



Comparison of Microwave-Assisted Extraction DLLME with Conventional DLLME for the Determination of PAHS in Vegetables

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Abstract

Microwave-assisted extraction coupled with for the extraction of polycyclic aromatic hydrocarbons (PAHs) in vegetables, dispersive liquid-liquid microextraction (MAE-DLLME) is already advanced. The analyses were segregated and detected using the ionic detector for gas-chromatography-flame (GC-FID). In this study, microwave-assisted pre-treatment was used to solve the problem of matrix interferences. The parameters affecting the MAE-DLLME performance have been developed and optimized in a previous work. Pre-treatment was done by heating the sample with acetone under 200 W of microwave power for 1.5 minutes and followed by DLLME extraction using 30 uL of 1-bromo-3-methylbutane as extraction solvent and 800 uL of acetone as dispersive solvent for one minute extraction time. Analytical parameters, such as sensitivity, detection limits (LOD), quantification limits (LOQ), relative extraction recovery (ER) and relative standard deviation (RSD), were already compared in this work with those obtained from modified DLLME and conventional DLLME techniques. MAE-DLLME showed the highest sensitivity, lowest LOD and LOQ values, the best related extraction recovery with the low RSD compared to the modified DLLME and the conventional DLLME. The MAE pre-treatment has improved the overall analytical performances of MAE-DLLME by eliminating the matrix interferences.

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1 Introduction

The compounds of polycyclic aromatic hydrocarbon (PAH) have showed the great raised awareness among the researchers because of their potential mutagenic or carcinogenic characteristics. There are hundreds of different PAHs have been identified. Some PAHs are graded by the European Food Safety Authority, 2008 and the International Agency for Research on Cancer, as carcinogens such as benzo[a]pyrene, benz[a]anthracene, chrysene[10]. Although certain PAHs have not been identified as carcinogenic, they are an effective tool in animal studies for causing non-genotoxic diseases[8] [5][20].

Literatures reported that air pollution caused the existence of PAHs in the raw fruits and vegetables [11][18][19]. The fruits and vegetables grown near urban area were contaminated with high concentration of PAHs [15]. With rapid developments in Malaysia, the level of urbanization has increased and thereby increasing the chance of contamination of PAHs on fruits and vegetables. Previous experiments have shown that vegetables grown near toxic areas are contaminated with higher rates of PAHs that are sufficient to risk human health [12][14]. Moreover, fruits and vegetables could be contaminated with PAHs during long-term distance transportation [1]. Hence, corruption of PAHs in fruits and vegetables needs to be investigated and examined.

There is impossible for the direct detection of PAHs from food samples without pre-treatment because the food samples contain complex components such as lipid, phytocomponents, and phenolic compounds which can cause severe interference and thereby misinterpreted results[21]. Because of this fact, possibly the best test planning is important before chromatographic analysis for the preconcentration and removal of PAHs from the unknown solution.

Recently, many types of new sample preparation techniques using minimum organic solvents have been developed. One of them is microextraction of dispersive liquid-liquid (DLLME). This approach has shown outstanding features like rapid, economical, quick, fast recovery and strong effective parameters. Increasing interest was received from researchers [3]. However, DLLME has several drawbacks such as the existence of matrix interference was reported for extraction of PAHs using DLLME technique [4]. While dilution was used to minimise the influence of matrix interaction, the presence of matrix factors had been available and combined with solvent extraction. This triggers the difficulties of moving the clean extraction process for chromatographic examination, and this issue has been minimised by high amounts of extraction solvent. It is important

to add a clean-up phase prior to DLLME extraction in order to address the matrix intervention of DLLME

Nowadays, one of the famous pre-treatment methods called microwave-assisted extraction (MAE) has been used by researchers due to its simplicity, usage low volume of organic solvent, cost effective, short extraction time and high recovery [2]. By utilizing appropriate parameters, unfavorable thermal-labile compounds such as polysaccharides, proteins and phenolic compounds can be degraded. Therefore, the matrix interference could be minimized. The combined MAE and DLLME method is unique to the results related and has not been used in samples of fruits and vegetables that extract PAHs.

