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Determination of Light−Medium−Heavy Polycyclic Aromatic Hydrocarbons in Vegetable Oils by Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode Array and Fluorescence Detection

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ABSTRACT: A new method for determination of 16 polycyclic aromatic hydrocarbons (PAHs)—naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene, and indeno[1,2,3-*cd*]pyrene—in vegetable oils was developed. Solid-phase extraction (SPE) prior to high-performance liquid chromatography with fluorescence detection could be used for all those PAHs except acenaphthylene. Acenaphthylene could be detected using a diode array detector at 228 nm. The parameters and variables that affect the extraction were investigated. Under optimum conditions: the extract reagent was centrifuged at 4 °C and evaporated. After that a SPE procedure was used for further cleanup. The limits of detection and limits of quantification were in the range of 0.01−2.35 and 0.04−7.00 μg kg^{-1} in vegetable oil, respectively. The relative standard deviations were under 5%.

KEYWORDS: *Polycyclic aromatic hydrocarbons (PAHs), high-performance liquid chromatography (HPLC), solid-phase extraction (SPE), vegetable oils*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a major class of environmentally hazardous organic compounds due to their known or suspected carcinogenicity, mutagenicity, and toxicity.[1] Sixteen PAHs are on the priority pollutants list of the US Environmental Protection Agency (EPA), which should be monitored in the environment. They are highly stable contaminants in air, water, soil, and food.[2] Food processing involving severe heat treatments can lead to the production of high concentrations of benzo[*a*]pyrene and other carcinogenic PAHs.[3] Benzo[*a*]pyrene has been found at variable concentrations in several foods and water, including cereals, oils, and smoked meats.[4−6,3] Possible sources of PAHs contamination of vegetable oils are (1) direct drying of oilseeds with combustion smoke, (2) contamination through the extraction solvent, (3) uptake by the oilseed plants through contaminated soil, or (4) atmospheric deposition onto the plant material.[7] On the other hand, edible frying vegetable oil can also produce PAHs during high-temperature and long-time frying. There were many food-process enterprises and street stalls using their frying vegetable oils for extended periods because of a lack supervision and administration, which causes the quality of the oil to drop and the harmful matter in the oil to increase in China. For these reasons, it has become an important problem for detecting and monitoring. This has also led to the development of new analytical methods with improved selectivity and sensitivity.

High-performance liquid chromatography [with diode array detection (DAD) or fluorescence detection (FD)] and gas chromatography (with flame ionization or MS detection) are the methods of choice for the analysis of PAHs.[8−12] The preconcentration and extraction of PAHs from different matrices involve a saponification step followed by extraction (liquid−liquid extraction,[13] Soxhlet extraction,[14] accelerated solvent extraction,[10] microwave-assisted solvent extraction[3]). The cleaning up of the extracts is a very important step for some complex matrices. One of the techniques widely applied for the purification purposes to get rid of any interferences is solid-phase extraction (SPE).[15,16]

SPE is a sample treatment technique which passes a liquid sample through a sorbent. This technique can fulfill two functions: First, the analytes are eluted in a small volume of a solvent and so, the analytes are concentrated. Second, the function of the solid-phase extraction is to cleanup the sample. One of the benefits of using this method is that only small volumes of the solvent are required and the purification time is short. Another advantage is that SPE could perform under a wide variety of extraction conditions.

In this work, we described a new competitive method for the effective extraction and cleanup of 16 PAHs from vegetable oils based on HPLC-DAD-FD following SPE. Acenaphthylene was determinated by DAD at 228 nm, and other PAHs were analyzed by FD at optimal emission and excitation wavelengths at different time. The aim of this paper was to investigate an applicable method for the determination of 16 PAHs in some vegetable oils.

