Food Additives & Contaminants: Part B: Surveillance
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tfab20

Simultaneous analysis of phthalates, adipate and polycyclic aromatic hydrocarbons in edible oils using isotope dilution-gas chromatography-mass spectrometry

Min-Seok Oh[ab], Seon-Hwa Lee[ab], Myeong Hee Moon[b], Dong Soo Lee[b] & Hyun-Mee Park[a]
[a] Advanced Analysis Center, Korea Institute of Science and Technology, Seoul, Republic of Korea
[b] Department of Chemistry, Yonsei University, Seoul, Republic of Korea
Published online: 05 Feb 2014.

Food Additives & Contaminants: Part B, 2014
http://dx.doi.org/10.1080/19393210.2013.869770

Simultaneous analysis of phthalates, adipate and polycyclic aromatic hydrocarbons in edible oils using isotope dilution-gas chromatography–mass spectrometry

Min-Seok Oh[a,b], Seon-Hwa Lee[a,b], Myeong Hee Moon[b], Dong Soo Lee[b] and Hyun-Mee Park[a]*

[a]*Advanced Analysis Center, Korea Institute of Science and Technology, Seoul, Republic of Korea;* [b]*Department of Chemistry, Yonsei University, Seoul, Republic of Korea*

(*Received 28 August 2013; accepted 24 November 2013*)

A method for simultaneous determination of 12 priority phthalates, adipate and polycyclic aromatic hydrocarbons (PAHs) in edible oils by isotope dilution-gas chromatography–mass spectrometry (ID-GC–MS) was developed for fast, accurate and trace analysis. The extraction and clean-up procedures were optimised, and using stable isotope-labelled internal standards for each analyte, relative standard deviations (RSDs) of 0.92–10.6% and spiked sample recoveries of 80.6–97.8% were obtained. Limits of detection for PAHs were in the range of 0.15–0.77 μg/kg and those for phthalates were in the range of 4.6–10.0 μg/kg. The calibration curves exhibited good linearities with regression coefficients of $R^2 \geq 0.99$. Twelve edible oils were examined to evaluate the efficiency of this method. Among the 12 analytes, dibutyl phthalates (DBP), diethylhexyl phthalates (DEHP), diethylhexyl adipate (DEHA), benzo[a]anthracene (B[a]A), chrysene (Chry) and benzo [b]fluoranthene (B[b]F) were detected in the range of 1.17–806 μg/kg.

Keywords: phthalates; adipate; polycyclic aromatic hydrocarbons; isotope dilution; GC–MS; edible oils; simultaneous determination

Introduction

Endocrine disrupting phthalates and carcinogenic polycyclic aromatic hydrocarbons (PAHs) are present not only in the environment but are also migrated into foods during packaging, storage (Aurela et al. 1999) and manufacturing (Phillips 1999). Phthalates are plasticisers that gently soften plastics (Steiner et al. 1998) and are widely used in industrial products, such as toys, vinyl flooring, medical devices and food packaging (Hauser & Calafat 2005; Guo et al. 2012). Because phthalates do not form stable bonds with plastic polymers, they easily accumulate in the fats of foods exposed to packaging and processing with plastic products (Witorsch 2002). Carcinogenic PAHs, which have more than two benzene rings, are formed during the incomplete combustion of organic substances, such as wood, fuel, tobacco and food; thus, PAHs can be incorporated into foods during production (Camargo & Toledo 2002; FAO/WHO 2005). In addition, humans may be exposed to PAHs through accumulation in soil, water, air, food chain and diol epoxide. Diol epoxide is a PAH intermediate that induces cytotoxicity, mutations and tumours by interacting with proteins, DNA and RNA (Burczynski et al. 1999; Mollerup et al. 2001). Because phthalates occur in plastic containers and PAHs in the course of food processing and cooking, such as during roasting and smoking, most of the exposure to these substances is associated with food (Kluska 2003). Lipophilic phthalates migrate into oils from plastic packaging and containers, whereas PAHs are produced by the incomplete combustion of plant seeds during oil production; therefore, edible oils are commonly contaminated with both phthalates and PAHs.

The Scientific Committee for Food (SCF, EU) has designated the tolerable daily intake (TDI) of dibutyl phthalates (DBP), benzylbutyl phthalates (BBP), diethylhexyl phthalates (DEHP) and diethylhexyl adipate (DEHA) as 0.1 mg/kg, 0.05 mg/kg, 0.05 mg/kg and 0.3 mg/kg per day, respectively (Petersen & Breindahl 2000; Kueseng et al. 2007). In vegetable oil, concentrations of DBP and DEHP were <0.01–0.30 mg/kg and 0.25–1.10 mg/kg (Liu et al. 2013) and in cooking oil were 4.00–17.9 μg/kg and 47.1–70.9 μg/kg (Guo et al. 2012), respectively. For PAHs, the United States Environmental Protection Agency (USEPA) manages 16 species, especially benzo[a]pyrene (B[a]P), benzo[a] anthracene (B[a]A), dibenzo[a, h]anthracene (DB[ah]A) and chrysene (Chry). The European Union Commission Regulation (835/2011 L215) (EPA 1984) permits a maximum level of B[a]P of 2 μg/kg and the sum of B[a]P, B[a] A, Chry and benzo[b]fluoranthene (B[b]F) of 10 μg/kg for oils and fats. Concentrations of B[a]P and B[a]A were in the ranges of 0.05–0.31 μg/kg and 0.05–2.83 μg/kg in smoked food, and B[a]P, B[a]A, Chry and DB[ah]A were detected in the ranges of 0.7–1.3 μg/kg, 0.8–9.0 μg/kg, 0.7–6.4 μg/kg and ND–0.1 μg/kg in edible oils, respectively (Moreda et al. 2001).

