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Development of Multiresidue Analysis for Twenty Phthalate Esters in Edible Vegetable Oils by Microwave-Assisted Extraction–Gel Permeation Chromatography–Solid Phase Extraction–Gas Chromatography–Tandem Mass Spectrometry

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ABSTRACT: A novel multiresidue analysis method is developed for the determination of twenty phthalate esters at the μg/kg level in edible vegetable oils by microwave-assisted extraction–gel permeation chromatography–solid phase extraction–high resolution gas chromatography–tandem mass spectrometry (MAE-GPC-SPE-HRGC-MS/MS). The samples were extracted with methanol under microwave incubation. Cleanup was carried out with GPC followed by a further C18 SPE column and then separated by the HP-5MS capillary column under a temperature program. The eluents were qualitatively and quantitatively determined by tandem mass analyzer with selected reaction monitoring (SRM) type and positive ion mode. The calibration curves showed good linearity in the range 5 μg/kg to 2.50 mg/kg with correlation coefficients larger than 0.999. Low detection limits (LODs) of 0.218–1.367 μg/kg and quantification limits (LOQ) of 0.72–4.51 μg/kg were achieved. The mean recoveries were in the range from 93.04% to 104.6% at 5, 15, and 40 μg/kg spiked levels, and the relative standard deviations (RSDs) were in the range of 1.01% and 5.26% (n = 7). This method could potentially overcome the interference from large amounts of lipids and pigment. The real sample test showed this method can be used for sensitive and accurate determination and confirmation of phthalate ester residues in high-fat and complex samples.

KEYWORDS: *phthalate esters, microwave-assisted extraction, gel permeation chromatography, solid phase extraction, gas chromatography–tandem mass spectrometry, multiresidue analysis, vegetable oil*

INTRODUCTION

Phthalate esters are di- and monoesters of phthalic acid, an ortho-dicarboxylic acid derivative of benzene, for example: dimethyl phthalate (DMP), diethyl phthalate (DEP), dipropyl phthalate (DPP), diisobutyl phthalate (DIBP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBzP), dicyclohexyl phthalate (DCHP), di-*n*-hexyl phthalate(DHP), di-2-ethylhexyl phthalate (DEHP), di-*n*-octyl phthalate (DOP), diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), di-2-ethylhexyl adipate (DEHA), and diisononyl adipate (DINA). These compounds are widely used as industrial plasticizers to coat polyvinyl chloride surfaces of plastics used in food packaging and medical devices. Consequently, they are ubiquitous environmental contaminants due to volatilization and leaching from their widespread applications.[1] The contamination of the environment has become another important source for phthalates in foods in addition to migration from packaging materials. So humans have significant exposures to plasticizer. These phthalate esters are not allowed to be used in food additives,[2] but DEHP, as cloudy agents, was used unlawfully in drink and caused a severe food security crisis in Chinese Taiwan.

Edible vegetable oils are one of the most important components of the human diet. Therefore the presence of toxic residues constitutes a significant health risk. The Bundersverband NaturKost Naturwaren has established guidelines for phthalate residues in edible oils, taking into account that they are ubiquitous substances: for DEHP the recommended value is 3 mg/kg, while for benzyl butyl phthalate (BBP), DINP, DIDP, and others it is 5 mg/kg.[3] Human exposure to phthalate esters has been an increased concern due to the findings from toxicology studies in animals. Since foods are the major source of exposure to phthalates, information on levels of phthalates in foods is important for human exposure assessment. Consequently, development of accurate and reliable methods for the determination of phthalates is required for the assurance of food safety.

There is a need for an analytical procedure which would determine all phthalate forms in one run. The majority of publications deal with some phthalate determination in water,[4−6] biological fluids,[7] soil samples,[8] and sediment and some fish species.[9] In 2010, Cao[1] reviewed traditional and new methodologies for the determination of phthalates in foods. Phthalates are semivolatile and stable compounds; liquid chromatography (LC) is not essential, and it is rarely used for phthalate analysis. GC was used for the determination of diisooctyl phthalate (DIOP), DEHP, and DEHA in cheese, meat, poultry, sandwiches, edible oil, and milk with the detection limit (LOD) of 30–70 mg/kg using flame ionization

