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MULTIRESIDUE ANALYSIS FOR TWENTY-FOUR POLYCYCLIC AROMATIC HYDROCARBONS IN EDIBLE OILS BY ULTRASOUND-ASSISTED EMULSIFICATION MICROEXTRACTION AND SOLID-PHASE EXTRACTION CLEAN-UP FOLLOWED BY GC-MS/MS

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ABSTRACT

In this study, an analytical method for simultaneous determination of 24 polycyclic aromatic hydrocarbons (PAHs) comprising of 16 Environmental Protection Agency (EPA) priority PAHs and 15+1 European Union (EU) priority PAHs in edible oil was proposed by gas chromatography-tandem mass spectrometry (GC-MS/MS). An efficient ultrasound-assisted emulsification microextraction (USAEME) method was adopted by using dimethyl sulfoxide (DMSO) as extracting solvent followed by solid-phase extraction (SPE) clean-up. Under optimum conditions, the calibration curves showed a good linearity in the range of 1 to 50 µg/L with correlation coefficients greater than 0.995. The limit of detections (LODs) for 24 PAHs were 0.12 – 0.32 µg/kg. Using the spiked oil sample at three levels, the mean recoveries were 81 – 115% and the relative standard deviations (RSDs) in term of reproducibility were below 17.7%. Trueness assessment was further participated in international olive oil proficiency test (FCCE1-OIL22-06145) organized by the Food Analysis Performance Assessment Scheme (FAPAS) with satisfaction result (z-score < 2). The method was successfully applied to real oil samples and the results suggested that an extension to large-scale study on the PAHs contamination in edible oil in Vietnamese market is encouraged.

Keywords: GC-MS/MS, edible oil, PAHs, USAEME.

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are large groups of organic compounds which contain two or more aromatic rings. Some of them are found to be strong mutagenic and carcinogenic to human [1]. Based on the occurrence and carcinogenicity of PAHs compounds, 16 of them are listed as priority pollutants by the United State Environmental Protection Agency (16 EPA priority PAHs) [1]. In addition, the European Food Safety Agency (EFSA) has replaced eight high molecular weight PAHs by eight low molecular weight PAHs (listed in 16 EPA priority PAHs) and they are called as the 15 + 1 EU priority PAHs list [2]. To meet food safety regulations for human consumption, the European Commission has set the maximum residue limits (MRLs) in edible oils and fats at 2 µg/kg for benzo[a]pyrene, and at 10 µg/kg for sum of 4 PAHs (called as PAH4 including: benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene) [3].

For non-smoker occupants, food consumption especially edible oils and fats is known as main contributor of intaking PAHs during human diet. The PAHs contamination in edible oils

and fats is found to be from the processing such as direct smoke-drying and solvent extraction, and from raw materials as a consequence of environmental pollution [4, 5]. The PAHs levels and health risk assessment have been studied in previous works [6, 7].

Determination of PAHs in oil sample is challenge due to their low levels and high similarity in non-polarity with oil components. The PAHs analysis is commonly comprised of two parts: sample preparation and measurements usually by HPLC-FLD (high performance liquid chromatography – fluorescence detector) and GC-MS. Based on literature review, the extraction of PAHs from oil matrix are usually conducted by liquid-liquid extraction [6], solid-phase extraction [8], ultrasonication extraction [9], pressurized liquid extraction [10], etc... After extraction, further purification is necessary to remove any co-extract from complex oil matrices. This step is often completed by using solid-phase extraction (SPE) [8] and gel permeation chromatography [9]. To gain a purpose of green analytical chemistry during chemical analysis, ultrasound-assisted extraction has been applied [11]. The advantages of this technique are approved in previous studies [11, 12]. To minimize the consumption of organic solvents, ultrasound-assisted emulsification microextraction (USAEME) using micro volume of extracting solvent has been proposed [12]. However, most previous studies have been conducted for water samples [13-15]. Currently, there is a little information regarding this method (i.e., USAEME) for edible oil samples.

The objective of this work was to develop an effective and reliable analytical method based on USAEME method and SPE clean-up followed by GC-MS/MS to simultaneously measure 24 PAHs in edible oil. The 24 PAHs in this work consist of all 16 EPA priority PAHs and 15+1 EU priority PAHs lists. In USAEME, dimethyl sulfoxide (DMSO) was selected as effectively extracting solvent due to its high affinity to PAHs compounds (based on π-π interaction between sulfur atom in DMSO and the aromatic π electron system). The extraction conditions including ultrasonication time, solvent volume, sample amount and numbers of extraction were optimized. For trueness assessment, olive oil reference material (FCCE1-OIL22-06145) was used. The validation method was assessed in accordance to AOAC 2016 Appendix F [16]. Finally, primary results on PAHs concentrations in edible oil collected from local market were also presented.

2. MATERIALS AND METHODS

2.1. Materials

Twenty-four PAHs standards (including 16 EPA priority PAHs and the 15+1 EU priority PAHs lists) and six internal standards are purchased from LGC group (UK) with > 96% purity (Table 1). All organic solvents including acetonitrile (ACN), n-hexane, DMSO and methanol are HPLC grade and purchased from Merck (German). The C18 SPE cartridge from Merck is used for purification. Olive oil sample (code: FCCE1-OIL22-06145) for proficiency testing are from Food Analysis Performance Assessment Scheme (FAPAS, UK). All edible oil samples are obtained from local market (Ho Chi Minh City, Vietnam).