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Table 1. Wavelength Changes of RF-10Axl

time (min)	excitation wavelength (nm)	emission wavelength (nm)
0.01	270	324
20.8	248	375
23.6	270	440
27.5	260	420
31.0	280	462
43.0	270	324

MATERIALS AND METHODS

Instrumentation. A high-performance liquid chromatograph (Shimadzu) coupled with a diode array detector (SPD-M20) and a fluorescence detector (RF-10Axl) in series was used. The column was a C_{18} reversed phase, SUPELCOSIL LC-PAH (250 mm × 4.6 mm, particle 5 μm) (Sigma-Aldrich). A centrifuge from Tianmei Biochemical Instrument Plant (Shanghai, China) was used to accelerate phase separation and control the temperature. The Chroma-bond ProElut C_{18}-SPE cartridges (2 g/12 mL) were purchased from Dikma (Beijing, China).

Reagents and Chemicals. The 16 polycyclic aromatic hydrocarbons (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene, and indeno[1,2,3-*cd*]pyrene) were purchased from Accustandard (New Haven, CT). All purities were above 98.2%. An initial solution of the PAHs was prepared in methanol/dichloromethane (50/50, v/v). A standard mixed stock solution with a concentration of 200 μg mL^{-1} was obtained. A fresh 20 μg mL^{-1} mixed standard solution was prepared in methanol and stored at 4 °C. Working mixed standard solution was obtained by diluting intermediary mixed standard solution, and it was renewed every 4 days. Acetonitrile and methanol were purchased from Anhui Fulltime Specialized Solvents & Reagents Co. (Anqing, China). Acetone was purchased from Lingfeng Chemical Reagents Co. (Shanghai, China). They were all HPLC grade.

Procurements of Vegetable Oils. Several vegetable oils including soybean oil, sunflower oil, sesame oil, groundnut oil, corn oil, and olive oil were all purchased from local retail markets of Shanghai. They are all common, edible vegetable oils in China.

HPLC Experiment. In order to acquire the optimum conditions for analysis of 16 PAHs, the mobile phase selected for HPLC determination consisted of acetonitrile and water at a flow rate of 1.5 mL min^{-1}. The column was initially equilibrated with a 40/60 (v/v) of acetonitrile and water mixture (held for 5 min). Then gradient elution program started with 40% acetonitrile and rose linearly to 87% after 30 min (held for 10 min). From 40 to 45 min, the mobile phase returned back to its initial value. The total time for one HPLC run was 53 min. The temperature of two detectors was maintained at 35 °C. The eluted components were detected by DAD and FD in series. The setting of fluorescence wavelength at different times is shown in Table 1. Quantification was carried out by the external standard method.

Extraction and Analysis. In a typical extraction experiment, 2.5 g of vegetable oil sample was placed in a 50 mL screw-capped centrifuge tube, and 5.0 μL of mixed standard solution containing 16 PAHs was added to it. Then 10 mL of acetonitrile/acetone (60/40, v/v) as the extract reagent was added in sequence. The solution was stirred for 5 min on the vortex and then placed in an ultrasonic wave generator for 5 min. After that, it was centrifuged for 5 min at 10 000 rpm at 4 °C. The two phases were separated. The above organic phase was sucked out with the aid of a syringe and transferred to another 50 mL rotary evaporation bottle. The oil phase was extracted by 10 mL of extract reagent two more times. In the end, all the liquid was transferred to a 50 mL rotary evaporation bottle and evaporated to nearly dry.

A solid-phase extraction method was used to clean up the above samples. The cartridge (ProElut C_{18}) was previously prerinsed by flushing with 20 mL of acetonitrile. The contents of the above rotary evaporation bottle were dissolved with 4 mL of acetonitrile/acetone (60/40, v/v) and transferred to the solid-phase extraction cartridge. This step was repeated two times. At last, all the solution that eluted out from the cartridge was