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Table 1. Optimized Parameters for Analysis of Twenty Phthalate Esters Using MS/MS with SRM Mode

					ion pair (m/z)		
no.	compound	CAS no.	mol wt	t_R (min)	for qualitative	for quantification	collision energy (V)
1	dimethyl phthalate (DMP)	131-11-3	194	9.076	163/77	163/77	25
					163/135		10
2	diethyl phthalate (DEP)	84-66-2	222	10.251	149/93	149/93	15
					149/121		10
3	diisopropyl phthalate (DIPrP)	605-45-8	250	10.879	149/65	149/65	25
					149/93		15
4	dipropyl phthalate (DPrP)	131-16-8	250	12.329	149/65	149/65	25
					149/93		15
5	diisobutyl phthalate (DIBP)	84-69-5	278	13.919	149/65	149/65	20
					149/93		15
6	dibutyl phthalate (DBP)	84-74-2	278	15.512	149/65	149/65	25
					149/93		15
7	bis(2-methoxyethyl) phthalate (DMEP)	117-82-8	282	16.123	149/65	149/65	25
					149/93		15
8	diisopentyl phthalate (DIPeP)	605-50-5	306	17.043	149/65	149/65	30
					149/93		15
9	bis(4-methyl-2-pentyl) phthalate (BMPP)	146-50-9	334	17.144	149/65	149/65	30
					149/93		15
10	bis(2-ethoxyethyl) phthalate (DEEP)	605-54-9	310	17.653	149/65	149/65	25
					149/93		10
11	dipentyl phthalate (DPeP)	131-18-0	306	18.051	149/65	149/65	25
					149/93		15
12	dihexyl phthalate (DHXP)	84-75-3	334	20.080	149/65	149/65	25
					149/93		15
13	benzyl butyl phthalate (BBP)	85-68-7	312	20.164	149/65	149/65	25
					149/93		15
14	bis(2-*n*-butoxyethyl) phthalate (DBEP)	117-83-9	366	21.282	149/65	149/65	25
					149/93		15
15	dicyclohexyl phthalate (DCHP)	84-61-7	330	21.664	149/65	149/65	25
					149/93		15
16	di-*n*-heptyl phthalate (DHP)	3648-21-3	363	21.805	149/65	149/65	25
					149/93		15
17	diphenyl phthalate (DPhP)	84-62-8	318	21.870	225/77	225/77	30
					225/153		15
18	bis(2-ethylhrxyl) phthalate (DEHP)	117-81-7	390	21.941	149/65	149/65	25
					149/93		15
19	di-*n*-octyl phthalate (DNOP)	117-81-0	391	23.344	149/65	149/65	30
					149/93		15
20	dinonyl phthalate (DNP)	84-76-4	419	24.905	149/65	149/65	30
					149/93		15

detection,[10] and DEHP in milk with LOD of 0.05 to 0.2 mg/L using electron-capture detection.[11] Mass spectrometry (MS) now is almost the routine detection method for phthalates after separation by GC. The GC−MS method has been selected as Chinese standards for the determination of phthalate esters in food, but the LOD was 1.5 mg/kg for fat-containing samples.[12] A series of GC−MS methods have been reported for the determination of some phthalates in samples with fatty matrices.[13−19] The sensitivity for these methods was not high enough, with LOD value ranging from 5 to 500 μg/kg. A GC−MS method combined with a solid phase microextraction method was used for the determination of DMP, DEP, DBP, BBzP, DEHP, and DOP in cow milk and human milk with low LOD of 0.12−1.8 μg/kg.[20,21] A LC/MS/MS method was developed for the determination of DBP, BBzP, DEHP, DINP, and DIDP in milk and milk product with low LOD of 4−9 μg/kg.[22] Recently, a GC-FID method was presented for determination of sum of phthalate esters with LOD of 400 μg/kg.[23] Phthalate, adipate, and sebacate ester contamination in olive oils was investigated by HRGC−MS with acetonitrile as solvent; in the method LODs ranged from 3 μg/kg (DIBP) to 1200 μg/kg (DIDP).[24] Of the methods reported above, not more than 10 kinds of phthalate esters in food were determined.

Choice of analytical technique is also dependent on the method of sample preparation. Extraction and cleanup are the most challenging parts for phthalate ester analysis in foods and are often the critical steps in deciding the levels of detection limits of the overall methods. Solvent or liquid−liquid extraction is the most frequently used method for extraction of phthalates from foods, especially fatty food samples.[1] Oil samples were also occasionally analyzed directly or after being dissolved in a solvent without any extraction and cleanup.[25,26] A new technique, microwave-assisted extraction (MAE), has been used for the determination of nonylphenols and phthalate esters in sediment samples.[27] After solvent extraction, further

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cleanup of the extracts is always necessary for the fatty foods by size-exclusion chromatography.[11,13−16] Cleanup was also performed using solid phase extraction (SPE) with columns packed with Florisil or silica gel.[18,22] Solid-phase microextraction (SPME) was investigated for the determination of phthalates in vegetable oil.[28] For achieving multiresidue analysis with high throughput and high sensitivity, it is important to develop a new and effective extraction and cleanup system.

