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Determination of 23 phthalic acid esters in food by liquid chromatography tandem mass spectrometry

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ABSTRACT

A rapid and sensitive method was developed for the determination of 23 phthalates in food samples including milk-based products, distilled liquor, wine, beverage, grain, meat, oil, biscuit (cookie), and canned food by liquid chromatography tandem mass spectrometry (LC–MS/MS). Liquid samples were exacted by acetonitrile, while solid samples were prepared by QuEChERS or glass-based SPE methods. The 23 phthalates were separated on Poroshell 120 EC-C_{18} column and followed by positive electrospray ionization as well as multi-reaction monitoring provided by a triple–quadrupole tandem mass spectrometer. To reduce contamination, the plastic materials were avoided in sample handling and preparation . The LODs were between 0.8 and 15 $\mu g\,kg^{-1}$ and LOQs were between 10 and 100 $\mu g\,kg^{-1}$. By using different concentrations: 100, 500, and 1000 $\mu g\,kg^{-1}$) for DINP and DIDP; 50, 100, and 1000 $\mu g\,kg^{-1}$ for other 21 phthalate compounds, the spiked recoveries were within 75.5–115.2% and the relative standard deviations (RSDs) were in the range of 3.2–18.9%. The proposed protocol was then applied to the analysis of 623 real samples collected from the two sides of the Taiwan Straits, and the DEHP was found in almost all samples tested in this study, with levels ranging from 0.02 to 2685 mg kg^{-1}. The present study demonstrated a rapid, sensitive, and accurate method for determining 23 phthalates in foodstuffs.

1. Introduction

Phthalic acid esters (PAEs), also known as phthalates or phthalate esters, are widely used as plasticizers in a broad range of industrial and consumer products for improving plasticity, flexibility, and elasticity [1,2]. Previous studies have shown that detectable levels of PAEs in food samples [3], human mother's milk [3], dust and environmental samples [1], and textiles with bis(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate (DBP) recorded as the most abundant [3].

Previous studies reported that rodents exposed to the certain high dose phthlaltes had changed hormone levels and birth defects [1–3]. A recent British study reported similar results that the phthalate dibutyl phthalate (DBP) or its metabolite monobutyl phthalate (MBP) suppressed steroidogenesis by fetal-type Leydig cells in primates [4]. Based on previous studies, phthalates have been classified by their impact strength. DEHP was in Class B2 and has been shown to be embryotoxic and teratogenic, while butylbenzyl phthalate (BBP) was in Class C (possible human carcinogen). Di-*n*-butyl phthalate (DBP), di-ethyl phthalate (DEP) and dimethyl phthalate (DMP) were in Class D (not yet classified as human carcinogens) [5–7]. Human beings can be exposed to PAEs via water, air, soil or food; through ingestion (i.e., oral), inhalation, and dermal absorption. Phthalates are being phased out of many products in the United States, Canada, European Union, and China over health concerns [1,3,8].

Ever since industrial plasticizer was found in sports drinks in Taiwan of China in 2011 and in distilled spirits in mainland of China in 2012, PAEs have caught public attention. Lots of researchers have published methods for PAEs detection and quantitation. Currently, numerous efficient and accurate sample preparation methods are widely used on PAE detection, such as solid-phase extraction (SPE) [9], solid-phase microextraction (SPME) [10], headspace solid-phase microextraction (HS-SPME) [11], liquid-phase microextraction (LPME) [12], dispersive liquid–liquid microextraction (DLLME) [13], and polymer monolith microextraction (PMME) [14]. However, the major drawbacks of these sample prep methods are using too much organic solvents, time consuming, as well as expensive.

Previously, gas chromatography with flame ionization detection (GC–FID) was used in the determination of dimethyl phthalate (DMP), diethyl phthalate (DEP) and di-*n*-butyl phthalate (DnBP)

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[12]. Maria Polo et al. [10] have developed the method of gas chromatography–mass spectrometry (GC/MS) for the determination of the six phthalate esters. Brunella Cavaliere et al. [15] used gas chromatography–tandem mass spectrometry (GC–MS/MS) to detect DMP, DEP, DBP, BBP, DEHP, and DOP in olive oil. High performance liquid chromatography (HPLC) [16–18] and liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [19,20] were also reported in the detection of PAEs. However, no studies were reported to simultaneously detect over 20 different PAEs in food samples.

In any case, the majority of methods for determination of PAEs involve gas chromatography coupled with several detector as FID, ECD (electron capture detector) or MS [15].The former techniques do not provide unequivocal confirmation of identity and are often subject to matrix interferences, whereas the identification can be carried out by MS/MS. Under GC/MS conditions with electron impact (EI) ionization, the fragment at m/z 149 is the common ion for most PAEs. This is a major limitation in using GC/MS for the determination of PAEs isomeric mixtures by GC/MS, primarily because of the occurrence of coeluting isomers with varying composition of alkyl substitution [21]. In addition, the ubiquity of some phthalates causes severe problems in the determination of their content in food; special measures are required to keep the background levels low. Regarding the identification, blank values, and multi-analysis, the aim of this work was to develop an analytical method to detect and quantify 23 PAEs in food samples with different sample preparation methods for various matrices.

