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ARTICLE *in* ANALYTICAL AND BIOANALYTICAL CHEMISTRY · APRIL 2012

Impact Factor: 3.44 · DOI: 10.1007/s00216-012-5999-2 · Source: PubMed

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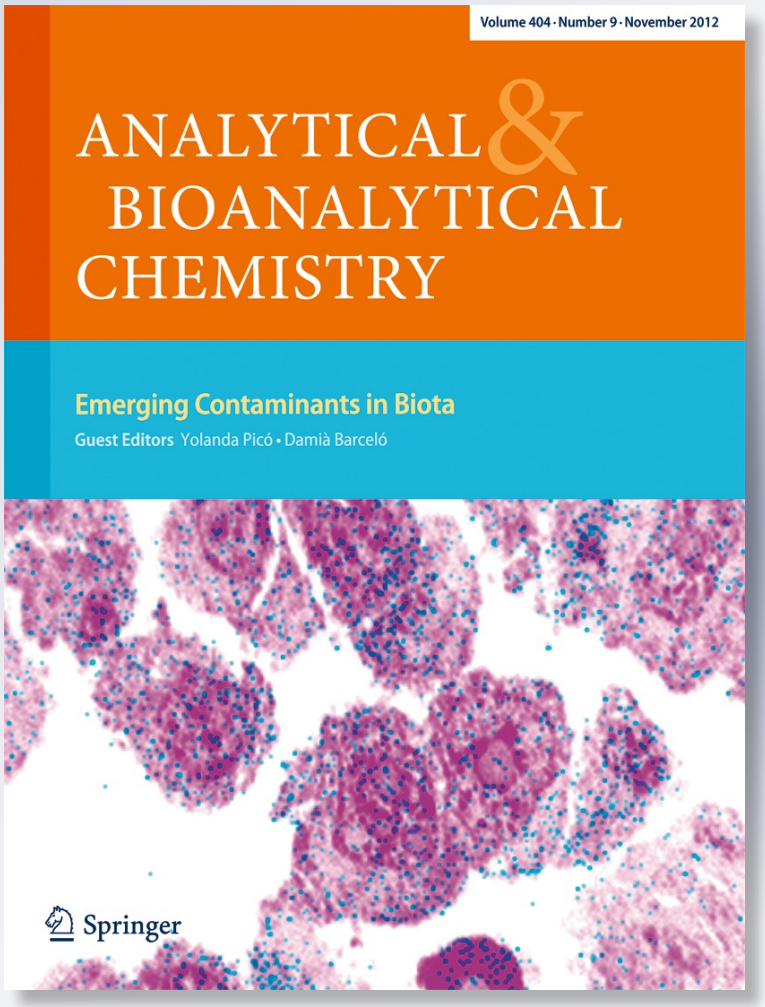
Analytical and Bioanalytical Chemistry

ISSN 1618-2642
Volume 404
Number 9

Anal Bioanal Chem (2012)
404:2539-2554
DOI 10.1007/s00216-012-5999-2



Springer

Anal Bioanal Chem (2012) 404:2539–2554
DOI 10.1007/s00216-012-5999-2

REVIEW

Challenges encountered in the analysis of phthalate esters in foodstuffs and other biological matrices

Ying Guo · Kurunthachalam Kannan

Received: 20 February 2012 / Revised: 28 March 2012 / Accepted: 29 March 2012 / Published online: 25 April 2012

Abstract Phthalate esters are ubiquitous environmental pollutants and are recognized as environmental endocrine disruptors because of their potential to elicit reproductive and developmental toxicity. Several phthalate esters have been listed by the US Environmental Protection Agency (EPA) as chemicals of concern. Determination of concentrations of phthalate esters in foodstuffs, typically present at sub to low nanogram-per-gram concentrations (between 0.1 and 100 ng g^{-1}), is essential for assessment of human dietary exposure. However, phthalate esters are commonly present as contaminants in several laboratory products, including organic solvents, that are used in sample preparation and analysis. Therefore, accurate analysis of phthalates in food samples is a challenging task. In this review, we summarize the methods available for the determination of phthalate esters in foodstuffs and report on concentrations of phthalates in foodstuffs and potential sources of contamination by phthalates in the analysis of foodstuffs. We offer suggestions to eliminate and/or reduce background levels of contamination by phthalates in the analysis of food and other biological samples. We also introduce methods that are suitable for trace analysis of phthalates in a variety of liquid and solid food samples, in particular, a liquid–liquid extraction method for removal of lipids from food samples, because these can substantially reduce background levels of phthalates in the analytical procedure.

Keywords Quality assurance · Pesticides · Endocrine disruptors · Plasticizers · Phthalate · Organic compounds · Foods · Beverages · Human exposure

Published in the topical collection *Emerging Contaminants in Biota* with guest editors Yolanda Picó and Damià Barceló.

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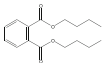
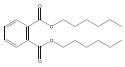
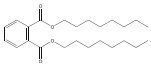
Introduction

Phthalic acid esters (or phthalates) are dialkyl or aryl alkyl esters of 1,2 benzenedicarboxylic (or phthalic) acid; a class of plasticizer used to impart flexibility and durability to poly(vinyl chloride) (PVC) and other plastics. Phthalates have been produced in large quantities since the 1930s, and, in 2010, the global production of phthalates was 4.9 million tons, which accounts for 84 % of total plasticizer production [1]. Most of the phthalates, in the pure form, are colorless liquids that are poorly soluble in water but soluble in organic solvents or oils (Table 1). Dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), di-*iso*-butyl phthalate (DIBP), benzyl butyl phthalate (BzBP), di-(2-ethylhexyl) phthalate (DEHP), and di-*n*-octyl phthalate (DNOP) are the common phthalates available in commerce. Specifically, DEHP accounts for 50 % of total phthalate production [2]. In addition to its use as a plasticizer, DMP is used as an insect repellent; DEP is used in shampoos, scents, soaps, lotions, and cosmetics, and in industrial solvents and medications; DBP and DIBP are used in adhesives, caulks, cosmetics, industrial solvents, and medications; DEHP is used in soft plastics, including tubing, toys, home products, food containers, and food packaging; and BzBP is used in vinyl flooring, adhesives, sealants, and industrial solvents [3, 4].

Table 1 Properties of the major phthalate esters

Common Name	Acronym	CAS	Structure	M.W.	B.P. (°C)	Water Solubility (mg/L, 25 °C)	Log Kow (25 °C)
dimethyl phthalate	DMP	131-11-3		194.2	282	5220	1.61
diethyl phthalate	DEP	84-66-2		222.2	295	591	2.54
di-iso-butyl phthalate	DIBP	84-69-5		278.3	327	9.9	4.27
dibutyl phthalate	DBP	84-74-2		278.3	340	9.9	4.27
butyl benzyl phthalate	BzBP	85-68-7		312.4	370	3.8	4.7
di-n-hexyl phthalate	DNHP	84-75-3		334.5	350	0.159	6
di(2-ethylhexyl) phthalate	DEHP	117-81-7		390.6	384	0.0025	7.73
di-n-octyl phthalate	DNOP	117-84-0		390.6	390	0.0025	7.73

