due to their behaviour of physicochemical properties.

4. Conclusion

The modified DLLME was implemented on vegetable samples and reduced the interference problems by using optimal dilution method. The recovery percentages of PAHs were enhanced from 80.07% to 111.19% in radish, cabbage and cucumber. Different vegetable needs different optimal dilution factor due to its composition. The implemented extraction solvent and target analytes are non-polar groups. Both of them are insoluble in water and showed high extraction efficiency of target analytes on extraction solvent due to Van der Waals interaction.

Acknowledgment

The authors express special thanks to the Ministry of Higher Education of Malaysia Fundamental Research Grand No. "FRGS/1/2013/ST01/UNITEN/02/1" for the financial supports, UNITEN Renewable Energy Centre and Faculty Science of University Malaya for the laboratory analysis.

References

[1] M. Pena, M. Casais, M. Mejuto and R. Cela, Development of an ionic liquid based dispersive liquid-liquid microextraction method for the analysis of polycyclic aromatic hydrocarbons in water samples, *Journal of Chromatography A* 1216 (2009) (36) 6356-6364.

- [2] M. H. Hosseini, M. Rezaee, S. Akbarian, F. Mizani, M. R. Pourjavid and M. Arabieh, Homogeneous liquid-liquid microextraction viaa flotation assistance for rapid and efficient determination of polycyclic aromatic hydrocarbons in water samples, *Analytica Chimica Acta* 762 (2013) 54-60.
- [3] A. Dugay, C. Herrenknecht, M. Czok, F. Guyon and N. Pages, New procedure for selective extraction polycyclic aromatic hydrocarbons in plants for gas chromatographic-mass spectrometric analysis, *Journal of Chromatography A* 958 (2002) 1-7.
- [4] S. Agata, M. Lukasz, G. Miguel and N. Jacek, Green aspects, developments and perspectives of liquid phase microextraction techniques, *Talanta* 119 (2014) 34-45.
- [5] P. P. Bolanos, G. F. Antonia and M. V. Jose Luis, Polycylic aromatic hydrocarbons in food and beverages: Analytical methods and trends, *Journal of Chromatography A* 1217 (2010) 6303-6326.
- [6] L. Guo and H. Kee Lee, Low density solvent based solvent demulsification dispersive liquid-liquid microextraction for the fast determination of trace levels of sixteen priority polycyclic aromatic hydrocarbons in environmental water samples, *Journal of Chromatography A*, 1218 (2011) 5040-5046.
- [7] M. I. Leong, C. C. Chang, M. R. Fuh and S. D. Huang, Low toxic dispersive liquid-liquid microextraction using halosolvents for extraction of polycyclic aromatic hydrocarbons in water samples, *Journal of Chromatography A* 1217 (2010) 5455-5461.
- [8] N. L. Medicine, PubChem compound search, National Scientific for Biotechnology Information, US, 2009.
- [9] N. K. Nagpal, Ambient water quality criteria for polycyclic aromatic, Ministry of Environment, Lands and Parks, Columbia, September 29, 2014.
- [10] J. Clark, ChemGuide analysis chromatography, Chemguide Co, United Kingdom, September 29, 2014.
- [11] A. B. Harvey Lodish, *Molecular Cell Biology* (4th ed.), New York: W. H. Freeman, 2000.