The purpose of this work was to develop a new GC−MS/MS method for the determination of phthalate esters in vegetable oil samples. In this work, prior to GC−MS/MS determination, MAE followed by gel permeation chromatography (GPC)−SPE was used for effective extraction, cleanup, and preconcentation, overcoming the interference from lipids and pigments, and increasing sensitivity. The proposed method has the advantages of low limit of quantification (LOQ) and high-throughput analysis, and has been applied for the determination of twenty phthalate esters in vegetable oil samples with satisfactory results.

MATERIALS AND METHODS

Instrument. The gas chromatography−tandem mass spectroscopy analysis was performed with an Agilent 7890A-7000QQQ high resolution gas chromatography−mass spectrometer, using a 30 m × 0.32 mm i.d. HP-5MS quartz capillary column (0.25 μm film thickness) (Agilent, USA). Microwave synthesizer (CEM Inc., Matthews, NC, USA), gel permeation chromatography (J2 Scientific, USA), and solid-phase extraction equipment (Tianjin BNAJE Science and Technique Co. Lid.) were used for extraction and cleanup. Agilent C18 SPE column 6 mL, 500 mg (Agilent, USA), Oasis HLB SPE column (1 g, 6 mL, Waters, USA), and silica gel SPE column (6 mL, 500 mg) were used for comparing extraction and cleanup purposes.

Chemicals and Solutions. Phthalate ester standards (purity: >99%) were purchased from Dr. Ehrenstorfer company (Germany). Mixed stock solution of twenty phthalate esters at concentrations of 5 mg/mL of each phthalate ester was prepared in methanol. The stock and working standard solutions were stored at 4 °C in the refrigerator. A matrix-matched calibration curve was measured on GC−MS/MS within the concentration range of 5−2500 μg/kg. Water used in solution preparation was purified on a MYQ-sub-boiling distilling water purification system (Changsha, China). Cyclohexane, *n*-hexane, ethyl acetate, methanol, acetone, and dichloromethane (Fisher Scientific, Germany) were HPLC grade reagents. Other reagents were analytical grade.

Extraction. The edible vegetable oil samples were taken from a local market in Shijiazhuang city. All samples were collected in small glass bottles and stored at 4 °C until analysis. Approximately 0.5 g (±0.001 g) of vegetable oil sample was weighed out and transferred quantitatively to the Teflon-lined extraction vessels added with 5 mL of methanol. The vessels were put in a microwave synthesizer. Microwave-assisted extraction was carried out for 15 min at 100 °C and 140 kPa. The vessels were taken out from the microwave synthesizer and allowed to cool down to room temperature before opening the caps. The extracts in the vessels were taken out and used for cleanup further.

Cleanup. The extract was evaporated to dryness in a rotary evaporator under a stream of nitrogen. A 10 mL mixture of ethyl acetate−*n*-hexane (1:1, v/v) was added, mixed by a vortex oscillator for 1 min, and then centrifuged at 15000 rpm for 10 min. The upper layer was moved out. GPC with glass column (700 mm × 25 mm i.d. BioBeads (S-X3)-200−400 mesh, 70 g) was used to cleanup using injection volume of 5 mL and with ethyl acetate−*n*-hexane (1:1, v/v) as an eluent at a flow rate of 4.7 mL/min. The fraction was collected within 8.5−15 min, and evaporated to 0.5 mL at 45 °C under a stream of nitrogen.

C18 SPE column was activated with 4 mL of methanol and 4 mL of methanol−water (1:3) in turn. The sample extracts were cleaned up on the C18 SPE column at a flow rate of 1 mL/min, and then eluted with 5 mL volume of methanol−dichloromethane (1:1). Anhydrous sodium sulfate of 2 g was added to the eluates. After standing for 15 min, the organic phase was evaporated to dryness at 40 °C with a rotary vacuum evaporator under nitrogen. The obtained dry residues were dissolved in *n*-hexane to 2 mL, and the final solution was used for GC−MS/MS analysis.

Conditions of GC−MS. Analytes were separated on an HP-5MS gas chromatographic column. The carrier gas was helium (purity: no less than 99.999%) at a flow rate of 1.5 mL/min. The GC conditions were as follows: injection volume 1.0 μL; injector temperature 280 °C; splitless time 1.0 min; initial oven temperature 50 °C for 1 min, increased to 180 °C at a rate of 15 °C/min with 4 min hold time, and a second ramp to 280 °C at a rate of 10 °C/min with 7 min hold time.

Quantification of phthalate esters was performed by GC/MS working in the EI positive ion mode, using the electron energy of 70 eV. Transfer line temperature and ion source temperature were maintained at 280 and 230 °C, respectively. Solvent delay time of 6 min was set. Optimized parameters for analysis of twenty phthalate esters using MS/MS with SRM mode are listed in Table 1.