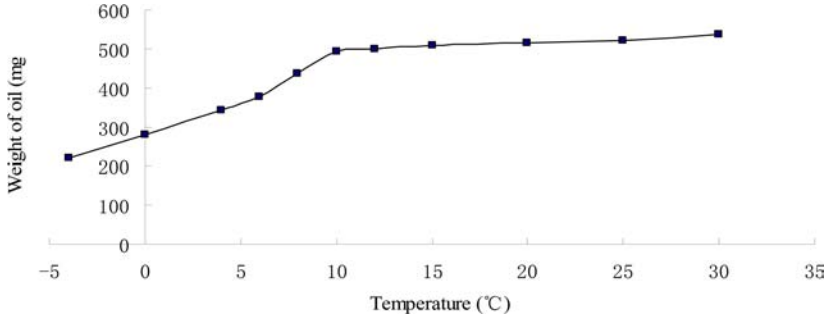

Figure 1. Effect of temperature on the oil extraction during the centrifugation process. Conditions: Extraction of 2.5 g of vegetable oil with 30 mL of extract reagent with a ProElut C_{18} cartridge (2 g/12 mL).

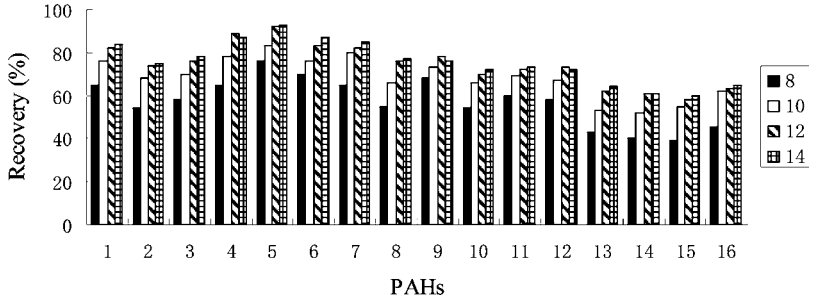

Figure 2. Effect of the SPE elution volume on PAHs recoveries. Conditions: 2.5 g of vegetable oil extracted with 30 mL of extract reagent. The extract was centrifuged at 4 °C and the upper phase evaporated. The residue was dissolved in different reagent volumes and passed through a ProElut C_{18} cartridge (2 g/12 mL). 1, Na; 2, Ace; 3, Ac; 4, Fl; 5, Ph; 6, An; 7, Flu; 8, Py; 9, BaA; 10, Ch; 11, BbF; 12, BkF; 13, BaP; 14, DahA; 15, BghiP; 16, Ind.