2.2. GC-MS/MS

The TSQ 9000 GC-MS/MS system from Thermo (US) are utilized for PAHs analysis. The separation of 24 PAHs is conducted on TG-PAH column (60 m × 0.25 mm; 0.1 μm). The conditions for GC-MS/MS analysis are as follows: solutions are injected in splitless mode with injection volume of 1 μL. The injector temperature is held at 300 °C. Helium is used as carrier gas with flowrate of 1 mL/min. The oven temperature program is held at 70 °C for 1 min, increased to 270 °C at a rate of 10 °C/min and then increased to 350 °C at a rate of 2 °C/min.

Finally, temperature is held at 350 °C for 10 min. The transfer line and ion source temperature are set at 300 °C and 300 °C, respectively. Electron ionization is maintained at 50 eV. Multiple reaction monitoring (MRM) mode is adopted for the quantitation. Detailed information on the MS parameters of 24 PAHs is shown in Table 1.

2.3. Sample preparation

Two g (2 ± 0.05 g) of oil sample was weighted into 50 mL glass centrifuge tube, then 100 µL of the mixed internal standard solution and 2 mL of hexane were added. The sample was mixing for 30 s on a vortex shaker. Next, 2 mL of DMSO was added and homogenized for 30 s by shaking on a vortex shaker. For extraction, the mixture was immersed into an ultrasonic water bath. After 10 min sonication at 40 kHz of ultrasound frequency, 100 W of power and 20 °C of the water bath temperature, the mixture was centrifuged at 10000 rpm for 10 min to separate two phases. Then, the bottom layer was carefully transferred into a new glass test tube using a Pasteur pipette, and the extraction was repeated two times with each of 2 mL DMSO. All extracts were pooled and mixed well with 12 mL of deionized water before passing through C18 SPE cartridge for purification.

For purification, the C18 SPE cartridge was preconditioned with 10 mL of methanol and 10 ml of DI water. Then, the extract was transferred into the SPE cartridge and 10 mL of water was used to wash the cartridge. Finally, 20 mL of hexane was used to elute the analytes. The eluant was evaporated to near dryness using rotary vacuum evaporator and then the residue was re-dissolved into 2 mL of ACN before injecting to GC-MS/MS.

2.4. Method validation

Parameters of the method validation including matrix effect, linearity, limit of detection (LOD), limit of quantitation (LOQ), recovery and precision were conducted in agreement with AOAC 2016 Appendix F [16]. Matrix effect (%ME) was determined by dividing slope coefficients from solvent-based ($a_{solvent}$) and matrix-matched (a_{matrix}) calibration curves (%ME = a_{matrix} /$a_{solvent}$ − 1). The mixtures containing 24 PAHs and internal standards of the working standard solutions were prepared by dilution of the stock standard solution in ACN with the range from 1 to 50 µg/L. Then, calibration curve was constructed by plotting the ratio (y) of individual PAHs peak area to IS peak area versus the PAHs concentration (x). LOD values were evaluated by analyzing the spiked samples (n = 12) with minimum concentration of each PAHs (1 µg/kg) which gave a signal to noise ratio of 3 (S/N ~ 3). Then LOQ values were calculated by multiplying LOD values with the factor of 10/3 (LOQ = 10/3*LOD). Recovery experiments were conducted by using spiked oil samples with PAHs mixtures at three levels (1, 2 and 10 µg/kg). In addition, precision in term of repeatability and reproducibility was performed at the same spiked levels. At each spiked level, the measurements were carried out by replicate injections (n = 10) of the extracting samples. Then, recoveries were calculated from differences between the spiked and found PAHs concentrations. Precision was expressed through the relative standard deviations (%RSD).

The trueness and suitability of this method were further elucidated by participating in international olive oil proficiency test (FCCE1-OIL22-06145). Finally, several oil samples from local market were analyzed.

3. RESULTS AND DISCUSSION

3.1. GC-MS/MS conditions

Column selection: In this work, we compared the separation ability of 24 PAHs by using TG-5MS and TG-PAH column. Figure 1 shows a comparison of separation ability of several PAHs between TG-5MS and TG-PAH columns. We found that traditional chromatographic column (i.e., TG-5MS) is difficult to separate several PAHs even though this column is good separation ability for most non-polar and weak polar organic compounds. In contrast, 24 PAHs compounds is found to be well separated by TG-PAH column. Therefore, TG-PAH column is suitable for completely separating 24 PAHs in oil sample.