Since 1970, when Jaeger and Rubin reported the occurrence of phthalates in blood stored in plastic bags, and in human tissues and organs of recipients of blood transfusions [5, 6], several studies have examined the toxicity of phthalates to laboratory animals [7–14] and to human health [15–18]. Phthalates have been classified as reproductive and developmental toxicants on the basis of their ability to interfere with the endocrine system, although the toxicity of phthalates varies somewhat, depending on the chemical structure. A few studies that have reviewed the toxicity of phthalates [16, 18] have indicated that these compounds and their metabolites have adverse effects on the reproductive system, especially in males. A negative correlation between semen volume and DBP and DEHP concentrations and a positive correlation between sperm malformation and DEHP concentrations have been reported [19]. Recent studies have revealed a negative relationship between environmental phthalate exposure and intelligence in children [20, 21]. A study investigated the association between urinary concentrations of phthalate metabolites and biomarkers of oxidative stress and inflammation, on the basis of data collected from 10,026 participants of the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2006 in the US [22, 23]. The results indicated that concentrations of DEHP, DBP, and BzBP metabolites were associated with increased oxidative stress and inflammation. Exposure to phthalates has been correlated with two metabolic abnormalities in men—abdominal obesity and insulin resistance [24]. In 2009, eight phthalate esters, including DBP, DIBP, BzBP, DEHP, DNOP, diisodecyl phthalate (DIDP), di-*n*-pentyl phthalate (DnPP), and diisononyl phthalate (DINP), were added to the list of chemicals of concern by the US Environmental Protection Agency (EPA) [25].

Phthalate esters can be rapidly metabolized to their corresponding monoesters within hours in the human body [26, 27]. The half-life of phthalate esters in the human body is in the range 6–20 h. Phthalates with a short alkyl chain (i.e., DMP, DEP, DIBP, DBP and BzBP) are metabolized mainly to monoesters [28], whereas long-alkyl-chain phthalates (i.e., DEHP, DINP, and DIDP) are metabolized, first, to the corresponding monoesters which are then further metabolized to more oxidized secondary metabolites [29–32]. Both monoesters and secondary metabolites can be conjugated with glucuronic acid in phase II metabolism [33]. All free and glucuronidated metabolites of phthalates are excreted in urine and feces. Urinary phthalate metabolite concentrations are widely used as a measure of recent human exposure to phthalate esters. As plasticizers, phthalates are not chemically bound to products and can, therefore, easily leach from the products into the environment. Consumer products containing phthalates can be a direct source of human exposure [3]. Several biomonitoring studies have reported widespread exposure to phthalates of humans in North America, Asia, and Europe [34–46].

Human exposure to phthalates via food

Several studies have shown that diet is the major source of human exposure to phthalates [3, 45, 47–49]. The significance of diet as a source of phthalate exposure has been demonstrated both by analysis of direct exposure (involving total diet and food monitoring) [47, 48] and by indirect analysis, by extrapolation from urinary phthalate metabolite concentrations [46, 50–52]. A study from Korea showed that, after following a

strict vegetarian dietary regimen for five consecutive days, urinary concentrations of DEP, DBP, and DEHP metabolites decreased significantly [46]. This suggested that dietary intervention can change human exposure to some phthalates. It is well documented that diet is the predominant source of human exposure to DEHP [35, 45, 47, 49, 50, 52, 53]. Nevertheless, other sources, for example inhalation and dermal exposure, are significant for some short-chain phthalates. Inhalation and dermal absorption have been suggested as important sources of human exposure to DMP, DEP, DIBP, and DBP [47, 50, 52]. Fasting for two days significantly reduced urinary concentrations of DEHP and DINP metabolites, whereas the concentrations of DBP, DIBP, and BzBP metabolites did not decrease [49]. These results suggested that foodstuffs were not the only sources of phthalate exposure. The major sources of exposure vary, depending on the type of phthalate.

Contamination of foodstuffs by phthalate esters can occur as a result of food-contact packaging and during production, processing, storage, and transport of foodstuffs [54]. Paper and plastic food packaging are important sources of phthalate contamination of foodstuffs [55, 56]. Several studies have reported migration of phthalate esters to foodstuffs from food-packaging materials, for example PVC containers [57–62], recycled paperboard [63], and glass jars with PVC lids [64, 65]. Migration of phthalate esters from food packaging material depended on the lipid content of the food matrix, the acidity and/or alkalinity of the food, alcohol content, temperature, and contact time [58]. A recent study showed that human exposure to DEHP was substantially reduced when the diet was restricted to foods that have limited contact with packaging materials [51]; this study documented the significance of packaging materials as a source of DEHP in foodstuffs. A field study estimated the migration of DEHP from greenhouse plastic films (PVC and polyethylene) through air to vegetables and showed that the higher the DEHP content of the plastic film, the lower the height of the greenhouse, and the newer the greenhouse, the higher the concentration of DEHP in the vegetables [66]. Foodstuffs can also be contaminated with phthalate esters during production, e.g., from PVC gloves used by food-production workers and farmers during handling of foods, from printing inks used on paper cartons [54], from food-chain transfer from the application of biosolids (contaminated with phthalates) as a fertilizer [67, 68], or during storage of beverages in PVC tanks in farms or in the food industry.

Despite the significance of foodstuffs as an important source of human exposure to phthalate esters, very few studies have reported the occurrence of these compounds. A report published in 2009 [54] reviewed the occurrence of phthalate esters in food samples and indicated that the concentrations and profiles of these compounds vary substantially, depending on the type of food and geographic location of samples. Nevertheless, most food-surveillance data were obtained a decade or two ago with the analytical methods available for determination of phthalate esters at that time [54]. Analytical techniques have improved substantially over the last 10 years in terms of accuracy, precision, and sensitivity.

A major problem encountered in trace level analysis of phthalates in foodstuffs is the high levels of background contamination that occur in laboratory procedural blanks. Two phthalate esters, DEHP and DBP, are ubiquitous in a variety of laboratory solvents and chemicals, and adequate precautions should be exercised when quantifying phthalate esters. For this review we compiled currently available information on DMP, DEP, DIBP, DBP, BzBP, and DEHP concentrations in beverages, milk and milk products, meat and meat products, cooking oils, cereals, and in total diet samples published since 2005 (Table 2). Total phthalate concentrations reported in foodstuffs typically range from 0.1 to 100 ng g^{-1} wet weight, and these values can be similar to the background levels of contamination encountered in analytical procedures. Reported studies have shown that DEHP and DBP are the two phthalate esters most frequently detected. The limited information available in the literature on phthalate esters in foodstuffs is because of the challenges faced in analysis of compounds that occur at substantial levels in procedural blanks (i.e., high background levels of contamination in the analytical procedures). Efforts to eliminate and/or reduce the background levels of phthalates in laboratory procedural blanks are the first steps toward preparing a laboratory for analysis of phthalates in food samples.

Procedural blanks in phthalate analysis

Blank values are defined as a reading or result originating from use of reagents, solvents, chemicals, laboratory ware, materials that come into contact with the samples in the analytical procedure, and any residual bias in the measurement device or analytical process, which contributes to the values [69]. Procedural blanks are processed simultaneously with every batch of samples in exactly the way that the samples are prepared, except that the blanks do not contain the sample matrix (but do use equal amounts/volumes of all solvents, reagents, chemicals, and glassware that come into contact with the samples). When the background levels of target analytes are expected to be high, more than one procedural blank is recommended for every batch of samples. Phthalates are ubiquitous laboratory contaminants [54, 69–71]. Phthalates have been found in laboratory solvents, sorbents (e.g., silica gel, alumina, activated carbon), plastic consumables (e.g., pipet tips), glassware, and in laboratory air and dust. The potential sources of contamination and concentrations of phthalates in reagents and materials used in the analysis are discussed in the following sections.