*Corresponding author. Email: phm4556@kist.re.kr

Extraction and clean-up are the most important steps for phthalates and PAHs analysis in food, especially in fatty food samples. Currently, several extraction, purification (clean-up) and detection methods are employed for the determination of phthalates and PAHs. Phthalate extraction methods include liquid–liquid extraction (LLE) (Wu et al. 2012; Liu et al. 2013), solid-phase extraction (SPE) (Nanni et al. 2011; Liu et al. 2013) and solid-phase microextraction (SPME) (Cao 2008). Extraction with polar or nonpolar solvents following silica and C_{18} cartridge clean-up has also been used for phthalates analysis, but the limit of detection (LOD) was relatively poor, being measured in the range of 0.01–0.1 mg/kg (Nanni et al. 2011; Wu et al. 2012; Liu et al. 2013). Soxhlet (Jaouen-Madoulet et al. 2000), pressurised-liquid extraction (PLE) (Jira 2004), microwave-assisted extraction (MAE) (Saim et al. 1997; Mooibroek et al. 2002) and LLE (Bogusz et al. 2004; Ballesteros et al. 2006) are typical PAH extraction methods, and clean-up methods such as gel-permeation chromatography (GPC), (Liguori et al. 2006) silica gel and florisil (Simon et al. 2006; Tfouni et al. 2007) are commonly used. The back extraction of PAHs with a polar solvent following a nonpolar solvent is time-consuming and yields low recoveries (Mottier et al. 2000; Diletti et al. 2005). The detection of phthalates is usually performed by gas chromatography-flame ionization detector (GC-FID) (Polo et al. 2005), gas chromatography-mass spectrometery (GC-MS) (Nanni et al. 2011; Wu et al. 2012; Liu et al. 2013) and liquid chromatography-mass spectrometery (LC-MS) (Jonsson & Boren 2002), whereas PAHs are detected by GC–MS (Bogusz et al. 2004; Ballesteros et al. 2006), GC–MS/MS (Helaleh et al. 2005) and high performance liquid chromatography-fluorescence detection (HPLC-FLD) (Pena et al. 2006; Tfouni et al. 2007). As an isotope dilution method that uses the deuterated or C^{13} isotope of analyte as the internal standard, which shows similar physical and chemical behaviour as the analytes, this can improve the accuracy by reducing recovery errors from the complex matrix and interferences during calibration for quantification (Boden & Reiner 2004). Usually, one or two isotopes are used as internal standards for the analysis of phthalates (DBP-d4, DEHP-d_4; Feng et al. 2005) and PAHs (phenanthrene-d_{10}, anthracene-d_{10}, benzo(a)pyrene-d_{12}; Wang & Guo 2010).

As mentioned above, the extraction and clean-up methods are different for phthalates and PAHs, and individual compounds are analysed separately. Thus, it is difficult to treat a large number of samples at the same time. Therefore, a simultaneous analysis method is needed for simple and fast analysis. The aim of this study was to develop a pretreatment and analytical method for edible oils with low LODs, good precision and accuracy for the simultaneous determination of three phthalates (dibutyl phthalates (DBP), benzylbutyl phthalates (BBP), diethylhexyl phthalates (DEHP)), one adipate (diethylhexyl adipate (DEHA) and eight PAHs (benzo[a]anthracene (B[a]A), chrysene (Chry), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), indeno[1,2,3-cd]pyrene (I[cd]P), benzo(ghi)perylene (B[ghi]P) and dibenzo[a, h]anthracene (DB[ah]A)). To analyse these compounds simultaneously, LLE and solid-phase clean-up were optimised, and an isotope dilution-GC–MS method, which uses isotopes for all analytes, was established for the improvement of the accuracy. The new method was applied to 12 samples, to demonstrate that it is able to determine phthalates, adipate and PAHs in edible oil.

Materials and methods

Chemicals and samples

All solvents were HPLC-grade and purchased from J.T Baker (Phillipsburg, NJ). Analytical standards of phthalates (purity > 99%) were DBP (Chem Service, West Chester, PA), BBP and DEHP (Wako Chemical, Osaka, Japan) and DEHA (Sigma Aldrich, Aldrich Chemical, USA). Analytical mixture standards of PAHs (2000 µg/mL), B[a]A, Chry, B[b]F, B[k]F, B[a]P, I[cd]P, B[ghi]P and DB[ah]A were obtained from Supelco (Bellefonte, PA).

Individual stock solutions of phthalate were prepared in methanol, and the PAHs were prepared in dichloromethane (DCM). Calibration standard mixtures of the four target analytes (DBP, BBP, DEHP and DEHA, 100 µg/mL) were dissolved in methanol. Their corresponding deuterated internal standards, DBP-d_4, BBP-d_4, DEHA-d_8, B[b]F-d_{12}, B[k]F-d_{12}, B[a]A-d_{12}, I[cd]P-d_{12}, DB[ah]A-d_{14} and B[ghi]P-d_{12} were purchased from Chiron As (Trondheim, Norway) and DEHP-d_4, B[a]A-d_{12} and Chry-d_{12} were obtained from Accustandard (New Haven, CT). Anhydrous Sodium sulphate (Na_2SO_4) for dehydration was obtained from Kanto chemical (Tokyo, Japan) and florisil® (60–100 mesh), for SPE, was purchased from J.T Baker (Phillipsburg, NJ).

Phthalates are widely present in the environment, such as in soil, rivers, drinking water, air and also in plants and animals. Furthermore, many solvents, chemicals, glassware, gloves, and vials contain phthalates, especially DBP and DEHP (Fankhauser-Noti & Grob 2007). This is a significant source for potential error in the results. To minimise the blank contamination, all the glassware was baked for 2 h at 200°C, Na_2SO_4 was baked for 7–8 h at 700°C and florisil was baked for 16 h at 130°C and then stored in a vacuum desiccator until analysis. Solvents were stored in bottles containing alumina adsorbent prior to use. Sample analyses were

conducted with 12 edible oils (rice bran, brown rice, canola, olive, grape seed, corn and soybean) that were purchased from a general retail market.