RESULTS AND DISCUSSION

Optimization of MAE Conditions. The extractability of solvents depends mainly on the solubility of the compound in

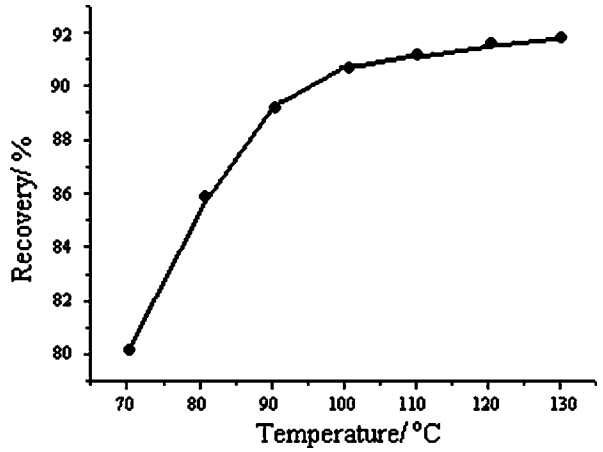

Figure 1. Effect of extraction temperature on the average recoveries of 10.0 μg/kg phthalate esters under extraction time of 30 min. Recovery data are the average values of three measurements and contain less than 3% of relative standard deviation.

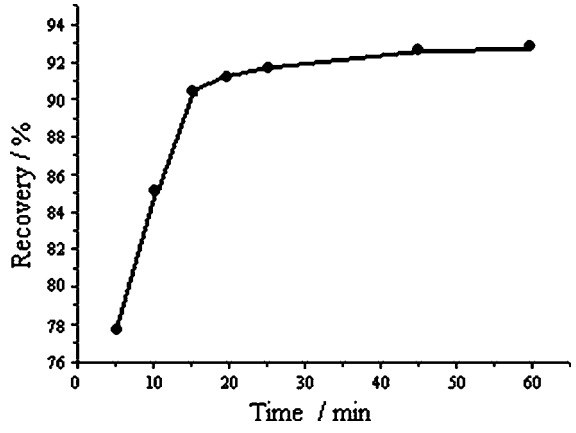

Figure 2. Effect of extraction time on the average recoveries of 10.0 μg/kg phthalate esters at extraction temperature of 100 °C. Recovery data are the average values of three measurements and contain less than 3% of relative standard deviation.

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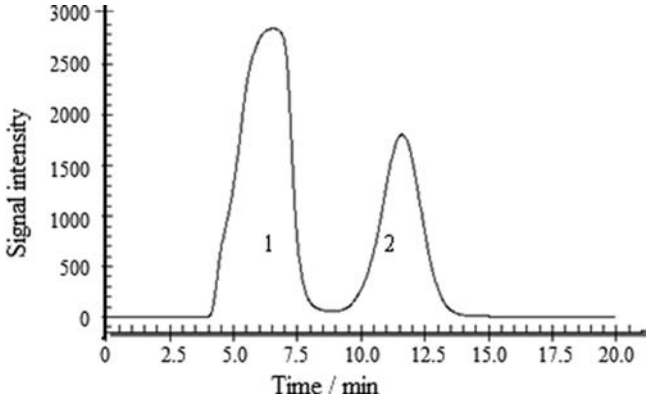

Figure 3. Gel permeation chromatogram of blank vegetable oil spiked with phthalate esters. (1) Pigment and lipids. (2) Phthalate esters. Injection volume: 5 mL. Flow rate: 4.7 mL/min. Detection wavelength: 254 nm.

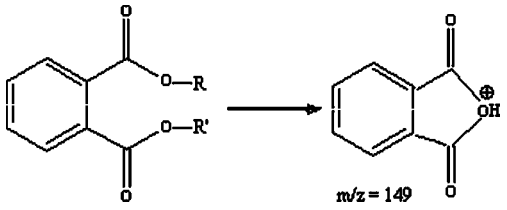

Figure 5. Primary structure of phthalates and major fragmentation of phthalate esters in EI ionization. The dominant ion in the EI spectrum is typically m/z 149. The two exceptions are for DMP and DPhP.