Table 2 Reported concentrations of phthalates in food samples from several countries

Sample (n)	Country	Unit	DMP	DEP	DIBP	DBP	BzBP	DEHP	Ref.
Beverages									
Drinking water (142)	Italy	ng mL^{-1}	0.02-0.06	0.02-0.22	0.03-0.32	0.04-0.23	NA[α]	ND	Montuori et al. [89]
Mineral water (1)	China	ng mL^{-1}	0.23	0.58	NA	5.62	1.25	0.93	Xu et al. [58]
Bottle water (1)	China	ng g^{-1}	ND	ND	0.011	0.046	ND	0.15	Guo et al. [81]
Bottle water (11)	Canada	ng mL^{-1}	ND	0.065-0.10	0.133-0.481	0.075-1.717	NA	0.052-0.338	Cao [90]
Soft drink (12)	China	ng g^{-1}	0.14	0.030	0.56	0.41	ND	0.80	Guo et al. [81]
Wine (4)	China	ng g^{-1}	3.34	0.07	26.0	31.8	ND	3.56	Guo et al. [81]
Wine (3)	Spain	ng mL^{-1}	NA	5.5	NA	4.7	NA	< 2	Carrillo et al. [91]
Wine (36)	Italy	ng mL^{-1}	ND	ND	76-119	ND −115	ND-40	57-76	Del Carlo et al. [92]
Wine (7)	Italy	ng mL^{-1}	ND	ND	NA	7.3-23.0	ND-7.0	2.4-16.0	Russo et al. [93]
Milk or milk products									
Human milk (86)	Canada	ng g^{-1}	NA	0.31	NA	0.87	NA	222	Zhu et al. [94]
Human milk (42)	Sweden	ng mL^{-1}	NA	1.22	NA	1.5	0.49	9.0	Högberg et al. [44]
Human milk (78)	Germany	ng mL^{-1}	NA	NA	1.2	0.8	ND	3.9	Fromme et al. [95]
Whole mike (24)	China	ng g^{-1}	ND-62	ND-35	NA	120-131	ND-24	NA	Li et al. [84]
Soybean milk (1)	China	ng mL^{-1}	NA	7.6	NA	8.8	NA	NA	He et al. [96]
Raw cow milk (6)	Canada	ng g^{-1}	ND	0.60-0.63	NA	5.79-6.39	ND	16.0-215	Feng et al. [97]
Raw cow milk (30)	South Korea	ng mL^{-1}	NA	ND	NA	NA	ND	57	Kim et al. [98]
Raw milk (18)	Sweden	ng g^{-1}	NA	NA	NA	< 9	< 4	7-30	Sørensen [80]
Infant formula, powdered (6)	Global range	ng g^{-1}	NA	NA	NA	< 9	<4	37-138	Sørensen [80]
Infant formula, liquid (2)	Europe	ng g^{-1}	NA	NA	NA	<9	<4	10-23	Sørensen [80]
Yoghurt with fruit (4)	Sweden	ng g^{-1}	NA	NA	NA	<9	<4	15-37	Sørensen [80]
Baby milk powders (27)	Global range	ng g^{-1}	NA	NA	NA	15-77	NA	34-281	Yano et al. [99]
Meat or meat products									
Sausage (3)	China	ng g^{-1}	1.25	1.27	12.8	6.99	ND	95.3	Guo et al. [81]
Fresh meat (3)	China	ng g^{-1}	0.71	2.17	10.8	6.84	ND	104	Guo et al. [81]
Sausage (3)	China	ng g^{-1}	NA	NA	NA	NA	NA	155	Guo et al. [61]
Cooking oils									
Oils (1)	China	ng mL^{-1}	3.18	6.72	NA	14.9	10.8	17.5	Xu et al. [58]
Vegetable oils (3)	China	ng g^{-1}	1.76	ND	4.70	6.20	12.5	62.4	Guo et al. [81]
Cereals									
Rice (3)	China	ng g^{-1}	0.12	0.83	6.21	5.53	ND	19.2	Guo et al. [81]
Flour or noodle (6)	China	ng g^{-1}	ND	0.72	19.1	12.2	ND	9.22	Guo et al. [81]
Instant noodle (5)	China	ng g^{-1}	6.60	1.89	3.92	7.17	ND	ND	Guo et al. [81]
Bread (20)	Italy	ng g^{-1}	NA	NA	NA	143	NA	270	Cirillo et al. [62]
Vegetables									
Vegetable in total diet (60)	Italy	ng g^{-1}	NA	NA	NA	38.1	NA	87.2	Cirillo et al. [62]
Fruit									
Fruits in total diet (20)	Italy	ng g^{-1}	NA	NA	NA	57.0	NA	77.3	Cirillo et al. [62]
Total diet									
School lunch meals (60)	Italy	ng g^{-1}	NA	NA	NA	86.8-169	NA	225-253	Cirillo et al. [62]

NA, not analyzed; ND, not detected

Phthalate contamination in plastic consumables

The plastic consumables commonly used in phthalate analysis are sample containers, solid-phase extraction (SPE) cartridges, pipette tips, and septa for autosampler vials. Most of these consumables are made from polyethylene or polypropylene polymers, and these materials can introduce phthalates into the analysis [70, 72]. Blank polypropylene SPE cartridges extracted with ethyl acetate contained 3 to 14 ng DIBP, DBP, and DEHP [73]. Washing of the SPE cartridges with an organic

solvent before use was found to reduce the background level of contamination only moderately [73]. In biological samples, for example urine or blood, phthalate metabolites are the preferred target compounds because the parent compounds are quickly (within a few hours) metabolized in the body. Laboratory/procedural blanks do not contain phthalate metabolites but do contain phthalates. Therefore, the issue of procedural blank contamination is not a concern for phthalate metabolite analysis. However, care should be taken during the collection and storage of blood or urine samples. We analyzed phthalate metabolites in 41 human serum samples collected in China then stored in polypropylene tubes at 4 °C for several months. High concentrations of mono-2-isobutyl phthalate (miBP, a metabolite of DIBP) and mono-*n*-butyl phthalate (mBP, a metabolite of DBP) were found in serum samples; respective median values were 305 and 99.8 ng mL^{-1}. These values in human serum are several orders of magnitude higher than values reported in other countries, which suggests the possibility of extraneous contamination. For example, concentrations of mBP and miBP were below the limits of detection in serum from US women [74], 0.54 and 0.50 ng mL^{-1} in serum from Swedish women [44], 0.43 and 0.72 ng mL^{-1} in serum from Danish men [75], and 24.9 and 3.44 ng mL^{-1} in serum from Taiwanese women [76]. mBP was not found in maternal blood or cord blood samples from Shanghai, China [77]. In our laboratory procedural blanks (n=6), only low concentrations of mBP and miBP were detected; average concentrations were 1.9 and 2.1 ng mL^{-1}, respectively. Therefore, the possible source of mBP and miBP contamination in Chinese serum samples is the containers used for storage of the samples. Plastic blood collection tubes and plastic syringes can introduce phthalates into the blood during sampling. Several medical devices have been reported to contain phthalate esters [6, 78, 79]. Testing of the sample-collection devices and storage containers before their use is very important. We also suggest that blank containers (from the same lot that was used for sampling and storage) should be analyzed to determine the extent of contamination possibly originating from sample collection and storage devices.