Optimisation of pretreatment

The solvent for the LLE and the eluent for the solid-phase extraction (SPE) used to extract and clean-up the phthalates and PAHs simultaneously were selected based on following reference results. Usually, the extraction solvents for phthalates and PAHs are different. Methylene chloride, *n*-hexane and acetonitrile (Wu et al. 2012; Liu et al. 2013) were used to extract the phthalates, whereas cyclohexane, dimethylformamide (DMF):H_2O (Diletti et al. 2005), dimethylsulfoxide (DMSO) (Mottier et al. 2000) and a mixture of *n*-hexane and acetonitrile were used to extract PAHs (Ballesteros et al. 2006). Therefore, it is important to select an appropriate extraction solvent for both substances. Nonpolar solvent extraction requires considerable time, and the loss of analytes is relatively large with back extraction. Conversely, the polar solvent extraction efficiency varies depending on the polarity of the solvent. In this study, extractions were performed using polar and nonpolar solvents as follows: methanol extraction, acetonitrile extraction, acetonitrile extraction after dissolving in hexane and hexane extraction after back extraction with DMF:H_2O (9:1). The extraction efficiencies are shown in Figures 1A and B.

After LLE, SPE helps to remove interferences from the complex matrix. Florisil, a polar stationary phase, was used as the SPE sorbent to purify the phthalates (Wu et al. 2012) and PAHs (Bartolome et al. 2005; Ehrenhauser et al. 2010) from interfering polar substances. One hundred grams of florisil, baked at 130ºC for 16 h, was mixed with 5.7 mL of water, and a 1 g aliquot was packed in a glass syringe with methanol. Instead of a commercial plastic cartridge, glass was used to avoid phthalate contamination from the plastic. The cartridge was conditioned with 10 mL of DCM after 15 mL hexane before clean up for preconditioning of the cartridge. Five solvents were compared for effective purification: *n*-hexane, *n*-hexane:DCM (3:1), *n*-hexane:DCM (1:1), *n*-hexane:acetone (9:1) and *n*-hexane:acetone (3:1). The results are shown in Figures 1C and D. The amount of solvent used during the extraction was also varied between 5 and 25 mL to determine the optimum volume.

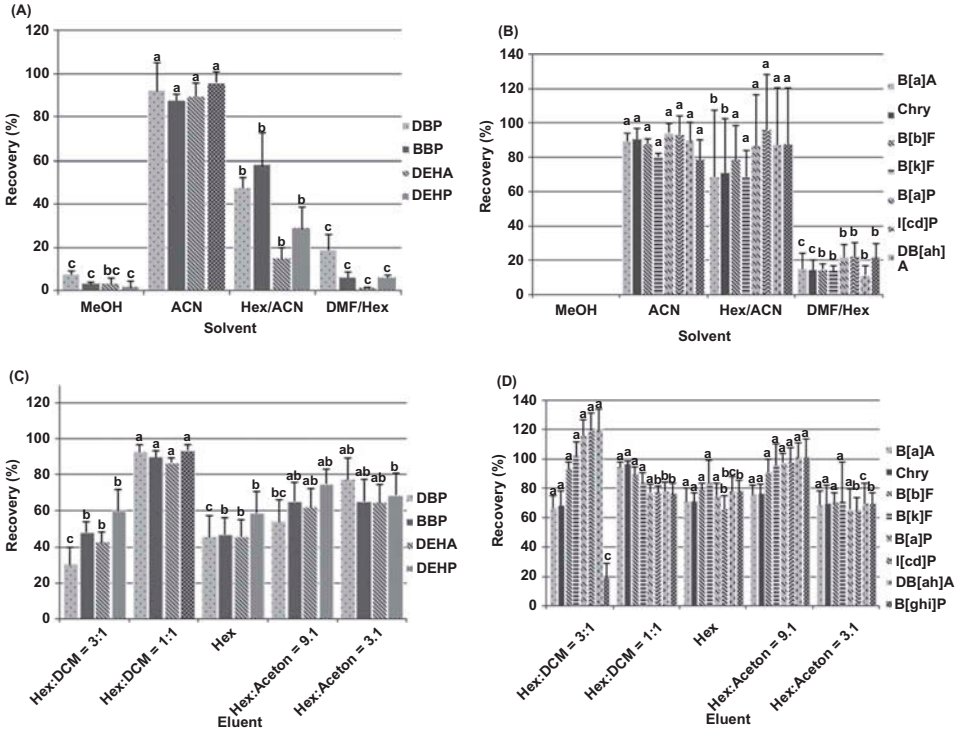

Figure 1. Extraction efficiencies of various solvents for (A) phthalates, (B) PAHs for the liquid–liquid extraction, (C) phthalates and (D) PAHs for the solid-phase extraction (ANOVA analysis, $P < 0.05$): dibutyl phthalates (DBP), benzylbutyl phthalates (BBP), diethylhexyl phthalates (DEHP), diethylhexyl adipate (DEHA), benzo[a]anthracene (B[a]A), chrysene (Chry), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), indeno[1,2,3-cd]pyrene (I[cd]P), dibenzo[a, h]anthracene (DB[ah]A), benzo(g,h,i)perylene (B[ghi]P), methanol (MeOH), acetonitrile (ACN), *n*-hexane (Hex), dimethylformamide (DMF) and dichloromethane (DCM); a, b, c across the group signifies that means with different letters differ significantly at $P < 0.05$ while means with the same letters do not differ significantly at $P < 0.05$.