The benefits exist improving the efficiency of MAE-DLLME in vegetable samples for the determination of PAHs were discussed in a existing article [17]. An objective of present paper reported here is to compare the analytical performances, such as sensitivity, Quantification Limit (LOQ), Detection Limit (LOD), Relative Recovery of Extraction (ER), and Relative Standard Deviation (RSD) the MAE-DLLME with others DLLME techniques. The matrix interference was reduced in the study thus the chromatogram was clear and extraction efficiency was increased. This microwave-assisted extraction coupled with DLLME was found to be more sensitive and time-saving than other conventional DLLME techniques.

2 Experimental

The highest purity and analytical reagent grade of solvents were used in this study. Acetone and methanol were bought fisher Scientific's (Loughborough, U.K.). (Loughborough, U.K.). Merck obtained a solvent of 1-bromo-3-methylbutane (extraction solvent) (Darmstadt, Germany). Initial mixture standard of 13 PAHs (EPA 525 PAH Mix B, 500 µg/mL per acetone) and the internal biphenyl standard (2000 µg/mL for methanol) were purchased from A local (U.S.A.)

The mixture standard of thirteen PAHs was diluted to 10 mg/L in acetone. It was used as stock solution and stored at 4°C in a refrigerator. The proper concentrations of working standard solutions of a mixture of 13 PAHs were freshly prepared daily from stock solution. Biphenyl was employed as an internal standard to counterpoise the volume changes of the sample.

2.1 Sample Preparation

The PAHs free vegetable was homogenized, sieved, filtered and centrifuged. After that, the supernatant was filtered using nylon membrane filters and the filtered substance was poured into bottles. Thereafter, the filtered substance was spiked with 13 PAHs and biphenyl (internal standard) prior to the extraction process of MAE-DLLME or others DLLME technique.

2.2 MAE-DLLME

Acetone was used for the extraction of PAHs in MAE process. The volume of 10 mL spiked sample was added into glass bottle containing 4 mL acetone and was microwaved in microwave oven (Samsung, ME711K) at 200 W power for 1.5 min. It had been refrigerated to room temperature and then centrifuged for 5 minutes at 4000 rpm. Then, the aqueous phase remained transferred directly to the centrifuge tube. In the sample solution, 0.8 mL acetone (dispersive solvent) and 30 μ L 1-bromo-3-methylbutane (extraction solvent) were added and gently shaken. The sedimented organic phase was designed at the bottom of the centrifuge tube after a centrifugation process at 4000 rpm for 3 minutes. Then, in a two mL vial, the sediment coating was moved to the crystal insert. For analysis, the amount of one using an autosampler, μ L of the organic process was extracted into GC-FID.

2.3 Conventional DLLME

The mixture of 100 μ L acetone (dispersive solvent) and 50 μ L tetrachloroethylene (extraction solvent) are quickly squeezed into the centrifuge tube containing the spiked sample and the internal norm by means of a microvolume syringe in the traditional DLLME. The mixture are shook at 3000 rpm for 10 minutes, homogenised (vortex) and centrifuged. The organic layer were removed using a syringe and a volume of one μ L of the organic layer is injected into GC-FID.

2.4 Modified DLLME

The modified DLLME was proposed by the previous paper [4]. In modified DLLME, the solvent of 100 μ L of 1-bromo-3-methylbutane was used as extraction solvent and there is no addition of dispersive solvent. Others parameter were performed using method described in Conventional DLLME.

2.5 Instrumentation

The separation and evaluation of 13 PAHs are performed out using a flame ionisation detector combined with an Agilent 7890A gas chromatograph (FID). The GC with a split/splitless injector system is coupled with an automatic liquid sampler (Agilent 7683B). The GC-FID instrument and its data analysis were controlled by Agilent Chemstation (B.04.02 version). Four types of gases were used namely helium, nitrogen, hydrogen and compress air and were purchased from Air products, U.K. For the separation of hydrocarbon compounds, oxygen gas and hydrogen in carrier gas (helium gas, 99.9995 basis points) with a flow rate of 1.67 mL/min, the Agilent Broad Universal Trap-Helium purge (model RMSN-2) was used. The splitless mode was used in injection port which held at 290°C with 40 mL/min purge flow to split vent at 3 min. The 13 PAHs and biphenyl (internal standard) was

separated by a Capillary column SLB-5ms (30 m x 0.25 mm ID, 0.25 μm thickness). The temperature of the oven was set as follows: from 70 °C (one min), increased to 120 °C at 20 °C/min (1 min); ramped at 10 °C/min to 258 °C and preserved for one min; increased again at 1 °C/min to 262 °C (two min); then ratcheted at 5 °C/min to 280 °C and kept for 5.1 min. The total time of the run was 36 minutes. The temperature of the FID detector was held at 300 °C. At a flow of 35mL/min, nitrogen gas and hydrogen gas were handled, while the forced air flow for FID was 350mL/min