Table 2. Linear Equations, Correlation Coefficient, LOD, and LOQ

no.	analyte	linear eq	correlation coeff (r)	LOD (μg/kg)	LOQ (μg/kg)
1	DMP	$y = 1.502x + 43.09$	0.9999	0.376	1.24
2	DEP	$y = 0.5594x + 44.18$	0.9995	0.382	1.26
3	DIPrP	$y = 1.592x + 96.08$	0.9995	0.337	1.10
4	DPrP	$y = 2.311x + 53.27$	0.9999	0.345	1.14
5	DIBP	$y = 2.184x + 51.09$	0.9999	0.566	1.87
6	DBP	$y = 1.872x + 46.75$	0.9999	0.390	1.29
7	DMEP	$y = 0.5806x + 8.048$	0.9999	1.367	4.51
8	DIPeP	$y = 0.8173x + 4.199$	1.000	0.345	1.14
9	BMPP	$y = 2.555x + 61.15$	1.000	0.955	3.15
10	DEEP	$y = 1.030x + 29.70$	0.9999	0.404	1.45
11	DPeP	$y = 2.642x + 85.31$	1.000	0.366	1.21
12	DHXP	$y = 2.109x - 17.34$	0.9996	0.218	0.72
13	BBP	$y = 5.088x + 64.36$	0.9999	0.511	1.69
14	DBEP	$y = 1.816x + 63.67$	1.000	0.901	2.97
15	DCHP	$y = 3.590x + 23.27$	0.9999	0.427	1.41
16	DHP	$y = 2.353x + 21.94$	0.9998	0.301	0.99
17	DPhP	$y = 1.640x + 11.91$	0.9994	0.741	2.45
18	DEHP	$y = 4.709x + 10.70$	0.9997	0.590	1.95
19	DNOP	$y = 2.860x + 74.24$	0.9999	0.526	1.74
20	DNP	$y = 2.960x - 18.99$	0.9996	0.833	2.75

the solvent, the mass transfer kinetics of the product, and the strength of the solute/matrix interactions. A correct choice of solvent is fundamental for obtaining an optimal extraction process. Solvent choice for MAE is dictated by the solubility of the target analyte, by the interaction between solvent and the pretreated matrix, and finally by the microwave absorbing properties of the solvent. The dielectric properties of the solvent toward microwave heating play an important role in microwave extraction. In this work, the effect of extracting solvent (methanol, acetone, and *n*-hexane) on the MAE recoveries of phthalate esters in a blank walnut oil sample spiked with 10.0 μg/kg was investigated for 3 parallel assays under same conditions. The mean percent recovery obtained with methanol was significantly better than with acetone or *n*-hexane.

The ability of a solvent to convert MW energy to heat depends on the properties of substance molecules in accordance with the dissipation factor (tan δ). Methanol, acetone, and *n*-hexane have dielectric constant (ε) of 32.6, 20.7, and 1.89, and dissipation factor (tan δ) of 0.64, 0.05, and 0.02, respectively.[29,30] Methanol has a higher dielectric constant and dissipation factor than acetone and *n*-hexane. This can be accounted due to the difference in dielectric properties of the solvent. Acetone and *n*-hexane are transparent to microwave, and so do not heat up under microwave. Although acetone and *n*-hexane are good extraction solvents, they are not good absorbers of microwave energy. Methanol has good microwave absorbing capacity and hence heats up faster and can enhance the extraction process. Thus methanol was selected as the extracting solvent and was employed for the rest of the recovery studies.

The volume of methanol used for the extraction in this work was not optimized, as the minimum amount of solvent of the MAE system was 5 mL. It was sufficient for complete immersion of the sample in the extraction solvent. Increasing the solvent volume could complicate the extraction procedure especially that lower recovery rates were obtained with

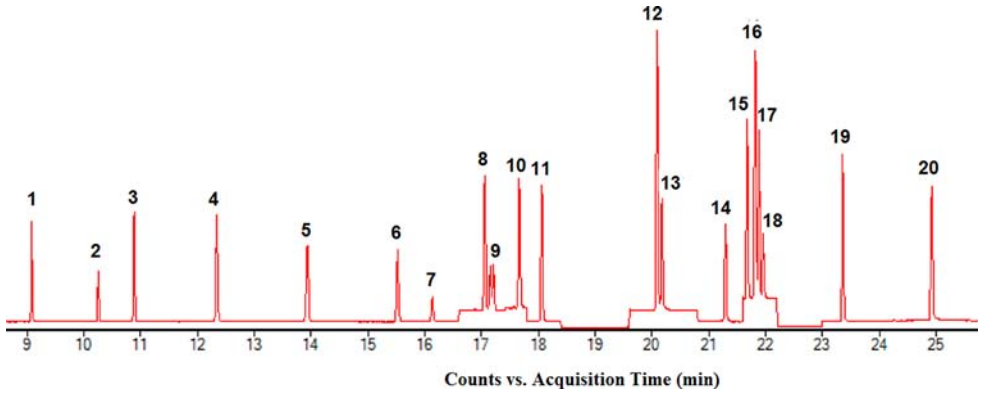

Figure 4. Total ion chromatogram of spiked blank matrix. Peak identification numbers correspond to compounds reported in Table 2.