2. Experiment

2.1. Materials

Unless specified, all reagents used were analytical grade and obtained from commercial sources. Water used was purified using a Milli-Q gradient A10 system (Millipore, Milford, MA, USA). Methanol, acetonitrile, *n*-hexane, acetone, magnesium sulfate and sodium acetate were HPLC-grade, and purchased from Merck (Germany). PSA powder (grain size 40 or 120 μm, opening 60 A) and C_{18} powder (grain size 40 or 120 μm, opening 60 A) were supplied by Agilent (USA). ProElut PSA cartridge (glass, 1 g, 6 mL) was bought from Dikma (China). 23 PAE analytical standards, including dimethyl phthalate (DMP), diethyl phthalate (DEP), diisopropylo-phthalate (DIPrP), diallyl phthalate (DAP), dipropyl hthalate (DPrP), diisobutyl phthalate (DIBP), dibutyl phthalate (DBP), bis(2-methoxyethyl) phthalate (DMEP), di-*iso*-amyl phthalate (DIPP), bis(4-methyl-2-pentyl) phthalate (BMPP), bis(2-ethoxyethyl) phthalate (DEEP), dipentyl phthalate (DPP), dihexyl phthalate (DHXP), benzyl butyl phthalate (BBP), bis(2-*n*-butoxyethyl) phthalate (DBEP), dicyclohexyl phthalate (DCHP), bis(2-ethylhexyl) phthalate (DEHP), di-*n*-heptyl phthalate (DHP), diphenyl phthalate (DPhP), di-*n*-octyl phthalate (DNOP), diisononyl ortho-phthalate (DINP), diisodecyl ortho-phthalate (DIDP) and dinonyl phthalate(DNP), were purchased from Dr. Ehrenstorfer (Germany, purity higher than 98%).

Individual standard solutions of each phthalate were prepared at a concentration of 100 mg mL^{-1} in methanol, and were stored in the refrigerator at 4 °C. The standard stock solution was diluted with methanol to the final concentration of 0.1–100 ng mL^{-1}, respectively. To minimize the risk of secondary contamination, all solvents were checked for the presence of phthalates before use.

2.2. Instrumentation and apparatus

The Applied Biosystems API 5000 triple–quadrupole mass spectrometer (AB SCIEX, USA), equipped with ESI ion source was used in this study, and the liquid chromatography was Agilent 1200 system equipped with G1322A degasser, G1312A binary pump, G1313A autosampler, and G1316A column oven (Agilent, USA). The HPLC separation was performed by a Poroshell 120 EC-C_{18} column (100 × 4.6 mm, 2.7 μm; Agilent). Electronic balance (accurate to 0.1 mg and 0.01 g; BSA224S-CW, SARTORIOUS, Germany), ultrasonic extractor (T25, IKA, Germany), Whirlpool mixer (MS1, IKA, Germany), centrifuge (≥4000 r/min; DT5-3, China) and nitrogen evaporators (N100DR, Peak Scientific, The UK) were used for sample pretreatment.

2.3. Sample preparation

2.3.1. Liquid samples

2.3.1.1. Liquid sample. For guava juice and wine, 5.0 g (accurate to 0.01 g) of the liquid sample without emulsion (carbon dioxide should be eliminated before extraction if contained) were weighted into a glass tube of 25 mL. After adding sodium chloride until the water layer saturated, 5 mL acetonitrile was added into the tube, and the mixture was immediately vortexed for 1 min. The tube was centrifuged for 2 min at the speed of 4000 r/min. Finally the acetonitrile layer was taken for LC–MS/MS analysis.

2.3.1.2. Liquid sample which is easy to emulsify. For papaya milk, an adequate amount of 5.0 g (accurate to 0.01 g) of the liquid sample associated to emulsion (carbon dioxide should be eliminated before extraction if contained) was transferred to a 25 mL glass tube, and 10 mL acetonitrile was added. The mixture was immediately vortexed for 1 min. The tube was centrifuged for 2 min at the speed of 4000 r/min. The upper layer was taken into a glass centrifuge tube, and sodium chloride was added until the water layer saturated. The mixture was vortexed for 1 min, then standing or being centrifuged to make it stratify. Then the upper acetonitrile layer was taken into another glass tube and was evaporated to nearly dry under nitrogen flow at a temperature below 50 °C. Finally, the extract was diluted to 5 mL with mobile phase for LC–MS/MS analysis.

2.3.2. QuEChERS method for solid sample

For seasoning powder, grape jelly, grapefruit sauce, and egg noodles, the QuEChERS was as follows: accurately weigh an adequate amount of 5.0 g (accurate to 0.01 g) of the mixed test sample into a glass tube with 50 mL ground glass stopper, add 5 mL water (there is no need to add water for aqueous sample), add 15 mL acetonitrile exactly. Add 6 g $MgSO_4$ and 1.5 g sodium acetate. Vortex for 1 min. Centrifuge for 2 min at speed 4000 rpm. Take the upper layer. Evaporate it at 40 °C to nearly dry, and then adjust to a constant volume of 5 mL with acetonitrile. Add 50 mg PSA powder, 50 mg C18 powder, 150 mg $MgSO_4$. Vortex for 1 min. Centrifuge for 2 min at speed 4000 rpm. Take the upper layer for LC–MS/MS analysis.

2.3.3. Glass-based SPE method for solid sample

For lemon flavored, the following method is optional for the samples failed in the protocol described in Section 2.3.2. The SPE procedure was as follows: weigh 1.0 g samples in glass tubes, add 2 mL *n*-hexane and 10 mL acetonitrile, vortex for 2 min to mix thoroughly, then centrifuge the sample under 4000 rpm for 2 min, collect the lower phase. Add 10 mL acetonitrile to sample, then repeat the extraction process, combine the twice lower phases and concentrated to dry under nitrogen at below 50 °C. Add 2 mL *n*-hexane to dissolve the residue (extract) for SPE cleanup. And then, add the extract, control the flow at about 1 mL/min and collect the elute; 5 mL *n*-hexane and 5 mL 4% acetone in *n*-hexane were used for elution, and collect the elute; collect the elutes and concentrated to approach dryness under nitrogen at below 50 °C, dissolve the residue with mobile phase for LC–MS/MS analysis.