3 Results and Discussions

The chromatogram and retention time for EPA 525 PAH Mix B standard mixture and biphenyl are shown in Fig. 1 and Table 1. All of the 13 PAHs and internal standard were eluted within 36 minutes. Based on the chromatogram (Fig. 1), the elution order for the 13 PAHs and biphenyl was in order of increasing molecular weight. Biphenyl (9.22 min) with lowest molecular weight was first eluted and IcP with largest molecular weight was last eluted (33.39 min). The elution order of PAHs was confirmed with the reference of certificate of analysis given by the supplier (Sigma-Aldrich, USA). All the peaks were successfully separated with good resolution except BbF(25.29 min) and BkF (25.44 min). The peaks of BbF and BkF were coeluted. The This same temperature is set to increase from 258 °C to 262 °C at a rate of 1 °C/min but perfect separation still could not be achieved. The similar physical properties of these two PAHs might cause the separation become difficult. In the future, narrow column could be used to obtain better separation[6].

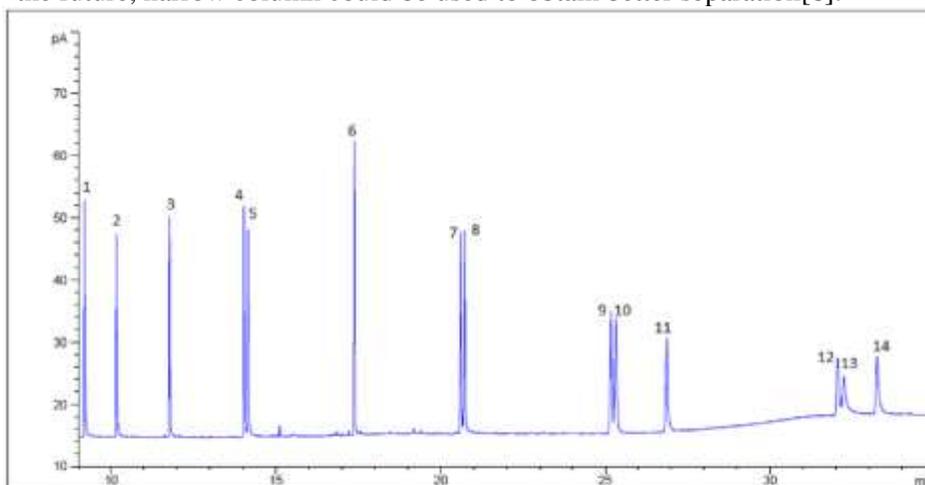


Fig 1. Chromatogram of EPA 525 PAH Mix B standard mixture and biphenyl (internal standard) by GC-FID.