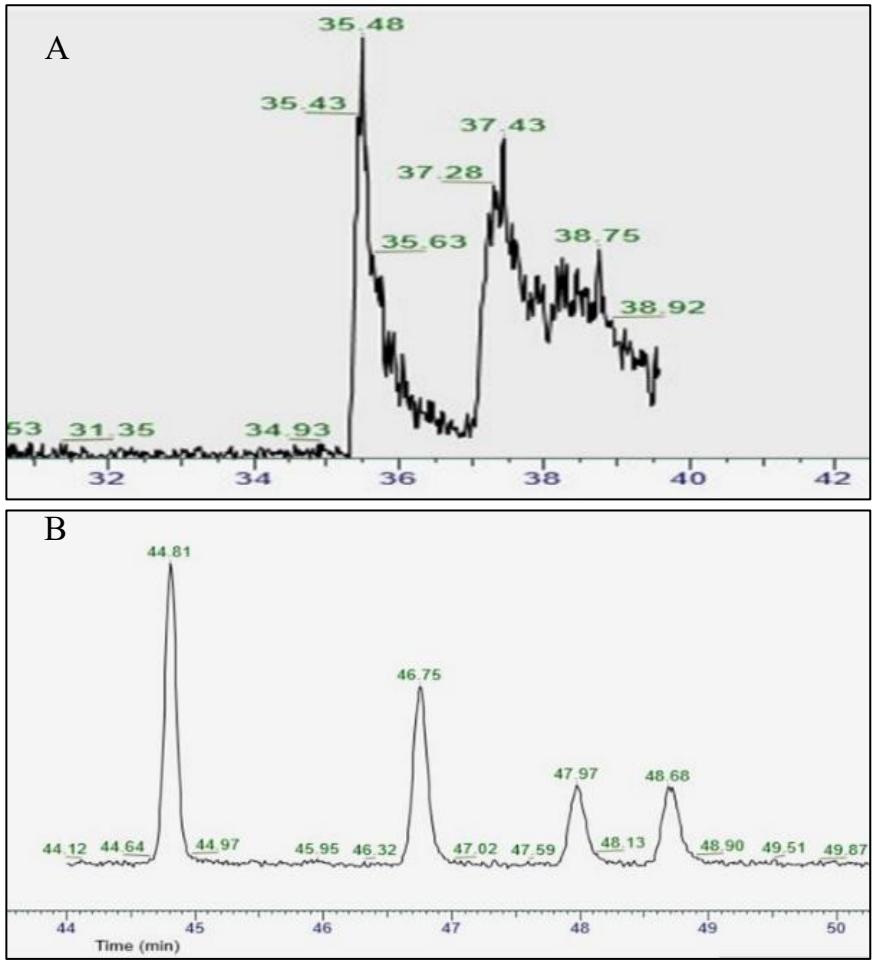

Figure 1. Chromatograms of dibenzo[a,h]pyrene, dibenzo[a,e]pyrene, dibenzo[a,i]pyrene and dibenzo[a,l]pyrene on TG-5MS (A) and TG-PAH (B) columns

Injection volume: Higher injection volume would improve the detection limit of the proposed method. Injection volume was increased from 1 µL to 3 µL; however, we found that the signal did not linearly increase with those of volume in the case of TG-PAH column. In contrast, when TG-5MS column was used, the signal linearly increased with increasing the injection volume. This result can be explained by the film of TG-PAH column (0.1 µm) is thinner than of TG-5MS column (0.25 µm). Figure 2 shows the effect of inject volume on chromatogram of pyrene as a typical example. Based on the result, injection volume of 1 µL is an optimum value, consistent with the previous study [17].

Table 1. GC-MS/MS acquisition parameters for 24 PAHs compounds

No	PAHs compounds	Abbr.	RT (min)	Quantitation ions		Confirmation ions			
				Transition MRM (m/z)	CE (eV)	Transition MRM (m/z)	CE (eV)	Transition MRM (m/z)	CE (eV)
1	Naphthalene	NAP	9.97	128 > 102	18	128 > 77.7	20	128 > 126.9	16
2	Acenaphthylene	ACE	13.87	152 > 150	40	152 > 151	40		
3	Acenaphthene	ACT	14.36	154.1>153.1	16	152.8 > 152.2	24	154.1 > 152.4	18
4	Fluorene	FLU	15.62	166.1>165.1	16	165 > 163	30	166.1 > 115	38
5	Phenanthrene	PHE	18.19	176 > 149.9	24	178 > 150.9	28	178 > 151.6	22
6	Anthracene	ANT	18.29	178 > 151.7	20	176 > 149.9	32	178 > 151	22
7	Fluoranthene	FLT	21.39	202 > 200	32	202 > 152.1	30	203.2 > 201.1	32
8	Pyrene	PYR	22.15	202.1 > 200	36	202.1 > 198.6	38	203.3 > 201	36
9	Benzo(c)fluoren	BcFL	23.18	216.1>215.1	22	216.1 > 189.1	30		
10	5-Methylchrysene	MCH	24.66	242 > 239	26	241 > 239	26	239 > 237	22
11	Benzo(a)anthracene	BaA	26.19	228.1>226.1	20	225.9 > 224.1	20	228 > 227.1	34
12	Chrysene	CHR	26.52	228 > 226.1	20	225.9 > 223.9	20	228 > 227.2	32
13	Benzo(b)flouranthene	BbFA	31.54	250 > 248.3	34	252.1 > 250.1	30		
14	Benzo(k)flouranthene	BkFA	31.66	250 > 248	32	252.2 > 250.1	25		
15	Benzo(j)flouranthene	BjFA	31.80	250 > 248	32	252.2 > 250.1	25		
16	Cyclopenta(c,d)pyrene	CCP	33.61	228 > 226.1	28	226 > 224.1	20	224 > 222.1	20
17	Benzo(a)pyrene	BaP	33.96	252.1 > 250	30	250 > 248	30	252 > 252	36
18	Indeno(1,2,3-cd) pyrene	IDP	41.16	274.1>73.3	10	274.1 > 271.8	35	276.1 > 275.3	25
19	Dibenz(a,h)anthracene	DBahA	41.20	278 > 276	35	278.1 > 277.4	10		
20	Benzo(g,h,i)perylence	BghiP	43.57	276.1 > 274	35	276.1 > 275.4	10		
21	Dibenzo(a,l)pyrene	DBalP	51.02	302.1>300.1	36	302.1 > 298.1	36		
22	Dibenzo(a,e)pyrene	DBaeP	53.50	302.1>300.1	36	302.1 > 276.1	28		
23	Dibenzo(a,i)pyrene	DBaiP	54.82	302.1>300.1	36	302.1 > 276.1	28		
24	Dibenzo(a,h)pyrene	DBahP	55.50	302.1>300.1	36	302.1 > 297.9	28		