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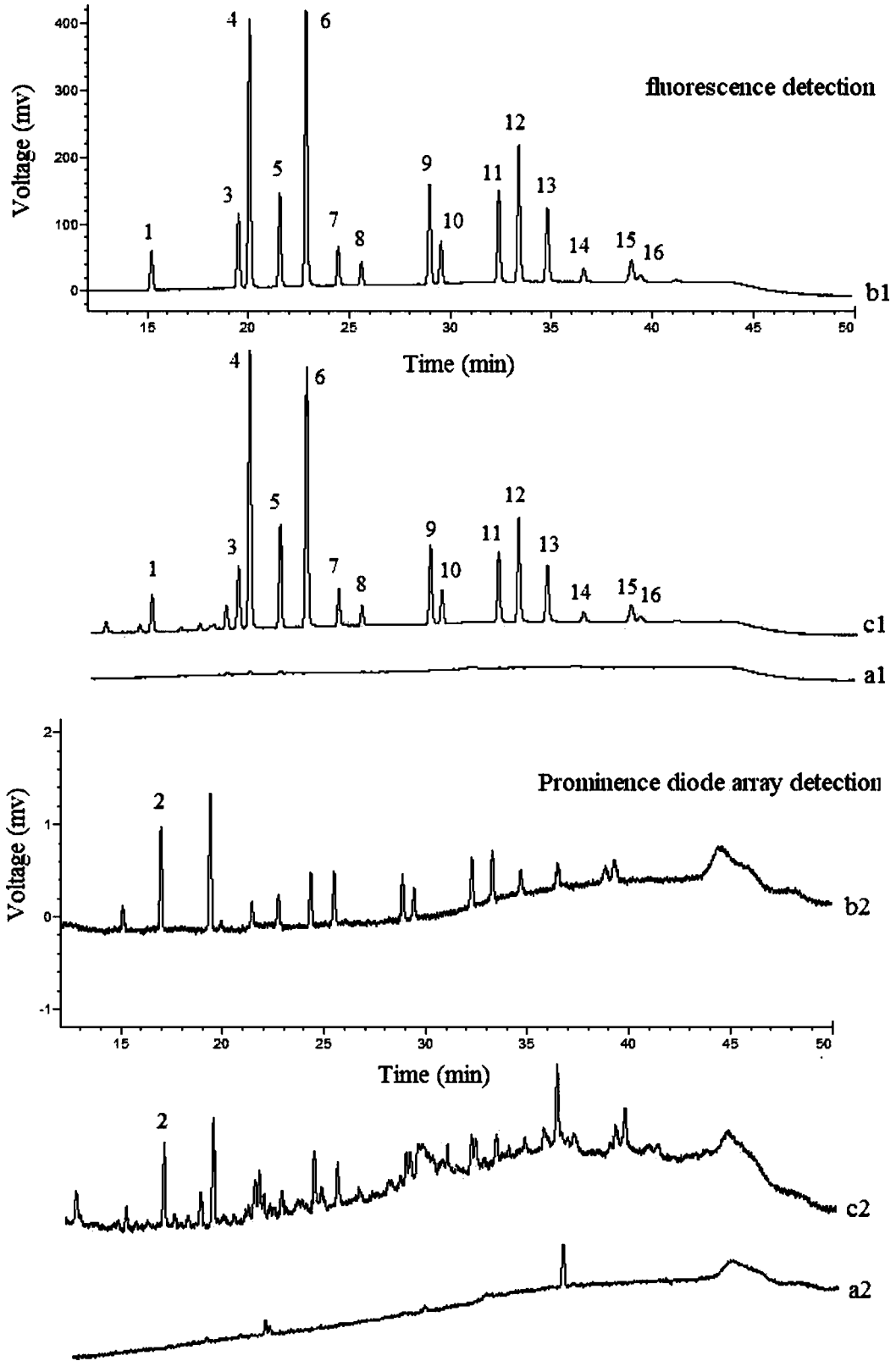

Figure 3. HPLC-DAD-FD chromatograms: soybean oil (a1 and a2), standards (b1 and b2), and soybean oil spiked with 40 μg kg^{-1} of working mixed standard solutions (c1 and c2). Peak numbers: 1, Na; 2, Ace; 3, Ac; 4, Fl; 5, Ph; 6, An; 7, Flu; 8, Py; 9, BaA; 10, Ch; 11, BbF; 12, BkF; 13, BaP; 14, DahA; 15, BghiP; 16, Ind.

collected. After the sample was loaded into the SPE cartridge, it was dried under vacuum. The eluted organic solvent was then evaporated to dryness at 35 °C. The residue was dissolved in 2.0 mL of acetonitrile and directly injected into the HPLC instrument after filtration.

RESULTS AND DISCUSSION

Extraction Solvents. The selection of extraction solvents was based on an earlier study.[17] Acetonitrile/acetone (60/40, v/v)

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was selected for the extraction of PAHs from oil samples. The recoveries of all PAHs were above 70%, except for that of BghiP in this step, which was above 60%.

Absorption Capacity of C_{18}-SPE Cartridge. It is necessary to optimize the absorption capacity of the C_{18} cartridge for the cleanup of target analytes during the SPE process. The effect of the absorption capacity of the C_{18}-SPE cartridge was determined by measuring a series of soybean oil (2.5 g) samples with added PAHs by using a ProElut C_{18} cartridge (2 g/12 mL), centrifugation at 4 °C, 12 mL of eluted solution, and different volumes of extract reagent.

In this experiment, the function of the SPE is to clean up the sample by adsorbing the redundant oil of the extraction process and other sample impurities. If the extract volume was less than 35 mL, there was nearly no oil eluted out from the cartridge. However, if the volume of extract reagent was above 35 mL, there was redundant oil that could not adsorbed by the cartridge. This proved that the adsorption capacity of this cartridge had reached its limit. The redundant oil could affect the detection PAHs in the sample. When the volume was less than 25 mL, the recoveries of BbF, BkF, BaP, DahA, BghiP, and Ind were all less than 60%. In order to obtain an optimized extraction volume and good recovery, 30 mL extraction solution was selected as a suitable extraction volume.

