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

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

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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 lineairities with regression coefficients of $R^2 \geq 0.99$. Twelve edible oils were examined and evaluated 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 the environment but are also migrated into foods during packaging, storage (Aurela et al. 1999) and manufacturing the environment but are also migrated into foods during packaging, storage (Aurela et al. 1999) and manufacturing the environment but are also migrated into foods during packaging, storage (Aurela et al. 1999) and manufacturing the environment but are also migrated into foods during packaging, storage (Aurela et al. 1999) and manufacturing the environment but are also migrated into foods during packaging, storage (Aurela et al. 1999) and manufacturing. 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; Kuesen & 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[a]H[A]) and chrysene (Chry). The European Union Commission Regulation (835/2011L215) (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[a]H[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).
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 C18 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 florisor (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 spectrometry (GC-MS) (Nanni et al. 2011; Wu et al. 2012; Liu et al. 2013) and liquid chromatography–mass spectrometry (LC-MS) (Jonsson & Boren 2002), whereas PAHs are detected by GC–MS (Bogusz et al. 2004; Ballesteros et al. 2006), GC–MS/MS (Helahel et al. 2005) and high performance liquid chromatography–fluorescence detection (HPLC–FDL) (Pena et al. 2006; Tfouni et al. 2007). As an isotope dilution method that uses the deuterated or C13 isotope of analyte as the internal standard, which shows similar physical and chemical behaviour as the analyte, 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, benzybutyl 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)perylen (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 (Bellevont, 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-d4, BBP-d4, DEHA-d8, B[b]F-d12, B[k]F-d12, B[a]A-d12, I[cd]P-d12, DB[ah]A-d14 and B[ghi]P-d12 were purchased from Chiron As (Trondheim, Norway) and DEHP-d4, B[a]A-d12 and Chry-d12 were obtained from Accustandard (New Haven, CT). Anhydrous Sodium sulphate (Na2SO4) 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, Na2SO4 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 performed in triplicate.
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):H2O (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:H2O (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 D. The amount of solvent used during the extraction was also varied between 5 and 25 mL to determine the optimum volume.

![Figure 1](image-url)

**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), benzyloctyl 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[a]h[A]), benzo(g,h,i)perylene (Bghi[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-d4 and B[a]P-d12 as internal standards and the 1:1 isotope dilution method of this study using 12 isotopes corresponding to 12 analytes: DBP-d4, BBP-d4, DEHA-d8, DEHP-d4, B[a]A-d12, Chry-d12, B[b]F-d12, B[k]F-d12, B[a]A-d12, I[c]dP-d12, DB[a]A-d14 and B[ghi]P-d12. 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®ChromaTOFTM 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 Na2SO4. 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).

<table>
<thead>
<tr>
<th>Compound</th>
<th>R.Ta (sec)</th>
<th>Q.I b (m/z)</th>
<th>Q.Ic (m/z)</th>
<th>Linearity (R2)</th>
<th>LOD (µg/kg, n = 9)</th>
<th>LOQ (µg/kg, n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phthalates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibutyl phthalates</td>
<td>558.1</td>
<td>121.150</td>
<td>149</td>
<td>0.9997</td>
<td>9.77</td>
<td>29.6</td>
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<tr>
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<td>556.7</td>
<td>125.154</td>
<td>153</td>
<td>0.9981</td>
<td>4.70</td>
<td>14.2</td>
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<td>Denzylbutyl phthalates</td>
<td>886.7</td>
<td>121.150</td>
<td>149</td>
<td>0.9961</td>
<td>4.70</td>
<td>14.2</td>
</tr>
<tr>
<td>Denzylbutyl phthalates-d4</td>
<td>885</td>
<td>125.154</td>
<td>153</td>
<td>0.9991</td>
<td>10.0</td>
<td>30.4</td>
</tr>
<tr>
<td>Diethylhexyl phthalates</td>
<td>1046.5</td>
<td>121.150</td>
<td>149</td>
<td>0.9991</td>
<td>10.0</td>
<td>30.4</td>
</tr>
<tr>
<td>Diethylhexyl phthalates-d4</td>
<td>1044.8</td>
<td>125.154</td>
<td>153</td>
<td>0.9991</td>
<td>10.0</td>
<td>30.4</td>
</tr>
<tr>
<td>Diethylhexyl adipate</td>
<td>920.1</td>
<td>112.130</td>
<td>129</td>
<td>0.999</td>
<td>4.56</td>
<td>13.8</td>
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<tr>
<td>Diethylhexyl adipate-d8</td>
<td>914</td>
<td>118.138</td>
<td>137</td>
<td>0.999</td>
<td>4.56</td>
<td>13.8</td>
</tr>
<tr>
<td>PAHs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>1000.9</td>
<td>226.229</td>
<td>228</td>
<td>0.9995</td>
<td>0.28</td>
<td>0.86</td>
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<tr>
<td>Benzo[a]anthracene-d12</td>
<td>995.3</td>
<td>236.241</td>
<td>240</td>
<td>0.9999</td>
<td>0.26</td>
<td>0.78</td>
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<tr>
<td>Chrysene</td>
<td>1009.3</td>
<td>226.229</td>
<td>228</td>
<td>0.9999</td>
<td>0.26</td>
<td>0.78</td>
</tr>
<tr>
<td>Chrysene-d12</td>
<td>1002.8</td>
<td>236.241</td>
<td>240</td>
<td>0.9999</td>
<td>0.26</td>
<td>0.78</td>
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<tr>
<td>Benzo[b]fluoranthene</td>
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<td>Benzo[b]fluoranthene-d12</td>
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<td>1272.6</td>
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<td>252</td>
<td>0.9987</td>
<td>0.44</td>
<td>1.34</td>
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<tr>
<td>Benzo[k]fluoranthene-d12</td>
<td>1267.8</td>
<td>260.265</td>
<td>264</td>
<td>0.9986</td>
<td>0.15</td>
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<td>Benzo[a]pyrene</td>
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<td>252</td>
<td>0.9966</td>
<td>0.15</td>
<td>0.44</td>
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<tr>
<td>Benzo[a]pyrene-d12</td>
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<td>260.265</td>
<td>264</td>
<td>0.9966</td>
<td>0.15</td>
<td>0.44</td>
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<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>1586.9</td>
<td>276.279</td>
<td>276</td>
<td>0.9987</td>
<td>0.32</td>
<td>0.96</td>
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<tr>
<td>Indeno[1,2,3-cd]pyrene-d12</td>
<td>1581.5</td>
<td>284.289</td>
<td>288</td>
<td>0.9987</td>
<td>0.32</td>
<td>0.96</td>
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<tr>
<td>Dibenzo[a, h]anthracene</td>
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<td>278</td>
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<td>1.53</td>
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<td>Dibenzo[a, h]anthracene-d14</td>
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<td>292</td>
<td>0.9917</td>
<td>0.50</td>
<td>1.53</td>
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<tr>
<td>Benzo(g,h,i)perylene</td>
<td>1641.9</td>
<td>274.277</td>
<td>276</td>
<td>0.9987</td>
<td>0.77</td>
<td>2.33</td>
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<td>Benzo(g,h,i)perylene-d12</td>
<td>1636</td>
<td>284.289</td>
<td>288</td>
<td>0.9987</td>
<td>0.77</td>
<td>2.33</td>
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</table>