Isotope dilution-GC–MS analysis

Accuracy was compared between typical isotope dilution methods using DEHP-d_4 and B[a]P-d_{12} as internal standards and the 1:1 isotope dilution method of this study using 12 isotopes corresponding to 12 analytes: DBP-d_4, BBP-d_4, DEHA-d_8, DEHP-d_4, B[a]A-d_{12},Chry-d_{12}, B[b]F-d_{12}, B[k]F-d_{12}, B[a]A-d_{12}, I[cd]P-d_{12}, DB[ah]A-d_{14} and B[ghi]P-d_{12}. Five hundred nanograms of the isotope mixture was added to 4 g of the oil sample before extraction.

Extracted samples were analysed with gas chromatography–time-of-flight mass spectrometry (GC–TOFMS: Agilent 6890N GC system, USA; Leco, PEGASUS IV MS system, USA). Data processing was performed with LECO®ChromaTOF™ and NIST MS search 2.0. A DB-5MS column (30 m × 0.25 mm × 0.25 μm, Agilent Technology, USA) was used with He (99.999%) carrier gas at a flow rate of 0.8 mL/min. The inlet was in the split (5:1) mode at 300°C with an injection volume of 1 μL. An oven gradient programme was used for the separation: 100–200°C with a 25°C/min ramp and a duration of 2 min, increase to 300°C with a ramp of 5°C/min and a duration of 1 min and finally increase to 315°C with a ramp of 5°C/min and a duration of 5 min. The operating parameters for the MS were as follows: electron impact (EI) ion source temperature, 220°C; electron energy, 70 eV; electron multiplier detector (EM) voltage, 1580 eV; transfer line temperature, 300°C; MS range, 30–500 amu. The parameters for the mass analysis of the analytes are listed in Table 1.

Sample analysis

All the 12 edible oils (rice bran, brown rice, canola, olive, grape seed, corn and soybean) purchased from a retail market were analysed by using the optimised analytical procedures. Four gram aliquots of homogenous oil samples were weighed in 50 mL centrifuge tubes, and 500 ng of the isotope solution and 15 mL acetonitrile were added. The tubes were sealed and shaken for 15 min at 200 rpm using a mechanical shaker (Chang-shin, Korea). This process was repeated twice, and then the acetonitrile extracts were collected following dehydration with anhydrous Na_2SO_4. The extract was evaporated in a nitrogen stream during 25 min to dryness, and the residue was dissolved in 1 mL *n*-hexane. Interferences, such as lipids and polar impurities, were removed from the residue using florisil. The florisil column was conditioned with 10 mL DCM

Table 1. Parameters for the GC–MS analysis, linearity, limits of detection (LOD) and quantification (LOQ).

Compound	R.T[a] (sec)	Q.I[b] (m/z)	Q.I[c] (m/z)	Linearity (R^2)	LOD (μg/kg, n = 9)	LOQ (μg/kg, n = 9)
Phthalates						
Dibutyl phthalates	558.1	121.150	149	0.9997	9.77	29.6
Dibutyl phthalates-d_4	556.7	125.154	153			
Denzylbutyl phthalates	886.7	121.150	149	0.9981	4.70	14.2
Denzylbutyl phthalates-d_4	885	125.154	153			
Diethylhexyl phthalates	1046.5	121.150	149	0.9991	10.0	30.4
Diethylhexyl phthalates-d_4	1044.8	125.154	153			
Diethylhexyl adipate	920.1	112.130	129	0.999	4.56	13.8
Diethylhexyl adipate-d_8	914	118.138	137			
PAHs						
Benzo[a]anthracene	1000.9	226.229	228	0.9995	0.28	0.86
Benzo[a]anthracene-d_{12}	995.3	236.241	240			
Chrysene	1009.3	226.229	228	0.9999	0.26	0.78
Chrysene-d_{12}	1002.8	236.241	240			
Benzo[b]fluoranthene	1265.8	250.253	252	0.9992	0.55	1.66
Benzo[b]fluoranthene-d_{12}	1260.2	260.265	264			
Benzo[k]fluoranthene	1272.6	250.253	252	0.9987	0.44	1.34
Benzo[k]fluoranthene-d_{12}	1267.8	260.265	264			
Benzo[a]pyrene	1339.8	250.253	252	0.9966	0.15	0.44
Benzo[a]pyrene-d_{12}	1334.6	260.265	264			
Indeno[1,2,3-cd]pyrene	1586.9	276.279	276	0.9987	0.32	0.96
Indeno[1,2,3-cd]pyrene-d_{12}	1581.5	284.289	288			
Dibenzo[a, h]anthracene	1596.5	274.277	278	0.9917	0.50	1.53
Dibenzo[a, h]anthracene-d_{14}	1589.1	288.293	292			
Benzo(g,h,i)perylene	1641.9	274.277	276	0.9987	0.77	2.33
Benzo(g,h,i)perylene-d_{12}	1636	284.289	288			

Notes: [a]Retention time; [b]Qualification ion; [c]Quantification ion.

following 15 mL of *n*-hexane. The elution solvent used for clean-up was 15 mL of a 1:1 DCM and *n*-hexane mixture. The eluate was dried in a nitrogen stream, and the residue was dissolved in 250 µL ethylacetate. One microlitre of this solution was injected into the GC–MS.

Statistical analysis

Recovery results of the extraction and clean-up procedures are presented as mean value ± standard deviation (SD) for four replicates. Multiple comparisons of means were performed by analysis of variance (ANOVA), and the means were separated by Duncan's multiple range test considering significant differences at $P < 0.05$.