Temperature. The effect of temperature was also investigated. In this experiment, it was observed that the temperature during the centrifugal procedure influenced the weight of oil that dissolved into the extract solution. Figure 1 shows the relationship between the temperature and the weight of oil dissolved in 30 mL of solution. It can be found out that with the increasing of temperature, the weight of oil gradually increased. The recovery of BaP, DahA, BghiP, and Ind were all less than 50% at temperatures under 0 °C. In this paper, in order to achieve high recovery of PAHs in SPE process, 4 °C was selected as centrifugal temperature.

Eluent Volume. There were two factors should be taken into account. First, the recoveries of the 16 PAHs were low if the volume of eluent was small. Second, there was a small amount of redundant oil that eluted out if the eluent volume was large, which can affect the detecting process. Figure 2 shows the relationship between the recovery and the volume of eluent. When the volume of eluent was increased from 8 to 12 mL, an increase of 34% in the recovery of some PAHs was observed. Further increase (from 12 to 14 mL) resulted in a slight increase. Therefore, 12 mL was selected as the eluent volume to acquire a good recovery.

HPLC-DAD-FD Analysis. HPLC combined with DAD or FD of PAHs has been successfully applied in many studies concerning different analytes.[18−21] In our work, we applied this technique to HPLC-DAD-FD with automatic injection. According to the optimal extraction and cleanup procedure, soybean oil and soybean oil spiked with 40 μg kg^{-1} of each PAH were studied to confirm this method. The chromatograms are shown in Figure 3. The chromatogram shown in Figure 3a1 presents a smooth baseline for the fluorescence spectral graph. On the other hand, there was no influence with determination of acenaphthylene at 228 nm for the UV spectral graph (Figure 3a2).

As can be seen from the Figure 3b1,2, 16 PAHs were separated in 53 min by the two detectors, and their peaks appeared free from any interference. Figure 3c1,2 shows in the case of the extract obtained from soybean oil spiked with 40 μg kg^{-1} of each PAH. There were 16 peaks corresponding with the peaks of PAHs in Figure 3b1,2.

Analytical Figures. On the basis of the method described above, the linear range was tested by varying the concentration of the mixed 16 standard solutions. The areas for the PAHs were proportional to their concentrations (μg kg^{-1}) in good linear relationships. The relative standard deviations (RSD) ranged between 0.48% and 4.98%. The limit of detection (LOD) of each PAH was calculated on the basis of a signal-to-noise ratio of 3:1. The limit of quantification (LOQ) was defined as three times the LOD, and the results are shown in Table 2.

Table 2. Analytical Parameters of the HPLC-DAD-FD Method

PAHs	R^2	RSD[a] (%)	linear range (μg kg^{-1})	(LOD)[b] (μg kg^{-1})	(LOQ)[c] (μg kg^{-1})
Na	0.9996	2.37	0.50−120	0.15	0.45
Ace	0.9999	2.56	10.0−2000	2.35	7.00
Ac	0.9996	1.09	0.20−120	0.03	0.10
Fl	0.9996	0.48	0.05−120	0.01	0.04
Ph	0.9995	1.05	0.15−120	0.03	0.10
An	0.9993	0.74	0.05−120	0.01	0.04
Flu	0.9994	1.75	0.50−120	0.15	0.45
Py	0.9997	1.25	0.70−120	0.17	0.50
BaA	0.9999	1.09	0.20−120	0.05	0.15
Ch	0.9996	1.47	0.50−120	0.15	0.45
BbF	0.9994	3.03	0.20−120	0.05	0.15
BkF	0.9995	3.54	0.10−120	0.03	0.08
BaP	0.9988	4.98	0.20−120	0.05	0.15
DahA	0.9995	2.61	2.00−120	0.50	1.50
BghiP	0.9995	3.02	1.00−120	0.25	0.80
Ind	0.9994	2.75	3.00−120	0.85	2.50

[a]Relative standard deviation, n = 3. [b]Represents the limit of detection. [c]Represents the limit of quantification.