The previous paper [17] has discussed the optimization work of MAE-DLLME for the extraction of PAHs in samples of vegetables. The proposed template MAE-DLLME has generated in reducing the extraction time and the utilization of organic solvent. The brominated solvent of 1-bromo-3-methylbutane (30 μ L) yielded highest average relative extraction recovery (ER) among the seven investigated solvents. It can be considered as a nonpolar solvent since it does Weakness to form polar correlations with hydrogen bonds and hydrophilicity. In contrast, the bromine atom will generate a halogen bond with PAHs. In general, 1-bromo-3-methylbutane is less toxic than other solvents investigated and was chosen in the study as an extraction solvent. Due to the dilution effect, the weighted mean enrichment factor (EF) decreased from 50 μ L with increasing volumes of extraction solvent. For the start of the class, however, 50 μ L was provided. However, after MAE, the content of the extraction solvent can be reduced from 50 μ L to 30 μ L pre-treatment because of the elimination of matrix interference. In the selection of dispersive solvent, no remarkable variation was obtained using acetone, acetonitrile and methanol. Acetone (0.8 mL) was chosen in this study because it is less toxic and low cost. The extraction time of 1 minute was selected because there On average, the standard relative ER was not meaningful for 1 to 3 minutes. That's because of the rapid formation of droplets of extraction solvent in the cloudy sample. The high dielectric constants and dissipation factor of acetone can rapid absorb microwave energy and turn the energy toward either thermal capacity. The heat degraded the involvement of the matrix and thus improved the extraction efficiency. In MAE, acetone was preferred as an optimum solvent. The average relative ER was an average of increased using 200 W and 300 W of microwave power. The due mainly to the pressure and temperature being too high, the sample was destroyed at 400 W. The power of 200 W was selected as it utilized less than 300 W of energy. The average relative ER was observed to increase from 0.5 min to 1.5 min of absorption spectra and then reduce. Short irradiation time may not be enough to degrade matrix interference and PAHs have also been degraded by too long irradiation time. The optimum irradiation time was set at 1.5 minutes in this experiment

3.1 Comparison of MAE-DLLME Performances vs. Modified DLLME and Conventional DLLME

In order to compare the method performances of MAE-DLLME with modified DLLME and conventional DLLME, different validation parameters including sensitivity, LOD, LOQ, relative ER and RSD were investigated. Triplicates analyses were run for each test.

Table 1. Peak Identification and retention time of EPA 525 PAH Mix B standard mixture and biphenyl

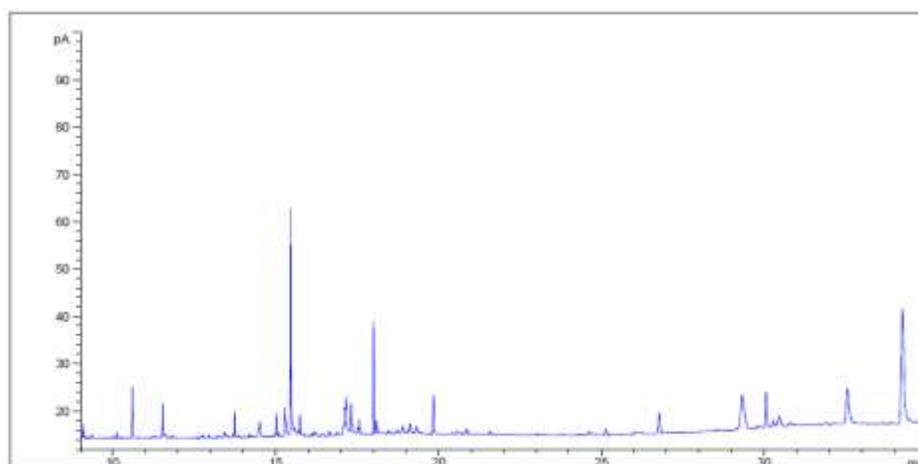
Peak No. ^a	PAHs	Abbreviation	Retention time (min)
1	Biphenyl	BIP	9.22
2	Acenaphthylene	ACY	10.19
3	Fluorene	FLU	11.80
4	Phenanthrene	PHE	14.06
5	Anthracene	ANT	14.18
6	Pyrene	PYR	17.41
7	benzo[a]anthracene	BaA	20.66
8	Chrysene	CHR	20.79
9	benzo[b]fluoranthene	BbF	25.29
10	benzo[k]fluoranthene	BkF	25.44
11	benzo[a]pyrene	BaP	26.97
12	Dibenz[a,h]anthracene	DahA	32.16
13	Benzo[ghi]perylene	BghiP	32.34
14	Indeno[1,2,3-cd]pyrene	IcP	33.39

^a Peak no is based on the number marked in Fig. 1.