Notes: *Retention time; †Qualification ion; ‡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:H2O (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 (87.0–94.7% 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 florasil 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-d4 and B[a]P-d12 as internal standards, all isopes corresponding to all analytes were added for the isotope dilution (1:1 isotope dilution). The accuracy using DEHP-d4 and B[a]P-d12 as internal standards.
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 (R²) 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, 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
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 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


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

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.</th>
<th>Packaging material</th>
<th>DBP²</th>
<th>DEHP</th>
<th>DEHA</th>
<th>B[a]A</th>
<th>Chry</th>
<th>B[b]F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice bran</td>
<td>S-1</td>
<td>PETᵇ</td>
<td>&lt;9.77</td>
<td>498 ± 6.47</td>
<td>&lt;4.56</td>
<td>4.16 ± 0.42</td>
<td>&lt;0.28</td>
<td>&lt;0.55</td>
</tr>
<tr>
<td>Brown rice</td>
<td>S-2</td>
<td>PET</td>
<td>&lt;9.77</td>
<td>804 ± 9.28</td>
<td>&lt;4.56</td>
<td>&lt;0.28</td>
<td>&lt;0.55</td>
<td></td>
</tr>
<tr>
<td>Canola</td>
<td>S-3</td>
<td>PET</td>
<td>&lt;9.77</td>
<td>34.2 ± 2.66</td>
<td>&lt;4.56</td>
<td>2.62 ± 0.28</td>
<td>&lt;0.26</td>
<td>&lt;0.55</td>
</tr>
<tr>
<td>Olive</td>
<td>S-4</td>
<td>PET</td>
<td>&lt;9.77</td>
<td>44.0 ± 5.21</td>
<td>&lt;4.56</td>
<td>&lt;0.28</td>
<td>&lt;0.55</td>
<td></td>
</tr>
<tr>
<td>Grape seed</td>
<td>S-5</td>
<td>PET</td>
<td>40.6 ± 2.30</td>
<td>214 ± 5.98</td>
<td>&lt;4.56</td>
<td>&lt;0.28</td>
<td>&lt;0.55</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>S-6</td>
<td>PET</td>
<td>13.2 ± 2.29</td>
<td>729 ± 4.87</td>
<td>5.46 ± 0.15</td>
<td>&lt;0.28</td>
<td>&lt;0.55</td>
<td></td>
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<tr>
<td>Soybean</td>
<td>S-7</td>
<td>PET</td>
<td>&lt;9.77</td>
<td>&lt;10.0</td>
<td>&lt;4.56</td>
<td>2.00 ± 0.08</td>
<td>&lt;0.26</td>
<td>&lt;0.55</td>
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<tr>
<td>S-8</td>
<td>PET</td>
<td>&lt;9.77</td>
<td>498 ± 5.67</td>
<td>&lt;4.56</td>
<td>2.35 ± 0.07</td>
<td>&lt;0.26</td>
<td>&lt;0.55</td>
<td></td>
</tr>
<tr>
<td>S-9</td>
<td>PET</td>
<td>&lt;9.77</td>
<td>498 ± 5.67</td>
<td>&lt;4.56</td>
<td>2.35 ± 0.07</td>
<td>&lt;0.26</td>
<td>&lt;0.55</td>
<td></td>
</tr>
<tr>
<td>S-10</td>
<td>PET</td>
<td>&lt;9.77</td>
<td>806 ± 10.1</td>
<td>&lt;4.56</td>
<td>2.35 ± 0.07</td>
<td>&lt;0.26</td>
<td>&lt;0.55</td>
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<tr>
<td>S-11</td>
<td>PET</td>
<td>&lt;9.77</td>
<td>25.0 ± 1.77</td>
<td>&lt;4.56</td>
<td>&lt;0.28</td>
<td>&lt;0.55</td>
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</tr>
<tr>
<td>S-12</td>
<td>PET</td>
<td>&lt;9.77</td>
<td>&lt;10.0</td>
<td>&lt;4.56</td>
<td>1.94 ± 0.08</td>
<td>&lt;0.26</td>
<td>&lt;0.55</td>
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</tr>
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References