Results and discussion

Optimisation of pretreatment

The recoveries of the phthalates and PAHs with methanol are shown in Figures 1A and B. The phthalate recoveries were poor, ranging from 1.8% to 7.6%, and PAHs were not recovered. The extraction efficiency using DMF:H_2O (9:1) following back extraction with hexane also showed poor recovery rates: 1.5–18.9% for phthalates and 11.5–22.6% for PAHs. Using acetonitrile, all phthalates and PAHs were extracted with good recoveries (87.5–95.8% and 78.8–94.3%) and repeatabilities (3.0–13.1% and 1.5–11.0%).

The residue oil remaining in the extract makes chromatographic separation poor due to the contamination of the column (column plugging). Therefore, it is important to clean-up, with high recovery efficiency, both phthalates and PAHs in edible oil. In Figure 1C, the *n*-hexane:DCM (1:1) solvent showed the highest recovery efficiency, from 87.0% to 94.1% for phthalates, whereas the recovery efficiency was relatively low with other eluents, ranging from 30.3% to 77.5%. As shown in Figure 1D, the extraction efficiencies for B[a]A and Chry were 94.7% and 96.6%, respectively, with the DCM:*n*-hexane (1:1) solvent, whereas other solvents showed relatively low recoveries for these two PAHs, ranging from 76.0% to 89.4%. The recovery efficiencies of the other PAHs (B[b]F, B[k]F, B[a]P, I[cd]P, B[ghi]P and DB[ah]A) were good with the *n*-hexane:DCM (3:1) and *n*-hexane:acetone (9:1) solvents. However, the recoveries for these PAHs using the *n*-hexane:DCM (1:1) solvent were similar to the other solvents.

From the statistical analysis of ANOVA, we can see significant efficiency differences between solvents and eluents in phthalates (Figures 1A and C) but no significant difference in PAHs (Figures 1B and D). However, because the goal of this research is the simultaneous determination of PAHs and phthalates, we chose acetonitrile as the solvent and *n*-hexane:DCM (1:1) as the eluent which gave good recoveries (87.0–95.8% for phthalates and 76.0–96.6% for PAHs) and repeatabilities (2.7–13.1% for phthalates and 1.5–11.0% for PAHs) for both phthalates and PAHs. Volume of 15 mL of this eluent showed maximum recoveries: phthalate (92.9–94.7%) and PAHs (78.3–93.7%), so the florisil clean-up was conducted with 15 mL of *n*-hexane:DCM (1:1) eluent for all the experiments.

Instead of the typical one or two isotope internal standards, all isotopes corresponding to all analytes were added for the isotope dilution (1:1 isotope dilution). The accuracy using DEHP-d_4 and B[a]P-d_{12} as internal

Table 2. Method validation results of phthalates, adipate and PAHs in oil samples.

	Recovery (%)			Accuracy (%)				Precision (%)	
	50	250	1250	50	250	1250	TID[a]	Interday	Intraday
Compounds	(µg/kg, n = 9)			(µg/kg, n = 9)				(n = 5)	(n = 3)
Phthalates									
DBP	91.6	92.1	91.2	107.5	97.6	94.2	91.4	4.1	3.8
BBP	87	94.6	83.9	93.2	98.1	91.4	74.1	4.7	3.6
DEHP	97.8	94.7	94.3	105.8	101.1	98.8	96.8	5.8	4.3
DEHA	89.7	89	81.7	96.8	99.1	90.7	88.3	6.3	7.8
PHAs									
B[a]A	96.9	92.9	96.9	94.3	99	115.6	63	4.0	2.9
Chry	92.1	92.7	96.9	94.3	93.7	106.4	78.2	2.3	2.5
B[b]F	96.7	81.3	87.7	97.1	92.5	103.6	78.2	4.4	3.7
B[k]F	93.8	90.3	88.3	97.1	98.4	102.4	78.9	6.0	6.1
B[a]P	91.7	87.3	95.8	93.1	94.7	95.8	87.3	5.8	4.9
I[cd]P	87.9	75.0	80.6	93.6	102.5	105.8	73.8	6.8	3.9
DB[ah]A	93.2	92.7	82.7	96.4	96.0	103.7	129.2	5.0	5.3
B[ghi]P	88.4	73.5	85.1	91.8	96.1	108.1	115	4.6	2.7

Note: [a]Typical isotope dilution (250 µg/kg).

standards was compared to that of the 1:1 isotope dilution method, where 12 isotopes corresponding to all analytes were spiked. The results are shown in Table 2. The accuracy of this method was 92.5–102.5%, whereas that of the typical isotope dilution was 63.0–129.2%. As expected, the accuracy was improved using the 1:1 isotope dilution method. Therefore, we used the 1:1 isotope dilution method for all the following experiments.

Method validation

As mentioned, phthalates are widespread throughout the experimental environment, such as vials, solvents, injector septa, gloves, glasses and even air; therefore, blanks have to be measured before each sample measurement. All analytical procedures for blanks were performed seven times, in which the sample was not included in the blank. The background levels of DBP and DEHP were determined to be 41.5 ± 5.5 μg/kg and 49.4 ± 7.5 μg/kg, respectively, and these values were subtracted in the sample concentration calculations.