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Table 3. Recovery of Twenty Phthalate Esters in Spiked Vegetable Oil (n = 7)

		added 5 μg/kg		added 15 μg/kg		added 40 μg/kg	
no.	analyte	recovery (%)	RSD (%)	recovery (%)	RSD (%)	recovery (%)	RSD (%)
1	DMP	103.3	2.34	99.17	2.65	102.3	3.61
2	DEP	99.65	3.56	98.76	2.62	96.23	1.01
3	DIPrP	98.45	2.78	94.36	2.45	97.67	2.71
4	DPrP	95.63	3.82	104.5	2.88	93.04	2.47
5	DIBP	99.17	4.40	95.17	1.31	102.3	3.59
6	DBP	93.65	5.06	100.5	1.87	96.30	2.85
7	DMEP	96.37	4.90	97.52	1.83	98.37	4.32
8	DIPeP	97.62	3.68	96.18	1.43	98.66	2.56
9	BMPP	96.86	2.66	104.6	2.56	99.29	3.19
10	DEEP	94.83	5.04	96.38	3.19	98.39	4.65
11	DPeP	101.6	4.46	99.11	3.32	97.19	2.62
12	DHXP	102.6	3.38	95.63	1.82	99.08	3.91
13	BBP	98.30	3.56	93.58	1.66	97.01	3.75
14	DBEP	95.78	4.22	96.09	2.14	97.32	1.38
15	DCHP	96.85	4.00	97.89	1.40	104.7	2.67
16	DHP	95.54	5.18	96.38	2.99	103.2	2.89
17	DPhP	102.2	3.92	98.09	1.57	101.6	4.90
18	DEHP	100.9	4.83	97.29	3.14	99.13	5.26
19	DNOP	103.2	2.43	98.67	1.91	97.38	1.53
20	DNP	101.6	3.51	97.54	2.84	99.63	3.56

increased solvent volumes. Based upon the above considerations, the minimum amount of solvent was used for extraction in this method.

Microwave power and temperature are very interrelated to each other and need to be given special attention particularly when working with a closed vessel system. In our work, as a representation, Figure 1 shows that recoveries for DMP differed markedly when compared under the same time (30 min) at different extraction temperatures. The recoveries increased with increasing temperature from 70 to 100 °C; no significant difference was observed in the mean recoveries of the analytes between 100 and 130 °C. As the time required to reach 100 °C during microwave irradiation was only half that required to reach 130 °C. The optimum extraction temperature was selected at 100 °C. For the other 19 esters, a similar result was obtained. The test result showed that an increase in the extraction temperatures resulted in an increase in the recoveries for the 20 phthalate esters. This elevated temperature does indeed result in improved extraction efficiencies since desorption of analyte from active sites in the matrix will increase. Additionally solvent has higher capacity to solubilize analytes at higher temperature while surface tension and solvent viscosity decrease with temperature, which will improve matrix penetration.

As in other extraction techniques, time is another parameter whose influence needs to be taken into account. The effect of extraction time from 5 to 60 min on recovery was investigated at 100 °C, as shown in Figure 2.

An increase in extraction time from 5 to 15 min gave a considerable increase in the mean recoveries. The differences in mean recoveries were insignificant when the duration of extraction was increased from 15 to 60 min. A longer extraction time did not result in any considerable increase in the extraction efficiency. For the other 19 esters, a similar result was obtained. A time of 15 min was selected, since it was sufficient to extract all the phthalate esters present in oil samples, which is shorter than the time required for conventional Soxhlet extraction and mechanical shaking.

Optimization of Cleanup System. In order to eliminate the coextractives from the extract obtained by MAE, GPC was chosen to remove some high molecular interference such as lipids and pigments based on the great difference in molecular size between coextractives and the target compounds. We investigated separation efficiency of GPC for the vegetable oil sample. One milliliter of the extracts of the vegetable oil mixed

Table 4. Determination of Edible Vegetable Oils ($X \pm$ SD, n = 3, mg/kg)[a]

		walnut oil		olive oil		mustard oil		soybean oil	
no.	analyte	std meth	this meth	std meth	this meth	std meth	this meth	std meth	this meth
1	DMP	nd	nd	nd	nd	nd	nd	nd	nd
2	DEP	nd	nd	nd	nd	nd	nd	nd	nd
3	DIPrP	–	nd	–	nd	–	nd	–	nd
4	DPrP	–	nd	–	nd	–	nd	–	nd
5	DIBP	nd	nd	nd	nd	nd	nd	nd	nd
6	DBP	nd	nd	nd	0.42 ± 0.06	nd	nd	nd	nd
7	DMEP	nd	nd	nd	nd	nd	nd	nd	nd
8	DIPeP	–	nd	–	nd	–	nd	–	nd
9	BMPP	nd	nd	nd	nd	nd	nd	nd	nd
10	DEEP	nd	nd	nd	nd	nd	nd	nd	nd
11	DPeP	nd	nd	nd	nd	nd	nd	nd	nd
12	DHXP	nd	nd	nd	nd	nd	nd	nd	nd
13	BBP	nd	nd	nd	nd	nd	nd	nd	nd
14	DBEP	nd	nd	nd	nd	nd	nd	nd	nd
15	DCHP	nd	nd	nd	nd	nd	nd	nd	nd
16	DHP	–	nd	–	nd	–	nd	–	nd
17	DPhP	nd	nd	nd	nd	nd	nd	nd	nd
18	DEHP	nd	nd	nd	0.70 ± 0.05	3.72 ± 0.32	4.93 ± 0.20	nd	nd
19	DNOP	nd	nd	nd	nd	nd	nd	nd	nd
20	DNP	nd	nd	nd	nd	nd	nd	nd	nd

[a]nd not detected, <LOQ; –, not determined.