Application to Vegetable Oils. The optimized procedure was used to analyze different vegetable oils from the Shanghai market in China. It could be found that the olive oil sample had the highest naphthalene load with a maximum concentration of 75.8 μg kg^{-1}. Large differences in PAHs contamination were also found in other oils. The olive oil sample showed the highest levels of total PAHs content (126 μg kg^{-1}). Among other vegetable oils showing total PAHs content of 36.8−96.4 μg kg^{-1} were groundnut oil (36.8 μg kg^{-1}), soybean oil (41.0 μg kg^{-1}), sunflower oil (58.5 μg kg^{-1}), corn oil (72.8 μg kg^{-1}), and sesame oil (96.4 μg kg^{-1}).

Reliability. In order to estimate the HPLC-DAD-FD method for determination of 16 PAHs involving SPE, the qualitative determination results in vegetable oils were obtained by the gas chromatography combined with mass spectrometry (GC/MS) method. Through qualitative analysis, the applicability of this proposed method was verified.

The GC/MS was operated under the following conditions: Before being injected into the GC/MS instrument, the residue was dissolved in 2.0 mL of methylene chloride. Helium was used as carrier gas at a constant flow of 1.2 mL min^{-1} with splitless injection. The temperature of the injection port and the detector was held at 290 °C. The oven temperature was set at 60 °C initially (1 min hold), increased to 200 °C at a speed of 20 °C min^{-1} (2 min hold), increased to 250 °C at a rate of 25 °C min^{-1} (0 min hold), and increased to 280 °C at 8 °C min^{-1} (10 min hold). The total time required for one GC run was about 26 min. Chromatographic separation was carried out

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Table 3. Comparing the Performance of this Method with ISO 15753-2006 in Soybean Oil Samples

		found (μg kg^{-1})/RSD[a] (%)		recovery (%)	
PAHs	added (μg kg^{-1})	HPLC-DAD-FD method	ISO 15753-2006 method	HPLC-DAD-FD method	ISO 15753−2006 method
Na	5	3.95/5.54	–[b]	78.9	–
	50	40.6/2.67	–	81.2	–
	100	80.5/1.73	–	80.5	–
Ace	5	nd[c]	–	nd	–
	50	37.6/1.99	–	75.3	–
	100	77.4/1.03	–	77.4	–
Ac	5	3.82/6.89	–	76.3	–
	50	37.5/3.02	–	75.0	–
	100	75.2/1.67	–	75.2	–
Fl	5	4.32/1.28	–	86.4	–
	50	44.0/0.97	–	87.9	–
	100	88.2/0.95	–	88.2	–
Ph	5	4.73/1.03	–	94.6	–
	50	46.3/1.21	–	92.6	–
	100	91.1/1.05	–	91.1	–
An	5	4.25/1.48	4.18/4.17	85.0	83.7
	50	42.4/1.14	42.2/3.78	84.7	84.5
	100	80.7/1.57	82.1/2.78	80.7	82.1
Flu	5	4.12/0.67	3.98/5.23	82.5	79.6
	50	41.5/0.58	39.2/4/72	83.0	78.3
	100	84.8/0.19	76.9/1.54	84.8	76.9
Py	5	3.80/2.22	3.87/1.29	75.9	77.4
	50	38.6/2.07	38.6/1.07	77.1	77.1
	100	78.5/1.98	76.4/3.55	78.5	76.4
BaA	5	3.81/1.88	3.79/2.89	76.2	75.8
	50	39.2/2.64	38.4/3.05	78.3	76.9
	100	76.9/2.03	78.5/3.79	76.9	78.5
Ch	5	3.48/3.01	35.6/4.01	69.7	71.3
	50	34.9/2.68	36.0/3.77	69.9	72.0
	100	70.1/2.54	70.9/2.56	70.1	70.9
BbF	5	3.50/2.75	3.58/3.35	70.0	71.7
	50	35.4/2.19	35.3/2.76	71.8	70.6
	100	70.5/2.07	70.2/4.09	70.5	70.2
BkF	5	3.65/2.76	3.62/4.54	73.0	72.4
	50	36.3/2.19	36.0/3.99	72.6	72.0
	100	72.5/3.47	71.6/4.31	72.5	71.6
BaP	5	3.13/3.34	3.28/5.71	62.6	65.6
	50	31.9/3.01	32.9/5.63	63.9	65.8
	100	65.8/3.36	64.9/5.17	65.8	64.9
DahA	5	3.06/4.23	3.18/4.40	61.1	63.7
	50	30.3/3.91	32.1/4.12	60.5	64.2
	100	61.2/2.79	65.5/3.47	61.2	65.5
BghiP	5	2.91/7.89	3.00/4.85	58.2	60.0
	50	28.7/5.12	29.8/4.54	57.3	59.9
	100	59.5/3.44	60.5/4.32	59.5	60.5
Ind	5	3.26/6.57	3.18/4.29	65.1	63.7
	50	32.3/4.03	32.0/4.05	64.6	64.1
	100	65.0/3.39	65.2/3.78	65.0	65.2