Phthalate concentrations in five brands of pipette tip were determined in our laboratory. All pipette tips were made of polypropylene. Pipette tips (n=30–98) were soaked in 2 mL hexane, and the hexane extract was analyzed for phthalates (n=15). Trace amounts of DEP (0.007±0.005 ng/tip), DIBP (0.03±0.04 ng/tip), DBP (0.01±0.02 ng/tip), BzBP (0.001±0.001 ng/tip), and dicyclohexyl phthalate (DCHP) (0.005±0.007 ng/tip) were found in pipette tips. DMP, di-*n*-hexyl phthalate (DNHP), DEHP, and DNOP were not found in pipette tips. An earlier study [71] reported the occurrence of DBP (0.15 ng) and DEHP (1–2 ng) in two types of autosampler-vial septum.

Phthalates in sorbents

Sorbents such as Florisil, alumina, and silica gel are commonly used in the purification of sample extracts in phthalate analysis. Anhydrous sodium sulfate is commonly used to remove moisture from the sample extracts. Phthalate esters have been found in these sorbents; one source of phthalates in sorbents is the plastic containers or glass bottles with plastic lids in which they are stored. Exposure of sorbents to indoor air can promote adsorption of phthalates from contaminated indoor air. Thus, previously opened, old adsorbents are a source of contamination of phthalates in laboratory procedural blanks. EPA method 8061A (for determination of DMP, DEP, DBP, BzBP, DEHP, and DNOP) reported that complete removal of phthalate esters from Florisil cartridges was not possible (Florisil was heated at 320 °C for several hours); phthalates were found in blank Florisil cartridges in amounts ranging from 10 to 460 ng per cartridge. We also measured phthalate concentrations in Florisil (60–100 mesh; Aldrich, St Louis, MO, USA), alumina (100–200 mesh; Bio-Rad, Richmond, CA, USA), and anhydrous sodium sulfate (J.T. Baker, Phillipsburg, NJ, USA); the results are shown in Table 3. All of these adsorbents were purchased in glass containers. Florisil contained DEHP at concentrations of 5–45 ng g^{-1}. DEHP was not removed by baking Florisil at temperatures of 450 °C and 650 °C. Alumina and anhydrous sodium sulfate contained DIBP and DEHP at low ng g^{-1} concentrations. Thus, a chromatography column packed with 10 g Florisil or alumina would introduce approximately 10 to 500 ng DEHP into the analytical method. Considering the low concentrations of phthalate esters (0.1 to 100 ng g^{-1}) typically found in food samples, high background concentrations of DEHP and/or DBP found in analytical grade sorbents preclude accurate determination.

Phthalates in organic solvents

High purity cyclohexane (relative polarity 0.006), *n*-hexane (0.009), pentane (0.009), methyl *tert*-butyl ether (MTBE) (0.124), ethyl acetate (0.228), acetone (0.355), dichloromethane (0.390), acetonitrile (0.460), and methanol (0.762) have been commonly used for extraction and purification of phthalates from foodstuffs. Phthalate esters have been found at ng mL^{-1} concentrations in reagent-grade, high-purity, organic solvents. It is expected that phthalate contamination arises mainly during the production or storage of these solvents in PVC tanks. Phthalate concentrations in cyclohexane were reported to be approximately 0.5 ng mL^{-1} for DEP and DEHP and 1.5 ng mL^{-1} for DIBP and DBP [73]; MTBE also contained similar concentrations of DEP and DEHP but lower concentrations of DIBP (0.4 ng mL^{-1}) and DBP (1.0 ng mL^{-1}) [73]. We also measured phthalate ester residues in the commercial solvents (all newly opened, high purity, glass-distilled solvents) hexane, acetone, dichloromethane, and acetonitrile

Table 3 Phthalate concentrations in selected laboratory sorbents—Florisil, alumina, and anhydrous sodium sulfate (ng g^{-1}, mean ± standard deviation)

Phthalate	Florisil		Alumina		Anhydrous sodium sulfate	
	Baked at 450 °C overnight (n=4)	Baked at 650 °C overnight and added 3 % water[a] (n=6)	Old bottle open for long time without baking (n=2)	Baked at 450 °C overnight (n=2)	New bottle opened without baking (n=2)	Baked at 450 °C overnight (n=2)
DMP	ND [b]	0.01±0.01	ND	ND	ND	ND
DEP	ND	ND	0.01±0.01	ND	0.007±0.003	ND
DIBP	ND	ND	0.14±0.01	0.04±0.02	0.05±0.09	0.05±0.11
DBP	ND	ND	ND	0.27±0.08	ND	ND
DNHP	ND	ND	ND	0.16±0.15	ND	ND
BzBP	ND	ND	ND	0.04±0.01	ND	0.003±0.05
DCHP	ND	ND	ND	ND	ND	ND
DEHP	4.81±1.33	44.6±15.9	6.55±3.41	1.03±0.01	2.70±2.32	5.08±1.66
DNOP	0.05±0.03	ND	0.24±0.26	ND	0.03±0.01	0.11±0.10

[a] Water was previously extracted with hexane

[b] ND, not detected

(Table 4). Phthalates were found in all the solvents analyzed; concentrations of DEP and BzBP were ~0.01 ng mL^{-1}, those of DBP and DIBP were ~0.1 ng mL^{-1}, and those of DEHP were ~0.1 to 1.0 ng mL^{-1}. Concentrations of other phthalate esters were generally below 0.01 ng mL^{-1}. The concentrations of DEHP and DBP found in hexane in our laboratory were a hundred times lower than those reported in an earlier study [71]; an earlier study showed that hexane contained DBP and DEHP at concentrations of approximately 100 ng mL^{-1} [68].

Although distillation can reduce phthalate levels in organic solvents [69], results from our laboratory showed that phthalate concentrations in high-purity, reagent-grade (purchased as glass-distilled) solvents were between 0.01 and 1.0 ng mL^{-1}. Further removal of phthalates from solvents by re-distillation can be ineffective, because distillation can introduce solvents to other sources of contamination. For example, after exposure of hexane to laboratory air, DBP and DEHP concentrations increased at rates of 0.13 and 0.42 ng cm^{-1} h^{-1} [71]. Organic solvents can be passed through sorbents to reduce phthalate contamination. Fankhauser-Noti and Grob [71] added 3 % deactivated alumina to hexane and reduced phthalate contamination by 99.8 %; nevertheless, DEHP and DBP were found at 0.1 ng mL^{-1} after purification with activated alumina. This highly efficient and simple purification method can be used for non-polar solvents, for example cyclohexane, hexane, and pentane. The sorption technique is not suitable for polar solvents, which would extract phthalates from alumina rather than the phthalates being adsorbed from the solvents.

Solvents are an important source of phthalate contamination in the analysis. Although concentrations of DBP and DEHP are low, approximately 0.1 ng mL^{-1}, because of the large volumes of solvents used (generally hundreds of milliliters), 10 to 100 ng phthalates can be introduced in the analytical procedure from the solvents alone. Again, considering the low concentrations of phthalates in food samples (typically, 0.1 to 100 ng g^{-1}), the contribution of solvents (10 to 100 ng) to

Table 4 Phthalate concentrations in selected high-purity organic solvents (ng mL^{-1}; mean ± standard deviation)

Phthalate	Hexane (n=6)	Acetone (n=3)	Dichloromethane (n=2)	Acetonitrile (n=2)
DMP	ND[a]	0.014±0.018	ND	0.022±0.004
DEP	0.029±0.024	0.010±0.005	0.019±0.017	0.017±0.006
DIBP	0.044±0.048	0.002±0.0007	0.026±0.034	0.21±0.009
DBP	0.45±0.53	0.010±0.005	0.27±0.35	0.080±0.068
DNHP	0.006±0.007	0.41±0.007	0.004±0.006	0.002±0.001
BzBP	0.057±0.064	0.023±0.001	0.074±0.10	0.027±0.010
DCHP	0.005±0.011	0.16±0.27	ND	ND
DEHP	0.59±0.69	0.58±0.004	6.39±0.64	0.28±0.089
DNOP	0.002±0.005	0.022±0.003	0.006±0.009	ND

[a]ND, not detected

procedural contamination is high, which can lead to false positive results or overestimated values. Minimization of usage and purification of solvents with sorbents can reduce introduction of phthalates in the analytical procedure. Procedural blank values must be carefully controlled and subtracted from sample values, when necessary.