Notes: Abbr.: abbreviation; RT: retention time; MRM: multiple reaction monitoring; CE: collision energy

MS conditions: Due to highly specific, selective and sensitive, MRM mode is adopted for quantitation of 24 PAHs compounds in this work. Firstly, full scan mass spectra of 24 PAHs compounds were applied to find the most abundant m/z ions. Then, mass confirmation was conducted by using NIST library. Based on ion cluster, abundance and m/z ratio, precursor ions for each PAH compound were selected. Collision energy for each product ions was also found in the product ion scan mode. The acquisition parameters of GC-MS/MS for 24 PAHs are shown in Table 1 and representative chromatogram is also depicted in Figure 3.

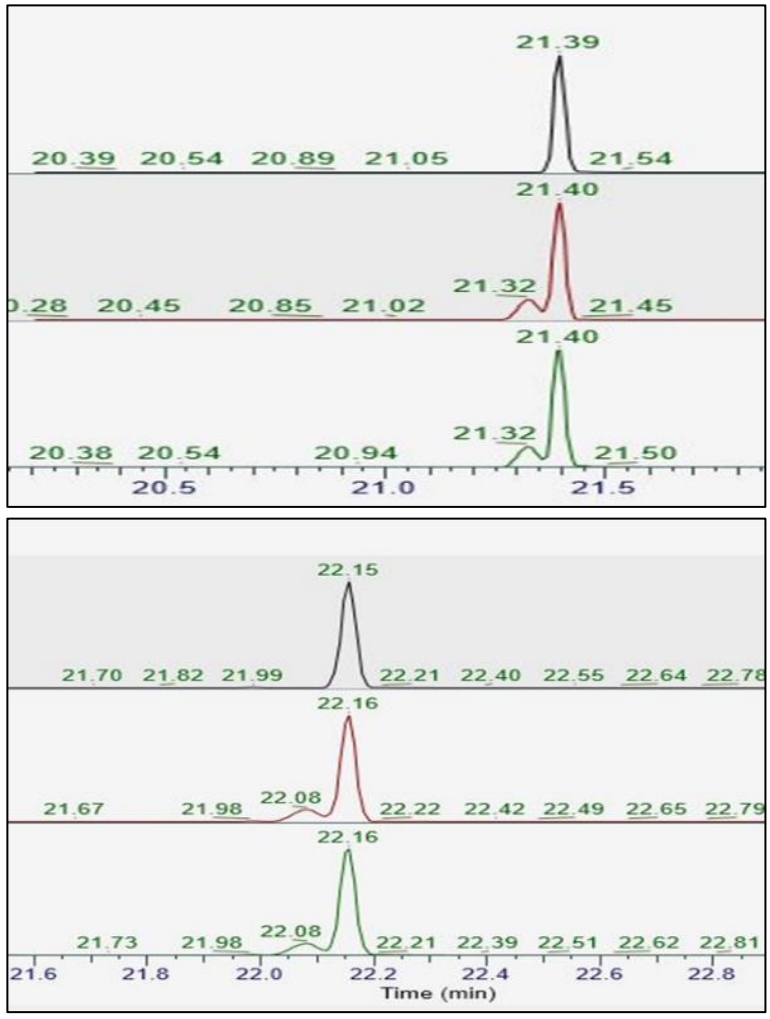

Figure 2. Chromatogram of fluoranthene (RT = 21.40 min) and pyrene (RT = 22.16 min) with different injection volume: 1 µL (top), 2 µL (middle), and 3 µL (bottom)