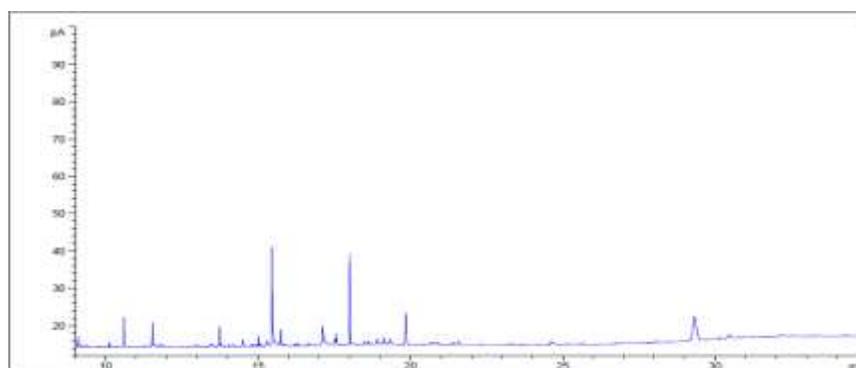
3.1.1 Sensitivity

According to the Western European Laboratory Accreditation Conference (WELAC), selectivity is characterised as a measure of the degree to which a certain analyte in tests could be calculated by the suggested protocol without intervention from the matrix substances in the device.

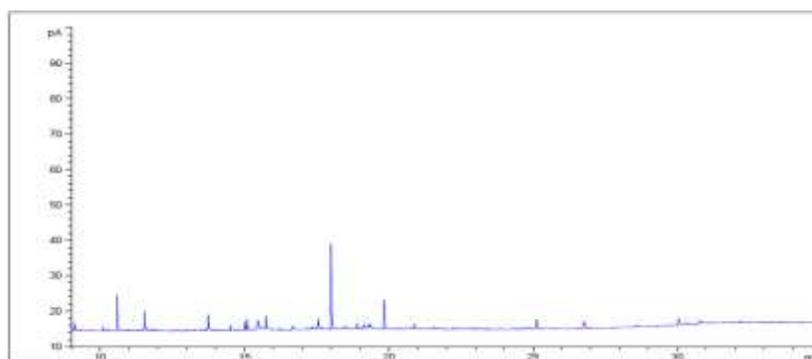
In this study, the selectivity of the three techniques was evaluated and the blank cabbage sample chromatograms (Fig. 2) using (a) generic DLLME (b) modified DLLME (c) MAE-DLLME and (d) spiked cabbage extracts using MAE-DLLME (b) modified DLLME (c) were compared. Based on Fig. 2 (a), (b) and (c), the number of the peak and the peak height of MAE-DLLME were much lesser and lower compare to conventional DLLME and modified DLLME although there are some interferences were still not fully eliminated. Moreover, when compared Fig. 2 (c) and (d), no interference has the same retention time with PAHs. Therefore, MAE-DLLME can be said to have the best selectivity among the three DLLME techniques. The chromatograms also proved the effectiveness of MAE on degradation of matrix interference and solved the common problem of matrix interference in conventional DLLME.



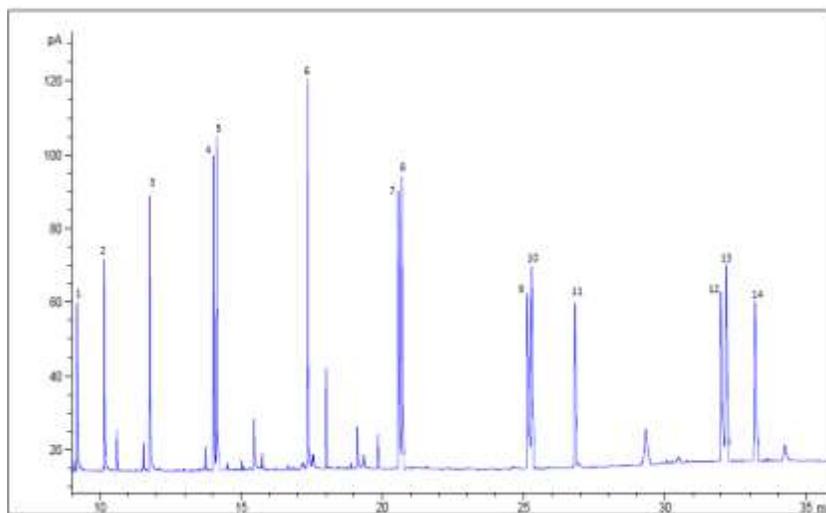
(a)



(b)



(c)



(d)

Fig 2. Blank cabbage sample chromatograms using (a) normal DLLME (b) modified DLLME (c) MAE-DLLME and (d) spiked cabbage sample using MAE-DLLME. Y-axes and X-axes were peak area and retention time (min), respectively. The peak order was followed as described in Table 1.