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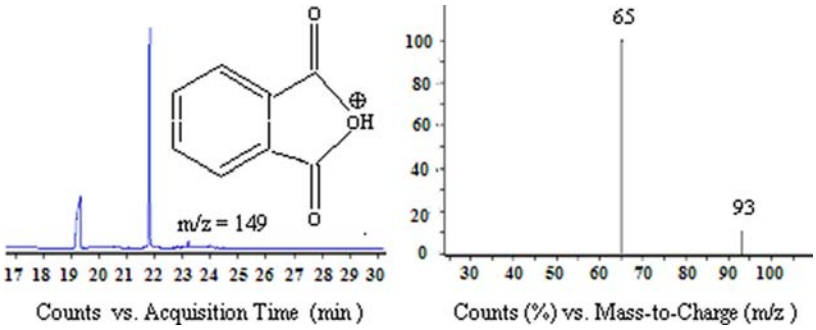

Figure 6. Mass spectra of the most abundant ion and characteristic fragment ions of DEHP in an olive oil sample.

with DMP standard of 10 μg/kg was injected into the GPC system. The elution was carried out with ethyl acetate−*n*-hexane (1:1, v/v) at a flow rate of 4.7 mL/min. The chromatograms were detected at a wavelength of 254 nm. It is shown in Figure 3 that the interferents were almost eluted in 8.5 min, the standards began to be outflowed from the GPC column at the 9.5 min, and the target analytes were completely eluted in 8.5−15 min.

The compounds of high size in vegetable oil samples can be eluted earlier than compounds of lower size phthalate esters. The GPC cleanup can decrease the presence of interferents in the final extract and also avoid the deterioration of the chromatographic column. If the extract was not treated through the cleanup steps, the interferents increased GC−MS/MS background noise and decreased the sensitivity of analytes. At the same time a lot of interferents also contaminated the ion source of MS/MS along with the analytic course of samples.

In order to achieve the goal of further separation and enrichment, the effect of SPE column (C18, silica, and HLB) and eluent on purification for the extract of blank sample spiked with 10 μg/kg DMP standard was compared for 3 parallel assays. The result showed that, for C18, silica, and HLB column with 5 mL of methanol as an eluent, the average recovery was 85.4%, 69.8%, and 80.2%, respectively, and with 5 mL volume of methanol−dichloromethane (1:1) as an eluent that was increased to 92.6, 73.5, and 86.8%, respectively.

The maximum efficiency was achieved with C18 SPE column and 5 mL volume of methanol−dichloromethane (1:1) for cleanup. The effective separation of matrices and enrichment of target compounds were achieved using GPC separation followed by cleanup with C18 solid phase extraction, with the recovery more than 92% for all analytes.

Optimization of Chromatography Separation. HP-5MS (30 m × 0.32 mm, 0.25 μm), DB-17MS (30 m × 0.32 mm, 0.25 μm), and DB-WAX (30 m × 0.32 mm, 0.25 μm) quartz capillary columns were compared for the separation of twenty analytes. Five heat programs were investigated under initial oven temperature 50 °C for 1 min, increased to 180 °C at different rates (10, 15, or 20 °C/min) with 4 min hold time, and a second ramp to 280 °C at different rates (8, 10, or 12 °C/min) with 7 min hold time. On the basis of the test result, HP-5MS quartz capillary columns were selected, and the heat program was used as follows: oven temperature increased to 180 °C at a rate of 15 °C/min with 4 min hold time, and a second ramp to 280 °C at a rate of 10 °C/min with 7 min hold time. The baseline separation was achieved and sample detection was completed within 25 min. Total ion chromatograms of blank matrix spiked with 100 μg/kg for each analyte are shown in Figure 4.

It was shown in Table 1 that there were 2 ion pairs for each compound. Total ion chromatogram for 20 analytes was a synthetical chromatogram of the 20 parent ions. Figure 4 shows that there was a closely eluting problem (DIPeP and BMPP; DHXP and BBP; and DHP, DPhP, and DEHP), which can be solved by extraction ion chromatogram. So their separation and quantification can be achieved.