Phthalate contamination in glassware and laboratory air

It is recommended that only clean glassware is used for analysis of phthalates in foodstuffs. EPA method 8061A indicates that phthalates are not removable from glassware by rinsing with hot water, followed by deionized water and acetone. Transfer of sample extracts into autosampler vials using glass pipettes resulted in addition of ~1 ng DBP and 6 ng DEHP into the sample extracts [71]. Baking of glassware at 450 °C, after water and solvent rinse, can significantly reduce phthalate levels (by more than 90 %). A major source of phthalates in glassware is laboratory air. Concentrations of DBP and DEHP in laboratory air are reported to be from 0.9 to 3.0 μg m^{-3} [71]. Concentrations of DIBP and DBP in the air in a laboratory in Belgium were approximately 500 to 1000 ng m^{-3}, and respective concentrations of DEP and DEHP were approximately 200 and 100 ng m^{-3} [69].

Although it can be a daunting task to completely eliminate phthalate contamination in organic solvents and materials used in the analysis, substantial efforts should be made in advance of the analysis to reduce background levels of phthalates in procedural blanks. The following tips may be used to reduce phthalate contamination in procedural blanks.

1. Avoid the use of plastic materials, for example PVC, for collection, storage, and analysis of samples.
2. Carefully select glassware. Wash all glassware with a soap solution, hot water, and Milli-Q water, then bake it at 450 °C overnight. Autosampler vials and glass pipettes should be baked at 450 °C overnight. After baking, store the items in a furnace (if possible, keep them wrapped in aluminium foil) until use.
3. Bake sorbents in a furnace at 450 °C for several hours. Purify Milli-Q water by shaking with hexane and use the hexane-extracted water for the deactivation of sorbents, when required.
4. Minimize sample-preparation time when possible. Design a clean room with stainless steel construction for processing the samples.
5. Check contamination levels of phthalates in solvents and avoid using solvents with high levels of phthalates; minimize solvent usage when possible; purify solvents with sorbents, if necessary.

Samples and blanks are spiked with isotopically labeled internal standards which are used for quantification of concentrations. Commonly, concentrations of phthalates reported in foodstuffs are corrected by subtraction of blank values. Total concentrations of phthalates in procedural blanks can be estimated by use of the equation:

$$BV_i = f_{i-1} \bullet BV_{i-1} + \Delta_i,$$

where BV ($i \geq 1$) is the total amount of phthalate in procedural blanks (ng), f is the recovery (%) of the internal standard, and Δ is the amount of phthalate contamination introduced in the different steps of the analytical procedure (ng). Thus, the fewer the analytical steps, the smaller the Δ value; when the recoveries of the internal standards and the Δ values in each of the analytical steps of the procedural blanks and actual samples are the same, correction for blank contamination is straightforward. However, recoveries of internal standards and procedural blanks vary substantially between and within batches of sample analysis, which emphasizes the need for simplification of the analytical procedure (to minimize such variations). The background levels of contamination contributed by different sources, including solvents, can vary from batch to batch and from day to day. This variability in background levels of phthalates in procedural blanks hampers the ability to confidently correct for the background levels and, consequently, accurately determine phthalates in foodstuffs.

Analytical methods for phthalates in foods

The procedure for analysis of phthalate esters in food samples is similar to that for many organic contaminants and involves extraction, purification, separation, and instrumental detection. For the purpose of discussion of analytical methods, we categorized food samples into two groups, liquid samples and solid/semi-solid samples. Because types of foodstuff vary widely, no one procedure can be used for all foods.

Methods reported for extraction of phthalates from liquid foods are summarized in Table 5. For liquid samples that do not contain lipids (for example water, juices, and other beverages), liquid–liquid (L–L) extraction with organic solvents, solid-phase extraction (SPE) with cartridges, and solid-phase micro-extraction (SPME) with commercially available fibers have been used in the extraction of phthalates (Table 5). L–L extraction is usually performed with non-polar organic solvents, for example hexane, dichloromethane, or isooctane, followed by phase-separation by centrifugation and removal of moisture by treatment with anhydrous sodium sulfate. SPME enables rapid, solvent-free extraction. SPME fibers can be directly immersed in a liquid food sample or placed in the headspace over a liquid sample placed in a vial. However, SPME fibers are usually expensive, and the time to reach equilibrium between the fiber and the target chemicals can be lengthy, which can lead to inaccurate measurements. The SPE

Table 5 Methods used for extraction and purification of phthalates in various liquid food samples

Extraction[a]	Further clean-up	Ref.
Water		
SPME extraction method. Sample was extracted by use of commercial fibers, for example polyacrylate (PA), polydimethylsiloxane (PDMS), polydimethylsiloxane–divinylbenzene (PDMS-DVB), Carboxen–polydimethylsiloxane (CAR-PDMS), and Carbowax–divinylbenzene (CW-DVB)	No	[89, 90, 100–103]
SPE extraction method. Samples were extracted with Oasis or C_{18} SPE cartridge, and eluted first with methane–hexane 4:1 (*v*/*v*), then with methanol–dichloromethane 9:1 (*v*/*v*)	No	[104]
SPE extraction method. Sample was extracted with electrospun nylon 6 nanofibers and eluted with acetone	No	[58]
L–L extraction method, sample was extracted with hexane three times by shaking for 30 min and centrifuged	No	[81]
Beverages		
Simultaneous steam distillation and extraction	No	[105]
L–L extraction method. 5 g sample and 500 mg sodium chloride, extracted with 2.5 mL hexane–dichloromethane 10:1 (*v*/*v*) twice and then dried over 500 mg sodium sulfate	No	[106]
L–L extraction method. Sample was extracted with hexane three times by shaking for 30 min and centrifuged	No	[81]
Wine		
SPME headspace extraction method. Sample was extracted with PDMS-DVB fiber	No	[91]
SPE extraction method. Sample was extracted with C_{18} SPE cartridge, eluted with dichloromethane, dried over sodium sulfate and sodium sulfate was washed with dichloromethane	No	[92]
L–L extraction method. Sample was extracted with hexane three times by shaking for 30 min and centrifuged	No	[81]
Milk		
Human milk. SPME headspace extraction method. Sample was extracted with PDMS fiber	No	[94]
Human milk. L–L extraction method. 10 g sample was extracted with 10 g acetone and 7 g hexane by shaking for 10 min, centrifuged, hexane layer was taken, and extracted again	Hexane was loaded on a selective pressurized liquid-extraction cartridge (8 g Florisil) and eluted with dichloromethane	[95]
Whole milk. L–L extraction method. 2 g sample was added with 10 mL acetonitrile, homogenized, 0.8 g sodium chloride was added and the sample was shaken for 15 min and sonicated for 10 min. Acetonitrile was concentrated to dryness and methanol was added	Frozen methanol solution to remove sodium chloride	[84]
Cows' milk. SPME headspace extraction method. Samples were extracted with 10 different types of fiber	No	[97]
Soybean milk. Molecularly imprinted solid-phase extraction (MISPE) method	No	[96]
Milk or milk products. L–L extraction method. 1.5 mL sample was mixed with 1.5 mL methanol, 2.0 mL hexane, and 2.0 mL MTBE, shaken for 1 min. After centrifugation, the hexane–ether phase was taken and the residue was extracted again. Combined extract was evaporated to dryness and re-dissolved in hexane	2 mL hexane solution and 2.0 mL acetonitrile and shaken for 1 min. Discard hexane layer, and add 1 mL hexane repeat again. The final extract was re-dissolved in acetonitrile.	[80]
Milk powder. L–L extraction method. 2 g sample and 1 mL acetonitrile, kept in a refrigerator overnight. After addition of 4.5 mL acetonitrile saturated with hexane, sample was mixed for 3 min, ultrasonicated for 20 min, and then centrifuged for 20 min. The upper solution was separated from the solid, and the solution was washed again with 0.5 mL hexane saturated with acetonitrile	No	[99]
Whole milk. SPE extraction method. 10 mL sample was added to 10 mL methanol and sonicated for 10 min. The solution	Florisil SPE cartridge was used for further clean-up.	[107]