3.1.2 Limits Of Detection (LOD) and Limits Of Quantification (LOQ)

The lowest concentration of proteins in a sample that can still be measured is LOD but not generally quantitated using the proposed procedure [9]. The LOD measurement was carried out by analysing blank samples that spiked until a peak height of 3 times greater than the noise level was obtained with a reduced concentration of PAHs. In a sample, the LOQ is the lowest quantity of analyte that could be quantitatively measured with sufficient precision and accuracy [9]. The LOQ measurement was done by evaluating blank samples that spiked until a peak height of 10 times higher than the noise level was reached with reduced PAH concentration.

The limits of detection (LOD) and quantifications (LOQ) for 13 PAHs using three DLLME techniques were determined. The results from Table 2 clearly show that under the present experiment conditions, MAE-DLLME is the most sensitive technique among the three techniques. The LOD range was between 0.02 to 0.08 $\mu\text{g/L}$ in MAE-DLLME, 0.05 to 0.20 $\mu\text{g/L}$ in modified DLLME and 0.08 to 0.30 $\mu\text{g/L}$ in conventional DLLME. For LOQ, the ranges were varied from 0.08 to 0.30 $\mu\text{g/L}$ in MAE-DLLME, 0.20 to 0.80

$\mu\text{g/L}$ in modified DLLME and 0.30 to 1.20 $\mu\text{g/L}$ in conventional DLLME. The LOD and LOQ in MAE-DLLME were two times and four times lower than that in modified DLLME and conventional DLLME, respectively. The highest sensitivity of the MAE-DLLME would be a considerable advantage after the microwave-assisted extraction (MAE) pre-treatment.

3.1.3 Relative Extraction Recovery (ER) and Relative Standard Deviation (RSD)

In this study, the relative extraction recovery (ER) was employed for expressing the results by calculating as the ratio between the ER resulting from the studied variable using vegetable sample and the ER achieved using distilled water within the working range. The relative ER and the RSD results are shown in Table 3. The results indicate that the three types of DLLME techniques were able to extract 13 PAHs from cabbage samples. MAE-DLLME yielded relative ER ranged from 83.65% to 98.42% with the RSDs values from 0.65% to 3.07%. Modified DLLME yielded relative ER ranged from 56.83% to 99.03% with the RSDs varied from 0.13% to 7.48%. In conventional DLLME, relative ER ranged from 55.55% to 89.67% with the RSDs ranged from 2.52% to 7.67%.

Most of the relative ERs and RSDs values between MAE-DLLME and modified DLLME were almost similar. However, modified DLLME extracted PYR, BaA and BbF with low relative ER (<70%) which were less than acceptable range (70%-120%). Overall, the relative ERs and RSDs obtained by MAE-DLLME and modified DLLME were better than conventional DLLME. This might caused by the appearance of the matrix interference obstructed the mass transfer of PAHs compounds into organic phase in conventional DLLME.

Table 2. Limits of Detection (LOD) and Limits of Quantification (LOQ) for MAE-DLLME, modified DLLME and conventional DLLME.

Analytes	LOD ($\mu\text{g/L}$, $\times 10^{-2}$)			LOQ ($\mu\text{g/L}$, $\times 10^{-2}$)		
	MAE DLLME	Modified DLLME	Conventional DLLME	MAE DLLME	Modified DLLME	Conventional DLLME
ACY	2	5	8	8	20	30
FLU	2	5	8	8	20	30
PHE	2	5	8	8	20	30
ANT	2	5	8	8	20	30
PYR	2	5	8	8	20	30
BaA	2	5	8	8	20	30
CHR	2	5	8	8	20	30
BbF	5	15	20	20	60	80
BkF	8	20	30	30	80	120
BaP	5	15	20	20	60	80
DahA	2	5	8	8	20	30
BghiP	4	10	15	15	40	60
IcP	2	5	8	8	20	30

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Table 3. Relative Extraction Recovery (ER, %) and RSD (% , n=3) for MAE-DLLME, modified DLLME and conventional DLLME