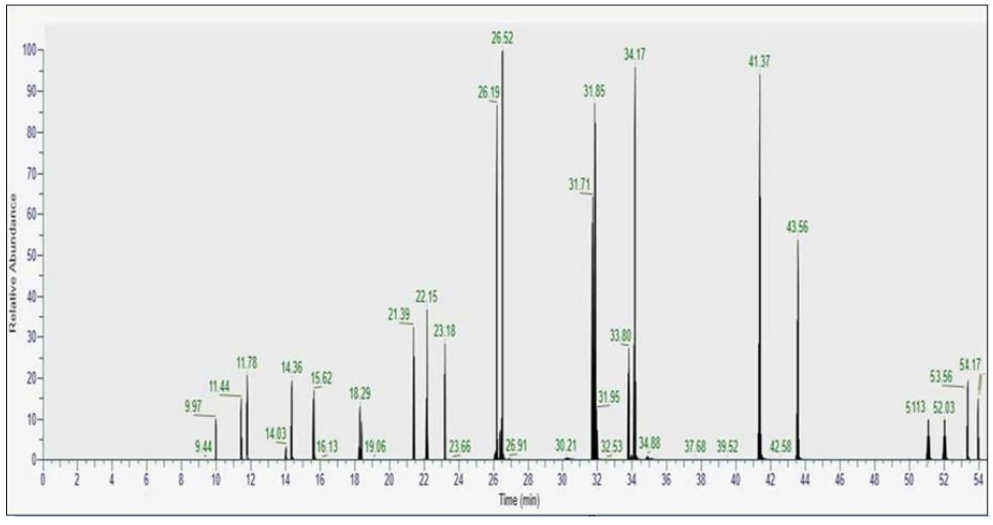

Figure 3. The TIC chromatogram of the PAHs standards

3.2. Optimization of sample preparation conditions

Ultrasonication duration: In this study, the extraction procedure comprised of liquid-liquid extraction and ultrasound-assisted emulsification microextraction; therefore, an ultrasonication duration is an important factor affecting on the recovery of the target compounds. As shown in Figure 4A, the recovery rates of the PAHs compounds are within 50 – 60% when no ultrasound (i.e., with 10 min for shaking) is applied. The recovery rates of the PAHs compounds increased with increasing the ultrasonication time. After 10 min of ultrasonic extraction time, the recovery rates reached around 90-112% for various PAHs, and no significant difference is obtained when extraction time is increased to 15 min (Figure 4A). Therefore, ultrasonication duration of 10 min is used as optimized condition.

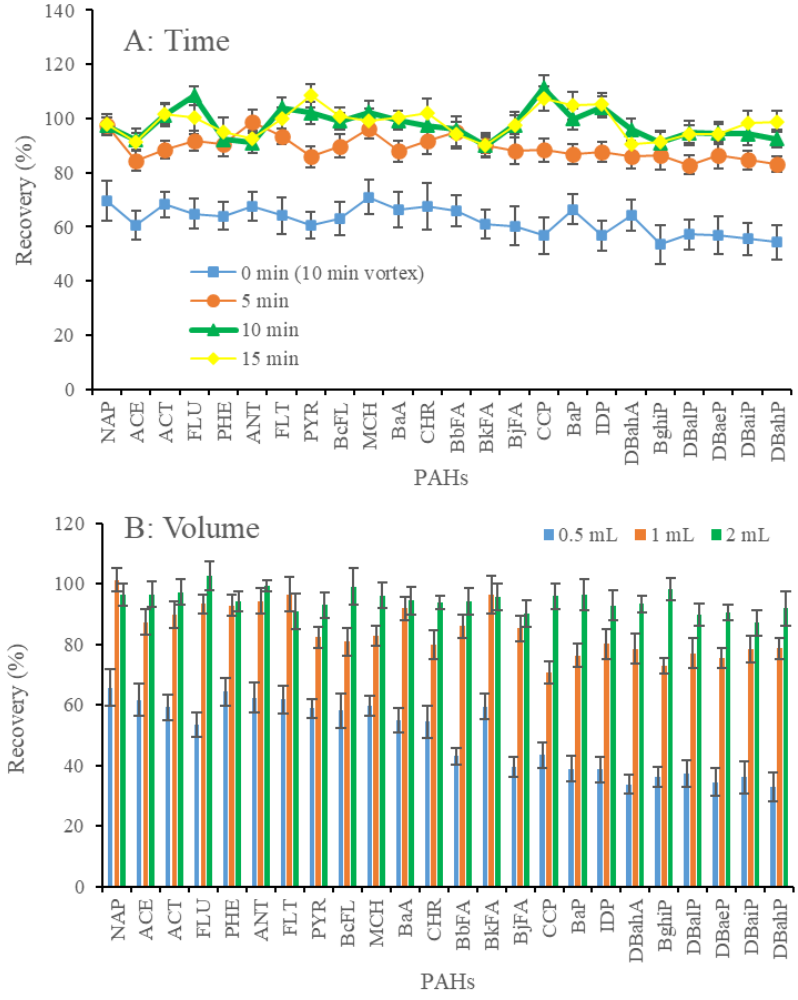

Figure 4. Effect of (A) the ultrasonication time; (B) the extracting solvent volume; (C) the sample amount; (D) the number of extractions. All experiments were repeated in triplicate (n = 3).

Solvent volume: Different volume of the extracting solvent (i.e., DMSO) is investigated to choose a suitable solvent volume. The DMSO volume of 0.5 mL is not effective to extract PAHs with the recovery rates of 30 – 60% (Figure 4B). When a solvent volume of 1 mL was used, the recovery rate was significantly increased for small molecular weight PAHs and medium molecular weight PAHs, but it was still low recovery for several high molecular weight PAHs. An improvement of the recovery rates for all PAHs is obtained when a solvent

volume is 2 mL. A volume of DMSO is expected to be a small as possible to reduce a viscosity of extract; therefore, a solvent volume of 2 mL is chosen for further study.