Table 5 (continued)

Extraction[a]	Further clean-up	Ref.
was added to 80 mL HPLC water. 0.5 g C_{18} SPE cartridge was used for extraction of phthalates from milk solution. Phthalates were eluted with 12 mL ethyl acetate		
Oil or cooking oil		
SPE extraction method. Sample was extracted with an electrospun nylon 6 nanofibers and eluted with acetone.	No	[58]
SPME headspace extraction method. Sample was extracted with PDMS, PA, PDMS-DVB and CAR-PDMS fibers	No	[108]
SPME extraction method. Samples was extracted with PDMS, PDMS-DVB and CAR-PDMS fiber	No	[109]
Sample was analyzed directly without extraction	No	[110]
L–L extraction method. 3.0 g oil was added to 40 mL of hexane-saturated acetonitrile, shaken for 15 min, and the hexane layer containing lipids was discarded. Three milli liters of hexane were added and the procedure was repeated twice to remove lipids	The extract was then concentrated to 5 mL and 25 mL HPLC water was added. Phthalates were extracted three times from the solution with hexane (7 mL), by shaking for 30 min	[81]

[a] SPE, solid phase extraction; SPME, solid phase microextraction; L–L, liquid-liquid extraction

method has been used for determination of phthalate esters in bottled water and wine (Table 5). Selection of SPE cartridges with appropriate sorbents is important for efficient and selective extraction of phthalate esters from liquid foods. A recent study used a nanomaterial (Nylon 6) SPE system for extraction of phthalates from water and oil [58].

For liquid foods that contain lipids, for example milk (milk powder) and oil (including cooking oil), phthalate analysis requires several additional steps (Fig. 1). The L–L extraction method would co-extract lipids and organic compounds with the phthalates (because of the lipophilic properties of phthalates). Removal of lipids from the extracts by gravity column chromatography or SPE can be cumbersome, because phthalates and lipids are of similar polarity [73]. Headspace SPME has been for determination of phthalates in milk samples (Table 5). Lipids from the extracts can be separated and removed by two techniques that have been commonly used in food analysis: gel-permeation chromatography (GPC) and L–L extraction. GPC is a size-exclusion chromatographic method that separates analytes on the basis of size (~molecular mass). In general, a GPC column with the dimensions 50 cm × 2.5 cm is packed with Biobeads S-X3 (Bio-Rad Laboratories, Hercules, CA, USA). Phthalates with molecular weights in the range of 200 to 400 can be separated from lipids (MW approx. 800) [73]. Dichloromethane–cyclohexane (1:1, *v*/*v*), cyclohexane–ethyl acetate (1:1), or dichloromethane–hexane (1:1) have been commonly used as the mobile phases in the GPC separation of lipids. Alternatively, L–L extraction can be used for removal of lipids from food extracts. L–L extraction with acetonitrile and hexane can separate lipids from phthalates [80]. In our laboratory, L–L extraction with hexane-saturated acetonitrile (prepared by shaking 4:1 acetonitrile–hexane) and hexane is used for removal of lipids from fatty food samples [81]. In comparison with the GPC method, L–L extraction consumes less solvent and therefore reduces background levels of phthalate contamination in the analytical procedure.

The traditional method of extraction of semi-volatile organic pollutants from solid samples is Soxhlet extraction, which was developed in 1879 by Franz von Soxhlet for separation of lipids from solid samples. However, Soxhlet

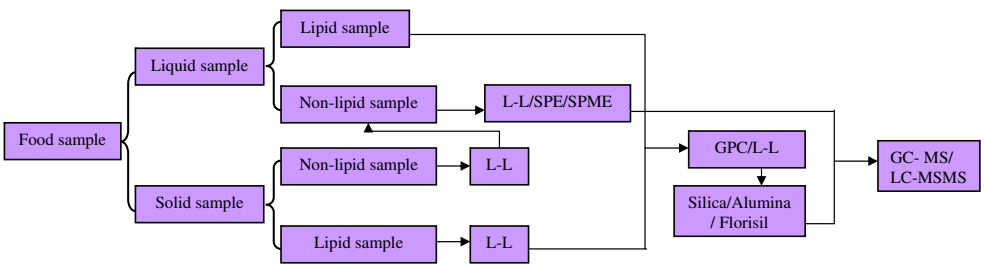


Fig. 1 Schematic illustration of typical analytical procedure used for analysis of phthalates in foods (*L–L*, liquid–liquid extraction; *SPE*, solid-phase extraction; *SPME*, solid-phase microextraction; *GPC*, gel-permeation chromatography; *GC–MS*, gas chromatography–mass spectrometry; *LC–MS–MS*, liquid chromatography–tandem mass spectrometry)

extraction is a time-consuming and labor-intensive procedure (4–72 h) and requires large amounts of solvent (100–500 mL). Phthalate contamination arising from exposure of samples and solvents to air is a concern in Soxhlet extraction. EPA method 8061A indicates that even after the heating the Soxhlet extractor under reflux with acetone for three days DEHP remained in the Soxhlet apparatus at a level of ~500 ng. Therefore, methods for the extraction of phthalates from solid or semi-solid food samples should be kept as simple as possible (liquid–solid extraction in a clean glass tube with mechanical shaking), followed by clean-up using GPC or L–L extraction [54, 69]. The methods available for the extraction of phthalates from food samples include mechanical shaking with solvents, ultrasonic extraction, and microwave-assisted solvent extraction. Before extraction of solid food samples, anhydrous sodium sulfate is commonly used to remove moisture and homogenize the samples. Solvents that have been used in the extraction include acetonitrile, dichloromethane, pentane, hexane–acetone, hexane–acetonitrile, hexane–methanol, hexane–dichloromethane, hexane–pentane–acetone–MTBE, and hexane–methanol–MTBE [54]. The extraction efficiencies of solvents have been shown to vary, depending on the combination of solvents and sample matrix [60].