Analytes	MAE-DLLME		Modified DLLME		Conventional DLLME	
	Relative ER	RSD%	Relative ER	RSD%	Relative ER	RSD%
ACY	94.74	1.251	96.16	0.165	89.67	4.798
FLU	88.45	2.773	99.03	0.958	88.99	5.509
PHE	92.60	3.072	84.05	0.132	84.52	6.280
ANT	94.09	2.197	78.67	0.882	86.94	5.428
PYR	98.42	2.426	56.83	1.416	76.26	4.646
BaA	94.06	1.790	66.89	1.790	78.09	3.587
CHR	84.12	1.790	87.45	1.790	70.90	4.108
BbF	86.78	2.107	59.25	2.107	74.15	7.629
BkF	93.49	0.645	82.71	0.645	80.85	4.736
BaP	86.46	1.301	84.61	1.301	69.01	2.521
DahA	92.38	1.610	90.13	1.610	70.05	5.577
BghiP	87.46	1.426	77.10	7.482	80.20	7.670
IcP	83.65	1.858	82.66	6.350	55.55	3.943

Table 4. Experimental conditions of MAE-DLLME, modified DLLME and conventional DLLME

	MAE-DLLME	Modified DLLME	Conventional DLLME
Extraction solvent	1-bromo-3-methylbutane (30 µL)	1-bromo-3-methylbutane (100 µL)	Tetrachloroethylene (50 µL)
Dispersive solvent	Acetone (0.8 mL)	-	Acetone (0.1 mL)
Extraction time (s)	60	120	120

Table 4 shows the experimental conditions of MAE-DLLME, modified DLLME and conventional DLLME. In MAE-DLLME and modified DLLME, the especially hazardous solvent tetrachloroethylene (LD50 2629 mg/kg) was accompanied by 1-bromo-3-methylbutane (LD50: 6150 mg/kg) as an extraction solvent to extract 13 PAHs. Brominated solvent for extraction, 1-bromo-3-methylbutane (C₅H₁₁Br) is low solubility in water (196 mg/L) because it is non-polar solvent and does not interact with water molecules.

This behavior prohibits the interaction of extraction solvent with the interferences from the water or complex matrix (vegetables). Furthermore, the targeted 13 PAHs contain alkyl aromatic ring substitutions which are non-polar compounds have large affinity on the implied solvent extraction (1-bromo-3-methylbutane). In modified DLLME, 100 μL of 1 as a dispersive solvent, 1-bromo-3-methylbutane was used as an extraction solvent with no organic solvent. To enable the dispersion of extraction solvent, Vortex was being used to boost. However, the extraction efficiency of the modified DLLME still affected by the matrix interferences. In conventional DLLME, the solvent of 50 μL of tetrachloroethylene and 100 μL of acetone were used. Although conventional DLLME required high dispersive solvent volume in order to receive acceptable extraction recovery, tetrachloroethylene is highly toxic compared to the 1-bromo-3-methylbutane.

The time interval between the injection into the sample of the extraction solvent and the dispersive solvent and before centrifugation is specified in DLLME as the extraction time [13],[7]. Due to the wide surface area, the fast mass transfer of PAHs into the organic phase is of droplets [16]. MAE-DLLME is much faster extraction method given that the results were obtained after 60 seconds instead of 120 seconds as it were in the case of modified DLLME and conventional DLLME.

4 Conclusion

Compared to the updated DLLME and traditional DLLME, the evolved MAE-DLLME has various benefits in the current analysis. Prior to sampling, no harmful materials were used for sample preparation. Matrix interference in vegetable samples can be minimised without chemical digestion by microwave heating. After MAE pre-treatment, the sensitivity, LOD and LOQ, relative recovery and RSD values were increased. In comparison, in the present the extraction solvent used was less toxic in the test than in past findings. In addition, in order to fix and extract PAHs from vegetable samples, small amounts of solvent were needed for MAE-DLLME. The extraction time is low and the process is quick, so the innovative MAE-DLLME-GC-FID used in this study is due to its speed, cost-effectiveness, simplicity and environmentally friendly extraction of PAHs from vegetable samples represents a powerful alternative to the traditional DLLME technique.

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Biographies



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