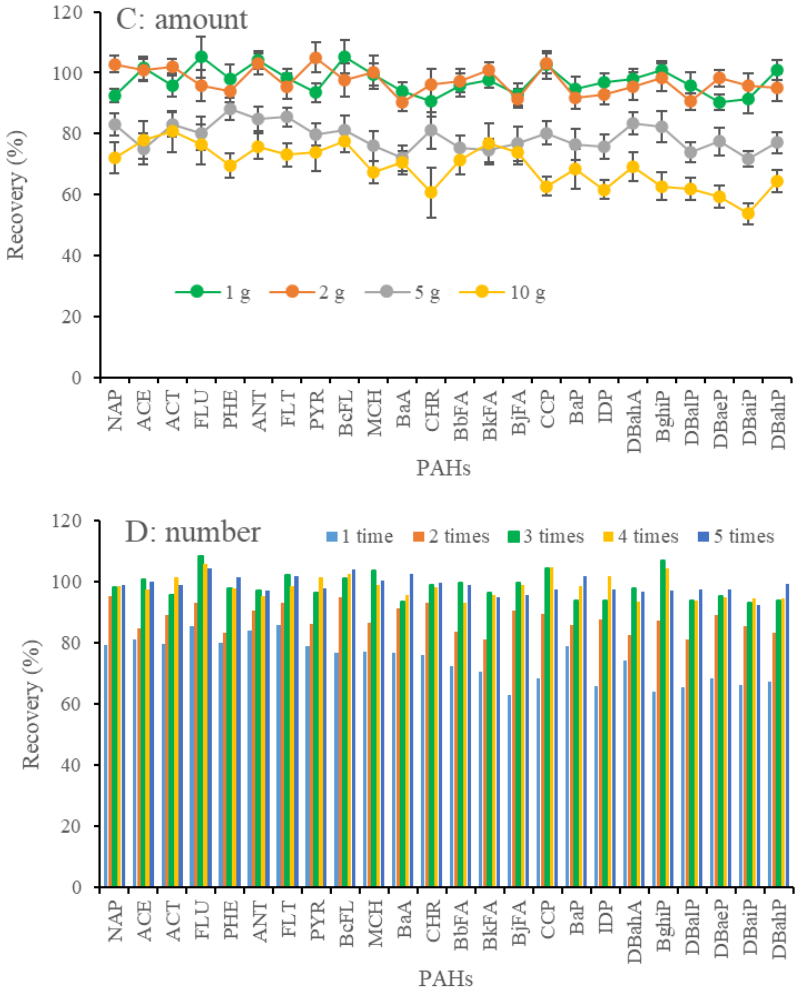

Figure 4. Effect of (A) the ultrasonication time; (B) the extracting solvent volume; (C) the sample amount; (D) the number of extractions. All experiments were repeated in triplicate (n = 3). (Cont.)

Sample amount: High amount of oil sample would improve the limit of detection; however, co-extract of several interfere compounds will occur stronger. Sample amounts of 1, 2, 5 and 10 g were studied. When sample amount increase, the recovery rates decrease as shown in Figure 4C, likely due to the impact of co-extract. In terms of detection limit and interference of co-extract, 2 g of oil sample was found to be suitable.

Number of extractions: PAHs and oil matrix have similar non-polarity in nature, while the extracting solvent (DMSO) has medium polar. Therefore, extraction needs to be repeated for several times to achieve a sufficient recovery. As shown in Figure 4D, the recovery rates of PAHs continuously increase with the increasing number of extractions. After three times of extraction, the recovery rates of all PAHs reached their values of 93 – 104%. Therefore, the extraction is repeated three times (3 x 2 mL of DMSO) as optimum conditions.

3.3. Method validation

Matrix effect: To investigate the effect of matrix effect (ME) on GC-MS/MS analysis, the value of %ME is estimated by dividing a slope coefficient from pure solvent calibration curve and a slope coefficient from matrix-matched calibration curve. The result shows that the %ME values ranged from 0.47% to 14.0% (Table 2). These values are within ±20%, indicating that co-extracting interferences are insignificant to affect the quantification of PAHs. Finally, the pure solvent standard solution is used to prepare the calibration curve.