After extraction, the extracts are purified by passage through sorbent-filled glass columns or cartridges. Silica, alumina, or Florisil have been commonly used for purification of extracts. Gas chromatography (GC) coupled with different types of detector, preferably mass spectrometric (MS) detection, is used in the separation, detection, and quantification of phthalate esters in food samples. Traditionally, flame ionization detectors (FID) and electron-capture detectors (ECD) were used for detection of phthalates in foods but are now replaced with more selective MS detectors. MS detection enables addition of isotopically-labeled phthalate internal standards before sample extraction; this internal standard enables more accurate quantification of phthalates in food samples. The isotopically labeled and native phthalate esters can be discriminated from each other by the use of an MS detector, which eliminates potential interferences from the analysis. In the MS analysis, molecular and fragment ions of phthalates can be targeted by use of selected ion monitoring (SIM) mode, and the relative ratios among the ion fragments can be used to confirm the detection of phthalates. Typical ion fragments of phthalate esters used in GC–MS quantification are listed in Table 6. As shown, except for DMP, which is monitored at *m/z* 163, the ion fragment *m/z* 149 $[C_8H_5O_3]^+$ is the most prominent ion in the spectra of all other phthalates (molecular ion); this ion is attributed to a McLafferty rearrangement followed by loss of an alkoxy radical and final stabilization as a cyclic oxonium ion. There have recently been reports of detection of phthalates in sediments and biota [82, 83] by use of liquid chromatography–mass spectrometry (LC–MS) and in milk and milk products [80, 84] and house dust [85] by use of liquid chromatography–tandem mass spectrometry (LC–MS–MS). The parent and product ions of phthalate esters monitored in LC–ESI–MS–MS analysis are listed in Table 6.

Table 6 Quantitation and confirmation ions used for GC–MS and LC–ESI–MS–MS analysis of phthalate esters

GC–MS SIM mode

Phthalate	Quantification ion (*m/z*)	Confirmation ion (*m/z*)
DMP	163	77, 194, 135
DEP	149	177, 76, 105
DIBP	149	223, 57
DBP	149	223, 205
DNHP	149	255
BzBP	149	206, 91
DCHP	149	167
DEHP	149	279, 167
DNOP	149	279, 150

LC–ESI–MS–MS–MRM mode

Phthalate	Parent ion (*m/z*)	Product ion (*m/z*)
DMP	195	163, 77, 135, 133
DEP	223	149, 121, 93, 65
DBP	279	149, 93, 121, 65
BzBP	313	149, 165, 205
DCHP	331	149, 121, 93, 65
DEHP	391	149, 167
DOP	413	301, 189, 317
DIDP	447	149, 415, 121, 335

The overall procedure for the analysis of phthalates in food samples is shown in Fig. 1. Because of the ubiquity of phthalates in the laboratory environment and in laboratory reagents and equipment, accurate and precise determination of low concentrations of phthalates in food samples can be a challenging task. Simplification of analytical steps and exercise of adequate precautions with quality assurance and quality-control procedures, with the objective of reducing background levels of contamination are important in the determination of phthalates in foodstuffs.

Liquid-liquid partitioning for the removal of lipids in food samples

As discussed above, GPC has been widely used for separation and removal of lipids from target organic analytes in food samples [54, 69]. However, GPC consumes large volumes of organic solvents (~300 mL). Because the solvents contain trace levels of phthalates as contaminants, GPC increases background levels of phthalates in the analytical procedure. For instance, if 300 mL solvent was used for elution of

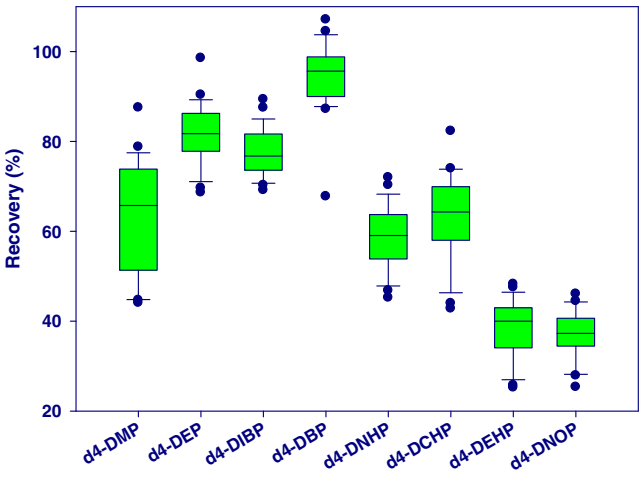


Fig. 2 Recovery of deuterated phthalate internal standards added to oil samples and passed through the analytical procedure. The *horizontal lines* represent 10th, 50th, and 90th percentiles and the *boxes* represent 25th and 75th percentiles. Outliers are shown as *individual points*

phthalates through a GPC column, and if the final solvent volume were concentrated to 0.5 mL, the concentration factor would be 600. If the average concentration of DEHP and DBP in the solvent were 0.1 ng mL^{-1}, the final concentration in 0.5 mL would be 60 ng mL^{-1}.

We investigated an L–L extraction method for removal of lipids from cooking oils in phthalate analysis. Five types of commercial cooking oil, soybean oil, safflower oil, canola oil, vegetable oil, and virgin olive oil, were mixed for 2 h. The oil sample (3 g) was then transferred to a 45-mL glass centrifuge tube containing 40 mL of hexane-saturated acetonitrile (150 mL hexane was mixed with 600 mL acetonitrile, and the mixture was shaken for 30 min). The oil sample was spiked with native phthalate standards (AccuStandard, New Haven, CT, USA; purity >99 %) at concentrations of 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 50.0 ng g^{-1}. After addition of 50 ng of isotopically labeled phthalate standards (except for BzBP), samples were shaken for 15 min in an orbital shaker (Eberbach, Ann Arbor, MI, USA) at 250 oscillations min^{-1}; the hexane layer containing the lipids was discarded. Hexane (3 mL) was added, and the procedure was repeated twice to remove lipids. The extract was then concentrated to 5 mL and transferred to a 35-mL glass centrifuge tube containing 25 mL HPLC-grade water (the water had previously been extracted with hexane to remove any residual phthalates present as contaminants). Samples were extracted three times with hexane (7 mL) by shaking for 30 min. After centrifugation at 4,500*g* for 10 min, the hexane layers were combined, concentrated by rotary evaporation, and transferred to a GC vial. The final extract was concentrated to 0.5 mL, under a gentle stream of nitrogen, for analysis by GC–MS. In total, 26 samples and seven blank samples were tested.

DNHP, DCHP, and DNOP were not found in any of the procedural blanks. Approximately 1 ng DMP, DEP, and BzBP, ~5 ng each of DBP and DIBP, and ~20 ng DEHP were found in the procedural blanks. Average recoveries of labeled internal standards added to all sample matrices and blanks were 39±5 %–95±5 % (Fig. 2) and 66±24 %–8 3±17 %, respectively. When recovery of the native compounds was corrected for recovery of the internal standards, phthalate recovery from samples was >80 %. In the L–L extraction method of lipid removal, recovery of d_4-DBP was >95 %, followed by d_4-DEP and d_4-DIBP (>75 %), d_4-DMP, d_4-DNHP, and d_4-DCHP (>60 %), d_4-DEHP (~40 %), and d_4-DNOP (>35 %). Addition of isotopically-labeled internal standards for each of the target analytes enabled correction for losses of analytes in the analytical procedure (Table 7). After correction for internal standard recovery, accuracy for DMP, DEP, DBP, DIBP, and DNHP, at a spike level of 0.5 ng g^{-1}, was ≥80 % (Fig. 3a); and accuracy for BzBP, DCHP, and DNOP, at a spike level of 5.0 ng g^{-1}, was >80 %. DEHP recovery, at a spike level of 20.0 ng g^{-1}, was approximately 60 %. The occurrence of DEHP at high concentrations in procedural blanks is the reason for the reduced accuracy. The coefficient of variation (CV) for