[a]Relative standard deviation, n = 3. [b]Not quantitative. [c]Not detected.

with a HP-5MS 5% phenyl−95% methyl siloxane capillary (30 m × 250 μm; 0.25 μm thickness). An automatic sample injector (HP 7890A; Agilent Technologies) was used to introduce 1.0 μL of each sample extract in an intermittent standard injection sequence and 5975C inert MSD with Triple-Aix detector (Agilent Technologies). The selected ion monitoring was then used for quantification in which three ions were selected for calculating the chromatographic peak area of each PAH.

Comparison Experiment. The levels of recovery and relative standard deviation (RSD) are given in Table 3. The average recoveries of the spiked standards for the analytes in soybean oil were 57.3−94.6% with average RSDs of 0.58−6.89%. The HPLC-DAD-FD method was compared with the international standard method of ISO 15753-2006. As summarized in Table 3, there was no significant difference between the proposed method and the reference method (ISO 15753-2006) except for PAHs with two or three aromatic rings. The extraction and purity procedure of ISO 15753-2006 are complex, time-consuming and relatively high temperature, so PAHs with two or three aromatic rings cannot be quantitative analyzed because of their high volatility. On the other hand, these disadvantages could be avoided by using the proposed method, and it could reduce use of toxic reagents.

In China, the national standard for determination of PAHs in vegetable oils was similar to ISO 15753-2006, so this new method may meet the national standard of China, and it can be a practical and economic analytical method of quality control for large-lot samples.

The following conclusions can be obtained from the present work.

For effective determination of PAHs in vegetable oils with complex matrix, SPE coupled with HPLC-DAD-FD with automated injection was successfully developed. This new method was sensitive, convenient, and practical.

This experimental process has low-temperature conditions, the extraction procedure was simple, and the extraction time was short, so 16 PAHs could be quantitatively analyzed after optimization of different extraction parameters.

However, whether this method is suitable for determination of other edible oils was not studied in this paper. The next work is to apply this method to detect PAHs in other edible oils.

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Notes

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ABBREVIATIONS USED

PAHs, polycyclic aromatic hydrocarbons; Na, naphthalene; Ace, acenaphthylene; Ac, acenaphthene; Fl, fluorine; Ph, phenanthrene; An, anthracene; Flu, fluoranthene; Py, pyrene; BaA, benz[*a*]-anthracene; Ch, chrysene; BbF, benzo[*b*]fluoranthene; BkF, benzo[*k*]fluoranthene; BaP, benzo[*a*]pyrene; DahA, dibenz[*a*,*h*]-anthracene; BghiP, benzo[*g*,*h*,*i*]perylene; Ind, indeno[1,2,3-*cd*]-pyrene; SPE, solid-phase extraction; HPLC, high-performance liquid chromatography.

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