Table 2. Recoveries, repeatability and reproducibility for the 24 PAHs

No	PAHs (*)	r	LOD µg/kg	LOQ µg/kg	%ME	Recovery % (repeatability %, reproducibility %)		
						1 µg/kg	2 µg/kg	10 µg/kg
1	NAP	0.9992	0.15	0.46	0.8	113.7 (2.92, 5.27)	103.1 (4.57, 5.91)	98.6 (5.66, 7.1)
2	ACE	0.9977	0.16	0.48	14.1	113.1 (3.12, 5.02)	103.2 (3.78, 3.24)	91 (4.05, 8.48)
3	ACT	0.9990	0.26	0.77	6.6	95.4 (8.27, 11.3)	92.1 (6.78, 7.91)	110 (5.78, 4.87)
4	FLU	0.9996	0.22	0.64	2.7	113.2 (2.48, 7.5)	104.4 (1.76, 2.62)	90.9 (4.89, 11.89)
5	PHE	0.9994	0.15	0.45	6.2	105.6 (3.87, 4.05)	99.3 (2.47, 4.09)	92.2 (4.95, 8.6)
6	ANT	0.9993	0.21	0.62	6.1	101.8 (6.15, 7.77)	80.9 (2.52, 3.33)	96.2 (5.8, 5.1)
7	FLT	0.9979	0.26	0.79	12.1	96.6 (5.24, 7.59)	99.3 (3.68, 4.62)	86 (5.31, 9.66)
8	PYR	0.9978	0.15	0.44	12	113.1 (2.2, 5.64)	105.6 (2.39, 2.69)	94.8 (4.48, 7.24)
9	BcFL	0.9991	0.26	0.79	2.1	113.1 (3.12, 10)	103.2 (3.78, 6.19)	91 (4.05, 7.22)
10	MCH	0.9993	0.12	0.36	0.9	113.1 (2.2, 3.56)	105.6 (2.39, 2.69)	94.8 (4.48, 5.28)
11	BaA	0.9978	0.30	0.89	12.9	90.5 (7.64, 10.3)	114.3 (2.53, 10.43)	83.6 (10.9, 15.4)
12	CHR	0.9993	0.22	0.67	5.1	98.6 (11.23, 9.35)	104.5 (6.5, 6.94)	83.7 (5.07, 14.8)
13	BbFA	0.9979	0.16	0.47	12.6	113 (2.82, 4.88)	104.7 (2.67, 2.79)	93.6 (4.1, 7.79)
14	BkFA	0.9988	0.32	0.96	2.6	105.3 (9.27, 10.6)	90.1 (12.5, 10.13)	103.9 (5.39, 4.94)
15	BjFA	0.9982	0.27	0.81	6.1	99.7 (7.96, 9.69)	97.2 (9.72, 9.81)	100.5 (6.36, 14.0)
16	CCP	0.9994	0.25	0.75	2.7	102.3 (8.23, 8.02)	91 (8.15, 9.65)	92.9 (7.33, 8.6)
17	BaP	0.9985	0.13	0.40	9.9	112.2 (2.43, 3.52)	103.2 (2.44, 2.76)	90.9 (5.19, 7.97)
18	IDP	0.9997	0.15	0.46	1.4	112.7 (2, 5.07)	105.7 (2.14, 2.42)	95.3 (4.45, 7.33)
19	DBahA	0.9991	0.32	0.96	12.2	89.9 (10.1, 12.8)	84 (2.23, 17.72)	98.4 (4.14, 7.33)
20	BghiP	0.9996	0.15	0.45	4.8	93.7 (2.42, 5.63)	114.7 (2.11, 13.02)	96.4 (4.15, 3.1)
21	DBalP	0.9996	0.31	0.92	2.3	98.6 (11.2, 10.2)	106.5 (7.44, 5.68)	83.7 (5.07, 15.4)
22	DBaeP	0.9987	0.17	0.50	3.6	108 (5, 4.95)	104.8 (2.93, 5.92)	103.5 (1.96, 9.11)
23	DBaiP	0.9994	0.14	0.41	12.2	108.8 (4.78, 4.78)	104.6 (2.71, 2.81)	100.2 (2.21, 3.13)
24	DBahP	0.9997	0.15	0.44	0.5	113.1 (2.2, 4.83)	105.6 (2.39, 6.1)	94.8 (4.48, 4.88)

Notes: (*): Abbreviation of PAH compounds refer to Table 1; r: coefficient of correlation; LOD: limit of detection; LOQ: limit of quantitation; %ME: matrix effect.

Method validation was performed in accordance with AOAC 2016 Appendix F [16]. Six standard solution concentrations in ranging from 1 to 50 µg/L are prepared in pure ACN

solvent containing internal standards. Good linearity of 24 PAHs compounds is obtained with correlation values of higher than 0.995. The method LODs and LOQs for 24 PAHs are in the range of 0.12 - 0.32 µg/kg and 0.36 - 0.96 µg/kg, respectively. When the tandem MS detector is used, the LOD values in this work are comparable to previous studies [8, 17]. Importantly, the obtained LOQs values are lower than those of MRLs in edible oil (2 µg/kg for benzo[a]pyrene and 10 µg/kg for PAH4). In contrast, the LOD values for several PAHs in this study are much lower when compared to fluorescence detector (HPLC-FLD) as found in previous studies (LOD value up to 27 µg/kg) [18, 19]. The recovery and precision were evaluated at three spiked levels (1, 2 and 10 µg/kg) for each PAHs. In general, the recoveries varied from 81% to 115% which were satisfactorily within the range of 70% - 120%. The precision of the proposal method was estimated in terms of repeatability and reproducibility. The obtained RSD values for both repeatability and reproducibility were below 12.5% and 17.7%, respectively. All experimental results are shown in Table 2. In conclusion, the results of validation method are in well agreement with the AOAC 2016 Appendix F, indicating that the proposal method is suitable for 24 PAHs analysis in edible oil.

3.4. Proficiency testing

The proposal method (USAEME-GC/MS/MS) in this work was participated to analyzing 4 PAHs during proficiency testing using the FAPAS olive oil (code: FCCE1-OIL22-06145). In addition, this method was compared to HPLC-FLD method developed by our laboratory (not published). The results are shown in Table 3. Overall, the 4 PAHs compounds concentrations measured by USAEME-GC/MS/MS are comparable to those of the certificated FAPAS concentrations and of the measured concentrations by HPLC-FLD. The z-score values are within ± 2. These results indicate that the proposal method is highly reasonable.