Table 7 Phthalate concentrations in an oil sample and in an oil sample spiked at different concentrations (ng g^{-1}, mean ± standard deviation)

Phthalate	Oil sample (n=5)	Spiked oil sample (n=3)						
		0.5	1.0	2.0	5.0	10.0	20.0	50.0
DMP	0.62±0.09	0.59±0.13	1.13±0.14	1.94±0.11	4.37±0.24	8.12±0.77	17.0±0.47	42.4±0.09
DEP	0.18±0.32	0.53±0.21	0.93±0.04	2.05±0.11	5.14±0.22	10.3±0.18	19.9±0.50	49.5±0.50
DIBP	2.10±0.38	0.68±0.15	0.98±0.02	1.83±0.18	4.23±0.24	8.56±0.14	16.3±0.34	40.3±0.63
DBP	7.05±0.59	0.65±0.16	0.95±0.34	1.70±0.44	4.61±0.30	8.12±0.96	14.6±1.10	33.8±0.46
DNHP	ND	0.51±0.01	0.96±0.06	1.78±0.06	4.30±0.06	8.27±0.25	16.7±0.18	41.5±0.51
BzBP	79.4±5.50	3.46±1.40	2.09±0.43	4.21±1.00	3.89±1.10	8.94±3.10	20.3±1.30	53.7±1.00
DCHP	ND	0.09±0.11	0.43±0.10	1.15±0.30	4.04±0.48	8.07±0.61	18.0±1.20	47.5±1.30
DEHP	86.9±5.30	1.71±3.30	ND	2.24±4.10	1.70±3.30	3.53±1.40	11.4±1.90	34.7±2.80
DNOP	ND	ND	ND	ND	3.81±0.94	8.98±2.40	19.5±1.20	44.9±4.50

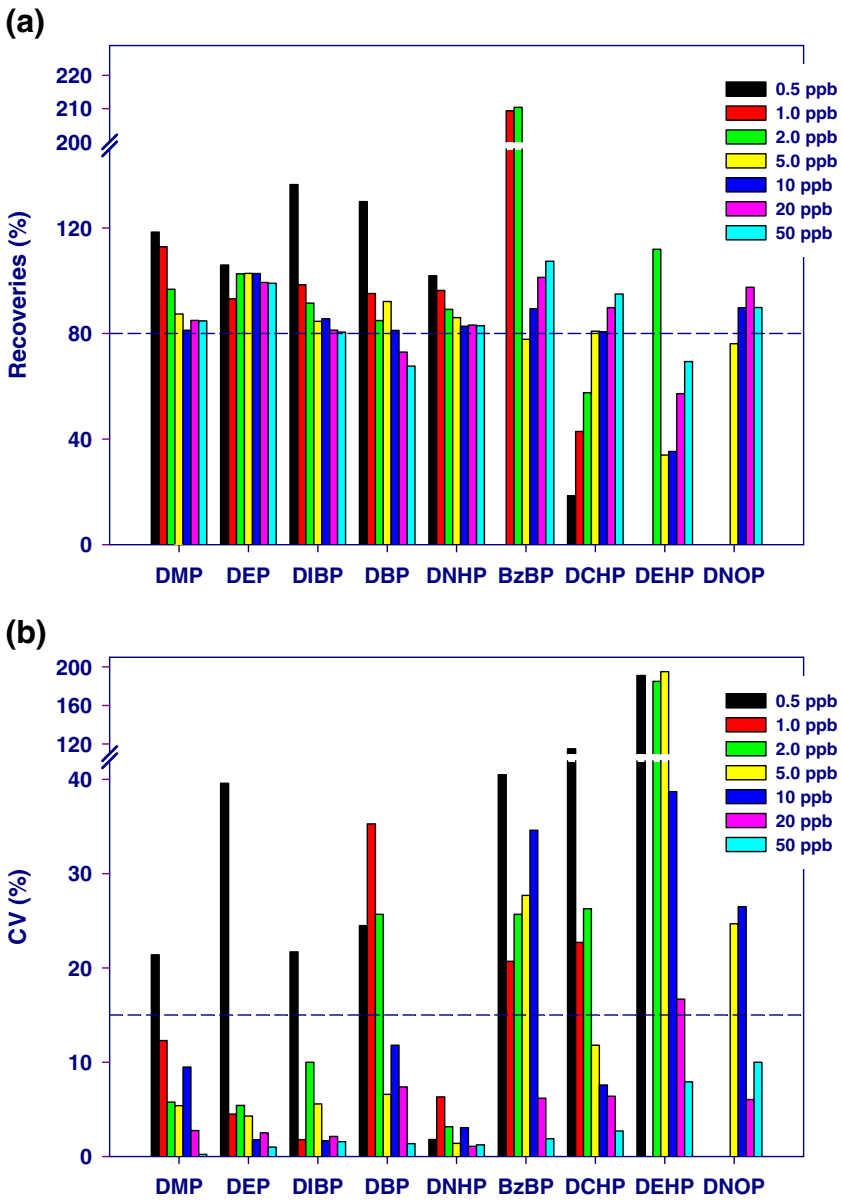


Fig. 3 Accuracy (reported as recovery) and precision (reported as coefficient of variation, *CV*) of phthalate esters added to oil samples and passed through the analytical methods

replicate analysis of samples (Fig. 3b) decreased with increasing concentrations of spiked target analytes. All CV values were below 15 % for DMP, DEP, DIBP, and DNHP, at a spike level of 1.0 ng g^{-1}; for DCHP, at a spike level of 5.0 ng g^{-1}; and for BzBP, DEHP, and DNOP, at a spike level of 20.0 ng g^{-1}. Of the nine target phthalate esters analyzed, an isotopically-labeled internal standard was not available for BzBP; this explains the high CV obtained for this compound DNOP was not detected in samples spiked at levels below 5.0 ng g^{-1}; the relatively high CV (~30 %) found for DNOP was because of the low recovery of d_4-DNOP (<35 %). The high CV found for DEHP was because of the combination of high background (blank) contamination and low recovery of d_4-DEHP (~40 %). The presence of high background levels of contamination of DEHP can introduce false positive results or overestimated concentrations if adequate precautions are not taken. In our study, we used 3 g oil for extraction, which is a large amount of lipid. If the volume of such oil samples used for analysis were limited to 1 g, better accuracy and precision could be expected. This is apparent from the high (>70 %) recovery of all the target analytes and their internal standards in procedural blanks. The L–L extraction method was also used for removal of lipids from meat, milk, and seafood [81], and the recovery of internal standards from milk was better than from oils. Considering the high solvent concentration factor encountered in the GPC method and the contamination of many organic solvents by phthalates, the L–L method is a better choice for lipid removal in the analysis of phthalate esters in foodstuffs.

Accurate measurement of concentrations of phthalates in foodstuffs is essential for assessment of exposure and risk.

Human exposure to phthalates can be estimated by analysis of urine by biomonitoring methods [35, 45, 46, 51, 52, 86–88] and by analysis of a variety of environmental and dietary sources (air, water, foodstuffs, dust, and personal care products) that contribute to exposure. A combination of biomonitoring and environmental monitoring approaches can yield better understanding of sources of human exposure to phthalates.

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