Selection of Characteristic Ion Pairs for MS/MS. The electron impact (EI) ionization source was used in the work. The ion scan chromatograms of molecular ion-induced characteristic pieces were observed. The parameters were optimized by combining the ion scan chromatograms of matrix blank and standard. The characteristic ion pairs for 20 analytes were confirmed for signal collection under SRM (see Table 1). The phthalate esters are mostly middle polar and strongly lipophilic compounds. These phthalate esters are based on the 1,2-benzenedicarboxylic acid structure. There are an infinite number of possible alkyl side chains (R) and an infinite number of combinations of the side groups (R and R′). For phthalate esters with saturated alkyl side chains (without oxygen), the most abundant ion in the EI ionization mass spectrum at 70 eV is always at m/z 149, with a signal-to-noise ratio approximately 10−100 times higher than that of other ions in the spectrum. It is due to the rapid formation and stability of the ion shown in Figure 5. One exception is for DMP where both R and R′ represent CH_3 and so the H on the oxygen is replaced by CH_3. Consequently, m/z 163 becomes the base peak. Another exception is for DPhP, where both R and R′ represent C_6H_5 and the base peak is at m/z 227.

The retention times of quantification ion for 20 analytes were obtained with EI source under positive ion mode and selected reaction monitoring (SRM) (see Table 2). The favorable separations and response for various components were achieved within 25 min.

Performance of the Method. In order to evaluate the matrix effect, a comparison between calibration curves obtained from standards prepared in pure solvent and calibration curves constructed using some matrix spiked with standards was performed. It was observed that the responses obtained were nearly equivalent in both cases. To ensure accuracy matrix-matched calibration curves were used in this work. The regression equations were obtained using the 7-points concentration of standard as abscissa and area of chromatogram peak as vertical coordinate. It is shown in Table 2 that good linearity was obtained in the range from 5 μg/kg to 2.50 mg/kg for the 20 analytes with a correlation coefficient of >0.999.

Method sensitivity was evaluated by measuring the limits of detection (LOD) and the limits of quantification (LOQ) of examined phthalate esters for the vegetable oil, calculated

according to the directives of IUPAC and the American Chemical Society's Committee on Environmental Analytical Chemistry, taken $S_{LOD} = S_{RB} + 3\sigma_{RB}$ and $S_{LOQ} = S_{RB} + 10\sigma_{RB}$ where S_{LOD}, S_{LOQ}, and S_{RB} are the signal at the limit of detection, at the limit of quantification, and of the reagent blank, respectively, while σ_{RB} is the standard deviation for the reagent blank. The method limit of detection (LOD) of twenty analytes was in the range of 0.218−1.367 μg/kg, and the limit of quantification (LOQ) was in the range from 0.72 to 4.51 μg/kg. The LOD values were better than those reported in the literature,[9−17,19,22−26,28] and for many analytes also lower than that in the literature.[18,20,21] With increase in sampling quantity, the LOD values could be improved further.

Recovery. The recovery experiment for phthalate esters was carried out by adding 5, 15, and 40 μg/kg for each analyte in real vegetable oil sample followed by MAE extraction, GPC−SPE cleanup, and GC−MS/MS analysis. The recovery and RSD are given in Table 3.

The data in Table 3 show that the recovery for the twenty analytes was in the range of 93.04−104.7% with RSD of 1.01−5.26%.

Application to Real Samples. The proposed method was applied for monitoring edible vegetable oils. Of forty soybean oil, ten walnut oil, six olive oil, and six flaxseed oil samples, DMP and DEHP in only two samples were found. The results obtained by this method were compared with the standard method.[12]

No analyte in the four classes of oil samples was detected using the standard method (LOD 1.5 mg/kg) except for DEHP in a mustard oil sample (Table 4). Using the proposed method, the contents of DBP and DEHP in an olive oil sample were found to be 0.42 ± 0.06 and 0.70 ± 0.05 mg/kg, respectively, and DEHP in a mustard oil sample was found to be 4.93 ± 0.20 mg/kg, and concentration of the rest of the analytes was lower than their LOQ.

DBP and DEHP in the olive oil and mustard oil were identified. The ion ratio of each analyte is effectively measured on each of the chromatograms. The retention time of DEHP was 21.9 min, the same as the retention time in the standard solution. The peaks of two transitions including the characteristic fragment ion (m/z 93) and characteristic fragment ion (m/z 65) were present, as shown in Figure 6. It illustrates that the GC−MS/MS method could be use for confirmatory studies in the detection of phthalate ester in vegetable oil samples.

Based on the above results, the developed MAE-GPC-SPE-GC-MS/MS method can ensure the confirmation and multiresidue analysis at low μg/kg level for the studied phthalate esters in vegetable oils.

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Notes

The authors declare no competing financial interest.

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