Table 3. The analytical results of the FAPAS olive oil (code: FCCE1-OIL22-06145) by GC-MS/MS and HPLC-FLD

PAHs (*)	The FAPAS conc. (µg/kg)	HPLC-FLD		USAEME-GC/MS/MS	
		Conc. (µg/kg)	Z-score	Conc. (µg/kg)	Z-score
BaA	3.00	2.48	-0.8	2.63	-0.6
CHR	3.70	3.51	-0.2	4.49	1.0
BbFA	1.37	1.00	-1.2	1.6	0.8
BaP	2.10	1.81	-0.6	2.25	0.3
ΣPAH4	10.10	8.79	-0.6	10.97	0.4

Notes: (*): Abbreviation of PAH compounds refer to Table 1

3.5. Real sample analysis

The proposal method is used to analyze PAHs in several edible oil samples. Oil samples were bought from local market. After extraction, PAHs were analyzed by GC-MS/MS. The PAHs concentrations are shown in Table 4. The total PAHs concentrations ranged from non-detectable to 5 µg/kg. Soy bean, sunflower and coconut are vegetable oils with non-detectable levels of PAHs. Among the tested oils, palm, olive and macadamia contain significant levels of PAHs (2.83 – 5.00 µg/kg), even though these levels are still below the permissible level of 10 µg/kg as given in Commission Regulation (EU) 2015/1125. The low level of PAHs found in this work can be explained that the analyzed vegetable oil samples are refined edible oil,

except for macadamia oil (cold-pressed oil only). Furthermore, we analyzed several fish oils to fill out the application of the developed method in analyzing oil sample matrix. It is noted that PAHs was found in three kinds of fish oils with the highest concentration of 11.42 µg/kg in herring oil. Currently, this is only a primary result on PAHs contamination in edible oil from Vietnamese market. Further study should be conducted to better understand the contamination of this highly toxic chemical group.

Table 4. The PAHs concentrations in different edible oils from Vietnamese market

No	Oil sample	BaP (*)	BaA	CHR	BbFA	PAH24
1	Soy bean	ND	ND	ND	ND	ND
2	Palm oil	2.29 ± 0.25	1.48 ± 0.32	1.14 ± 0.17	ND	4.91 ± 0.74
3	Sunflower	ND	ND	ND	ND	ND
4	Coconut	< LOQ	ND	ND	ND	< LOQ
5	Olive	1.33 ± 0.34	1.66 ± 0.43	2.01 ± 0.20	< LOQ	5.00 ± 0.98
6	Macadamia	< LOQ	1.78 ± 0.39	1.05 ± 0.21	ND	2.83 ± 0.60
7	Salmon	< LOQ	2.44 ± 0.44	1.80 ± 0.32	< LOQ	4.24 ± 0.75
8	Tuna	2.84 ± 0.34	4.04 ± 0.20	3.00 ± 0.22	1.54 ± 0.17	11.42 ± 0.76
9	Herring	< LOQ	1.17 ± 0.11	< LOQ	ND	1.17 ± 0.11

Note: ND: non-detectable; (*) Abbreviation of PAH compounds refer to Table 1.

4. CONCLUSION

This study developed a robust and reliable analytical method for analysis of 24 PAHs in edible oil based on GC-MS/MS. The importance of this method is capable of simultaneously measuring 16 EPA and 15+1 EU priority PAHs. For extraction, the USAEME method was adopted using less the volume of the DMSO extracting solvent than most previous studies. Then, C18 SPE clean-up was utilized to purify the sample and eliminate the DMSO solvent in the extract. After dissolving in ACN solvent, the sample was injected to GC-MS/MS for analysis of 24 PAHs with non-significant matrix effect. The limit of quantitation of the interested PAHs was below MRLs indicating that the proposed method was sensitive enough for trace-level determination of PAHs in edible oil. By participating in the international proficiency test, the trueness of this method was further approved. In conclusion, it is suggested that the developed method can be used for the routine determination of 24 PAHs at trace levels.

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TÓM TẮT

XÁC ĐỊNH 24 HỢP CHẤT HYDROCARBON ĐA VÒNG THƠM TRONG DẦU ĂN BẰNG KỸ THUẬT VI CHIẾT NHŨ HÓA VỚI SỰ HỖ TRỢ SIÊU ÂM, LÀM SẠCH SPE VÀ PHÂN TÍCH GC-MS/MS

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Trong nghiên cứu này, phương pháp phân tích đồng thời 24 hợp chất hydrocarbon thơm đa vòng (PAHs), bao gồm 16 USEPA PAHs và 15+1 EU PAHs, trong dầu ăn đã được phát triển sử dụng phương pháp sắc ký khí khối phổ hai lần (GC-MS/MS). Phương pháp vi chiết nhũ hóa có sự hỗ trợ siêu âm (USAEME) đã được áp dụng, sử dụng dimethyl sulfoxide (DMSO) làm dung môi chiết, sau đó được làm sạch bằng kỹ thuật chiết pha rắn (SPE). Tại điều kiện tối ưu, độ tuyến tính của đường chuẩn trong khoảng từ 1 đến 50 µg/L có hệ số tương quan lớn hơn 0,995. Giới hạn phát hiện (LOD) của 24 PAHs dao động từ 0,12 đến 0,32 µg/kg. Khi sử dụng mẫu dầu được thêm chuẩn ở ba mức thêm chuẩn, hiệu suất thu hồi trung bình đạt từ 81 – 115% và độ lệch chuẩn tương đối (RSD) nhỏ hơn 17,7%. Độ đúng của phương pháp còn được đánh giá thông qua việc tham gia thử nghiệm thành thạo quốc tế trên mẫu dầu ô liu (FCCE1-OIL22-06145) do tổ chức FAPAS thực hiện, với kết quả đạt yêu cầu (z-score < 2). Phương pháp đã được áp dụng thành công trên các mẫu dầu thực tế và kết quả cho thấy nên mở rộng nghiên cứu quy mô lớn về ô nhiễm PAHs trong dầu ăn trên thị trường Việt Nam.

Từ khóa: GC-MS/MS, dầu ăn, PAHs, USAEME.