To evaluate the effectiveness of this new method, the precision was measured in inter-day (n = 5) and intra-day (n = 3) intervals at low, medium and high concentrations (50, 250 and 1250 μg/kg). The relative standard deviations (RSDs) were as low as 3.6–7.8% for phthalates and 2.3–6.8% for PAHs. The accuracy experiment was performed by determining the recoveries of phthalates and PAHs in the blank oil spiked at three different concentration levels (n = 9). The data in Table 2 shows the good accuracy of the recoveries of 91.4–107.5% for phthalates and 91.8–115.6% for PAHs. The extraction recoveries were estimated by comparison of the peak area of spiked sample with the peak area of the standard solution. The recoveries of phthalates and PAHs were in the ranges of 81.7–97.8% and 80.6–96.9%, respectively. These results are not significantly different from previous studies where recoveries ranged from 64.0% to 109.0% for phthalates (Del Carlo et al. 2008; Wu et al. 2012; Liu et al. 2013) and 60.0–134.0% for PAHs (Mottier et al. 2000; Bogusz et al. 2004; Diletti et al. 2005; Ballesteros et al. 2006). For the quantification of phthalates and PAHs, calibration curves were fitted from eight points and showed good linearity; the correlation coefficients (R^2) of the phthalates were 0.9981–0.9997 and those of the PAHs were 0.9917–0.9999 (Table 1). The limits of detection (LOD, 3.3σ) and limits of quantification (LOQ, 10σ) for phthalates were 4.56–10.0 μg/kg and 13.8–30.4 μg/kg, respectively, and those of the PAHs were 0.15–0.77 μg/kg and 0.44–2.33 μg/kg, respectively. The LOD results of phthalate were 3 to 10 times lower than previous values in the literature (10–100 μg/kg), (Del Carlo et al. 2008; Nanni et al. 2011; Wu et al. 2012; Liu et al. 2013) and the values for the PAHs were similar (Bogusz et al. 2004; Diletti et al. 2005; Ballesteros et al. 2006). In a proficiency test with a sunflower oil simulant material, z-scores of –1.1 and –1.3 were achieved for DBP at 0.38 mg/kg and 0.36 mg/kg, and z-scores of –0.3 and –0.1 were achieved for DEHP at 0.86 mg/kg and 0.89 mg/kg, respectively, indicating good method performance (Food Analysis Performance Assessment Scheme 2012) These validation results show that this new method is sensitive and accurate for the quantification of both phthalates and PAHs at a low concentration levels (μg/kg) for the studied edible oils.

Application to samples

Twelve edible oils (rice bran oil, canola oil, olive oil, brown rice oil, grape seed oil, corn oil and soybean oil) were analysed to determine whether this method could be applied to oil samples. All samples were packaged in polyethylene terephthalate (PET) except sample 11 (polyethylene, PE). The food packaging industry has reduced its use of phthalates by employing polymers such as polyethylene (PE) and polypropylene (PP) instead of polyvinyl chloride (PVC). PVC contains up to 40% phthalates, usually DEHP (Aurela et al. 1999). Although the use of phthalates as plasticisers has decreased, they are still used in food packaging in many applications, such as adhesives, offset printing inks and lacquers (Aurela et al. 1999). Therefore, there still exists the possibility of contamination of phthalates from the packaging. In this study, DBP was detected in only two olive oil samples (16.7%) at concentrations of 13.2 ± 2.29 and 40.6 ± 2.30 μg/kg. Among the 12 analysed oils, a total of 9 samples (75%) were contaminated with DEHP, which accounts for more than 50% of plasticisers, at slightly high concentrations of 25.0 ± 1.77 to 806 ± 10.1 μg/kg. DEHA was detected only in S-6 (olive oil) at a concentration of 5.46 ± 0.15 μg/kg. Previous results for DBP and DEHP in Chinese vegetable oil were in the range of 250–300 μg/kg and 250–1100 μg/kg (Liu et al. 2013), respectively, which is higher than our results.

In the case of PAHs, B[a]A was found in the range of 1.17 ± 0.07–3.31 ± 0.42 μg/kg in seven oils (58.3%), whereas Chry was detected only in rice bran oil (S-1) at 2.95 ± 0.31 μg/kg and B[b]F was found in two corn oils (Table 3) at 2.12 ± 0.09 and 3.76 ± 0.16 μg/kg. In Spain, B[a]A, Chry and B[b]F were detected at <0.1–97, <0.2–217 and <0.2–67 μg/kg in edible oils, respectively (Barranco et al. 2003). Comparing our results with those from Spain, the concentrations of PAHs from our research are lower than those from Spain, and B[a]A is ubiquitous in edible oils. Other PAHs (B[a]P, B[k]F, I[cd]P, DB[ah]P and B[ghi]P) and BBP were not detected in the analysed oils. B[a]P, regulated at 2 μg/kg in edible oil in South Korea, was

Table 3. Concentrations of phthalates, adipate and PAHs in oil samples.

Sample	No.	Packaging material	Compound (μg/kg, n = 4)					
			DBP[a]	DEHP	DEHA	B[a]A	Chry	B[b]F
Rice bran	S-1	PET[b]	<9.77	498 ± 6.47	<4.56	3.31 ± 0.42	2.95 ± 0.31	<0.55
Brown rice	S-2	PET	<9.77	804 ± 9.28	<4.56	<0.28	<0.26	<0.55
Canola	S-3	PET	<9.77	34.2 ± 2.66	<4.56	2.62 ± 0.28	<0.26	<0.55
	S-4	PET	<9.77	44.0 ± 5.21	<4.56	<0.28	<0.26	<0.55
Olive	S-5	PET	40.6 ± 2.30	214 ± 5.98	<4.56	<0.28	<0.26	<0.55
	S-6	PET	13.2 ± 2.29	729 ± 4.87	5.46 ± 0.15	<0.28	<0.26	<0.55
Grape seed	S-7	PET	<9.77	<10.0	<4.56	2.00 ± 0.08	<0.26	<0.55
	S-8	PET	<9.77	806 ± 10.1	<4.56	2.35 ± 0.07	<0.26	<0.55
Corn	S-9	PET	<9.77	<10.0	<4.56	2.86 ± 0.20	<0.26	3.76 ± 0.16
	S-10	PET	<9.77	33.1 ± 1.35	<4.56	1.17 ± 0.07	<0.26	2.12 ± 0.09
Soybean	S-11	PE[c]	<9.77	25.0 ± 1.77	<4.56	<0.28	<0.26	<0.55
	S-12	PET	<9.77	<10.0	<4.56	1.94 ± 0.08	<0.26	<0.55

Notes: [a]Other phthalates and PAHs (BBP, Chary, B[k]F, B[a]P, I[cd]P, DB[ah]A and B[ghi]P) were not detected; [b]Polyethylene terephthalate; [c]Polyethylene.

not detected. The total sum of the examined PAHs was 1.94–6.6 μg/kg, which is lower than the EU commission regulation (835/2011 L215) of 10 μg/kg for the sum of four PHAs in oils and fats.

Conclusions

In this study, a new isotope dilution-gas chromatography–mass spectrometry (ID-GC–MS) method was developed for the simultaneous isolation and purification of 12 analytes from edible oils. The method showed excellent recoveries and precisions and a limit of quantification below the μg/kg level for most of the phthalates, an adipate and the PAHs. The entire procedure requires 85 min (including LLE, SPE and analysis by GC–MS) and does not require a large volume of solvents. These results demonstrate that this newly developed simultaneous analysis method could be used for the analysis of trace phthalates, adipate and PAHs in edible oil for routine analysis. This new method has many advantages, such as short time, labour and solvent savings, low LOD and good precision and accuracy compared to conventional analytical techniques, and the method can be used to control food safety and provide basic data for the risk assessment of phthalates and PAHs in edible oils.

Funding

Financial support from the Korea Food and Drug Administration [grant number 10162KFDA994, 2012] and the Korea Institute of Science and Technology [grant number 2VO2952, 2013] are gratefully acknowledged.

References

Aurela B, Kulmala H, Soderhjelm L. 1999. Phthalates in paper and board packaging and their migration into Tenax and sugar. Food Addit Contam. 16:571–577.

Ballesteros E, Sanchez AG, Martos NR. 2006. Simultaneous multidetermination of residues of pesticides and polycyclic aromatic hydrocarbons in olive and olive-pomace oils by gas chromatography/tandem mass spectrometry. J Chromatogr A. 1111:89–96.

Barranco A, Alonso-Salces RM, Bakkali A, Berrueta LA, Gallo B, Vicente F, Sarobe M. 2003. Solid-phase clean-up in the liquid chromatographic determination of polycyclic aromatic hydrocarbons in edible oils. J Chromatogr A. 988:33–40.

Bartolome L, Cortazar E, Raposo JC, Usobiaga A, Zuloaga O, Etxebarria N, Fernandez LA. 2005. Simultaneous microwave-assisted extraction of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phthalate esters and nonylphenols in sediments. J Chromatogr A. 1068:229–236.

Boden AR, Reiner EJ. 2004. Development of an isotope-dilution gas chromatographic-mass spectrometric method for the analysis of polycyclic aromatic compounds in environmental matrices. Polycycl Aromat Comp. 24:309–323.

Bogusz MJ, El Haj SA, Ehaideb Z, Hassan H, Al-Tufail M. 2004. Rapid determination of benzo(a)pyrene in olive oil samples with solid-phase extraction and low-pressure, wide-bore gas chromatography-mass spectrometry and fast liquid chromatography with fluorescence detection. J Chromatogr A. 1026:1–7.

Burczynski ME, Lin HK, Penning TM. 1999. Isoform-specific induction of a human aldo-keto reductase by polycyclic aromatic hydrocarbons (PAHs), electrophiles, and oxidative stress: implications for the alternative pathway of PAH activation catalyzed by human dihydrodiol dehydrogenase. Cancer Res. 59:607–614.

Camargo MCR, Toledo MCF. 2002. Polycyclic aromatic hydrocarbon contamination in different commodity groups. Braz J Food Technol. 5:19–26.

Cao XL. 2008. Determination of phthalates and adipate in bottled water by headspace solid-phase microextraction and gas chromatography/mass spectrometry. J Chromatogr A. 1178:231–238.

Del Carlo M, Pepe A, Sacchetti G, Compagnone D, Mastrocola D, Cichelli A. 2008. Determination of phthalate esters in wine using solid-phase extraction and gas chromatography-mass spectrometry. Food Chem. 111:771–777.

Diletti G, Scortichini G, Scarpone R, Gatti G, Torreti L, Migliorati G. 2005. Isotope dilution determination of polycyclic aromatic hydrocarbons in olive pomace oil by gas chromatography-mass spectrometry. J Chromatogr A. 1062:247–254.

Ehrenhauser FS, Wornat MJ, Valsaraj KT, Rodriguez P. 2010. Design and evaluation of a dopant-delivery system for an orthogonal atmospheric-pressure photoionization source and its performance in the analysis of polycyclic aromatic hydrocarbons. Rapid Commun Mass Sp. 24:1351–1357.

EPA, U. S. 1984. U.S. EPA Method 610, Methods for organic chemical analysis of municipal and industrial wastewater. Washington (DC): US Environmental Protection Agency.

Fankhauser-Noti A, Grob K. 2007. Blank problems in trace analysis of diethylhexyl and dibutyl phthalate: investigation of the sources, tips and tricks. Anal Chim Acta. 582:353–360.

FAO/WHO. 2005. Combined compendium of food additive specifications: Joint FAO/WHO Expert Committee on Food Additives: all specifications monographs from the 1st to the 65th meeting (1956–2005). FAO JECFA monographs, No. 1. Rome: Food and Agriculture Organization of the United Nations.

Feng YL, Zhu JP, Sensenstein R. 2005. Development of a headspace solid-phase microextraction method combined with gas chromatography mass spectrometry for the determination of phthalate esters in cow milk. Anal Chim Acta. 538:41–48.

Food Analysis Performance Assessment Scheme. 2012. Proficiency testing report 0585, Phthalates in sunflower oil. June–August 2012. Sand Hutton: The Food and Environment Research Agency.

Guo Y, Zhang ZF, Liu LY, Li YF, Ren NQ, Kannan K. 2012. Occurrence and profiles of phthalates in foodstuffs from China and their implications for human exposure. J Agri Food Chem. 60:6913–6919.

Hauser R, Calafat AM. 2005. Phthalates and human health. Occup Environ Med. 62:806–818.

Helaleh MIH, Al-Omair A, Nisar A, Gevao B. 2005. Validation of various extraction techniques for the quantitative analysis of polycyclic aromatic hydrocarbons in sewage sludges using gas chromatography-ion trap mass spectrometry. J Chromatogr A. 1083:153–160.

Jaouen-Madoulet A, Abarnou A, Le Guellec AM, Loizeau V, Leboulenger F. 2000. Validation of an analytical procedure for polychlorinated biphenyls, coplanar polychlorinated biphenyls and polycyclic aromatic hydrocarbons in environmental samples. J Chromatogr A. 886:153–173.

Jira W. 2004. A GC/MS method for the determination of carcinogenic polycyclic aromatic hydrocarbons (PAH) in smoked meat products and liquid smokes. Eur Food Res Technol. 218:208–212.

Jonsson S, Boren H. 2002. Analysis of mono- and diesters of o-phthalic acid by solid-phase extractions with polystyrene-divinylbenzene-based polymers. J Chromatogr A. 963:393–400.

Kluska M. 2003. Soil contamination with polycyclic aromatic hydrocarbons in the vicinity of the ring road in Siedlce City. Pol J Environ Stud. 12:309–313.

Kueseng P, Thavarungkul P, Kanatharana P. 2007. Trace phthalate and adipate esters contaminated in packaged food. J Environ Sci Health Part B. 42:569–576.

Liguori L, Heggstad K, Hove HT, Julshamn K. 2006. An automated extraction approach for isolation of 24 polyaromatic hydrocarbons (PAHs) from various marine matrixes. Anal Chim Acta. 573:181–188.

Liu YP, Wang SH, Wang L. 2013. Development of rapid determination of 18 phthalate esters in edible vegetable oils by gas chromatography tandem mass spectrometry. J Agri Food Chem. 61:1160–1164.

Mollerup S, Ovrebo S, Haugen A. 2001. Lung carcinogenesis: resveratrol modulates the expression of genes involved in the metabolism of PAH in human bronchial epithelial cells. Int J Cancer. 92:18–25.

Mooibroek D, Hoogerbrugge R, Stoffelsen BHG, Dijkman E, Berkhoff CJ, Hogendoorn EA. 2002. Comparison of various extraction techniques for the determination of polycyclic aromatic hydrocarbons in worms. J Chromatogr A. 975:165–173.

Moreda W, Perez-Camino MC, Cert A. 2001. Gas and liquid chromatography of hydrocarbons in edible vegetable oils. J Chromatogr A. 936:159–171.

Mottier P, Parisod V, Turesky RJ. 2000. Quantitative determination of polycyclic aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to mass spectrometry. J Agric Food Chem. 48:1160–1166.

Nanni N, Fiselier K, Grob K, Di Pasquale M, Fabrizi L, Aureli P, Coni E. 2011. Contamination of vegetable oils marketed in Italy by phthalic acid esters. Food Contr. 22:209–214.

Pena T, Pensado L, Casais C, Mejuto C, Phan-Tan-Luu R, Cela R. 2006. Optimization of a microwave-assisted extraction method for the analysis of polycyclic aromatic hydrocarbons from fish samples. J Chromatogr A. 1121:163–169.

Petersen JH, Breindahl T. 2000. Plasticizers in total diet samples, baby food and infant formulae. Food Addit Contam. 17:133–141.

Phillips DH. 1999. Polycyclic aromatic hydrocarbons in the diet. Mutat Res. 443:139–147.

Polo M, Llompart M, Garcia-Jares C, Cela R. 2005. Multivariate optimization of a solid-phase microextraction method for the analysis of phthalate esters in environmental waters. J Chromatogr A. 1072:63–72.

Saim N, Dean JR, Abdullah MP, Zakaria Z. 1997. Extraction of polycyclic aromatic hydrocarbons from contaminated soil using Soxhlet extraction, pressurised and atmospheric microwave-assisted extraction, supercritical fluid extraction and accelerated solvent extraction. J Chromatogr A. 791:361–366.

Simon R, Palme S, Anklam E. 2006. Single-laboratory validation of a gas chromatography-mass spectrometry method for quantitation of 15 European priority polycyclic aromatic hydrocarbons in spiked smoke flavourings. J Chromatogr A. 1103:307–313.

Steiner I, Scharf L, Fiala F, Washuttl J. 1998. Migration of di-(2-ethylhexyl) phthalate from PVC child articles into saliva and saliva simulant. Food Addit Contam. 15:812–817.

Tfouni SAV, Machado RMD, Camargo MCR, Vitorino SHP, Vicente E, Toledo MCF. 2007. Determination of polycyclic aromatic hydrocarbons in cachaca by HPLC with fluorescence detection. Food Chem. 101:334–338.

Wang JH, Guo C. 2010. Ultrasonication extraction and gel permeation chromatography clean-up for the determination of polycyclic aromatic hydrocarbons in edible oil by an isotope dilution gas chromatography-mass spectrometry. J Chromatogr A. 1217:4732–4737.

Witorsch RJ. 2002. Endocrine disruptors: can biological effects and environmental risks be predicted? Regul Toxicol Pharm. 36:118–130.

Wu PG, Yang DJ, Zhang LQ, Shen XH, Pan XD, Wang LY, Zhang J, Tan Y, Feng L, Ying Y. 2012. Simultaneous determination of 17 phthalate esters in edible vegetable oils by GC-MS with silica/PSA-mixed solid-phase extraction. J Sep Sci. 35:2932–2939.