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Article

Determination of Phthalates Released from Paper Packaging Materials by Solid-Phase Extraction–High-Performance Liquid Chromatography

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A solid phase extraction (SPE) high-performance liquid chromatography (HPLC) method was developed for the simultaneous determination of 10 phthalic acid esters (dimethyl phthalate, diethyl phthalate, dipropyl phthalate, benzylbutyl phthalate, diisobutyl phthalate, dicyclohexyl phthalate, diamyl phthalate, di-*n*-hexyl phthalate, di-*n*-octyl phthalate and di-2-ethylhexyl phthalate) released from food paper packaging materials. The use of distilled water, 3% acetic acid (w/v), 10% ethanol (v/v) and 95% ethanol (v/v) instead of the different types of food simulated the migration of 10 phthalic acid esters from food paper packaging materials; the phthalic acid esters in four food simulants were enriched and purified by a C18 SPE column and nitrogen blowing, and quantified by HPLC with a diode array detector. The chromatographic conditions and extraction conditions were optimized and all 10 of the phthalate acid esters had a maximum absorbance at 224 nm. The method showed limitations of detection in the range of 6.0–23.8 ng/mL the correlation coefficients were greater than 0.9999 in all cases, recovery values ranged between 71.27 and 106.97% at spiking levels of 30, 60 and 90 ng/mL and relative standard deviation values ranged from 0.86 to 8.00%. The method was considered to be simple, fast and reliable for a study on the migration of these 10 phthalic acid esters from food paper packaging materials into food.

Introduction

Phthalic acid esters (PAEs) are a family of compounds with a common chemical structure and dialkyl or alkyl/aryl esters of 1, 2-benzenedicarboxylic acid (1), which are used in many branches of industry and produced in huge amounts throughout the world. PAEs are used as plasticizers to impart flexibility and resilience to plastic products, and many consumer products and food packaging products contain PAEs (2). PAEs are utilized in the production of electrical cords, films, glues, paints, ink, varnishes, coatings, adhesives, cosmetics, pesticides, repellents, dielectric media and elastomers, and as a means of impregnation (3). PAEs of low molecular weight, such as dimethyl phthalate (DMP), diethyl phthalate (DEP) and dibutyl phthalate (DBP), are widely used in cosmetics and personal care products. PAEs with larger molecular weight, such as di-2-ethylhexyl phthalate (DEHP), diisononyl phthalate (DINP) and benzylbutyl phthalate (BBP), have wide applications as plasticizers in the polymer industry to improve flexibility, workability and general handling properties. Approximately 80% of all phthalates are used for this purpose, and DEHP is the most widely used of the PAE plasticizers (4).

Because PAEs are not chemically bound in plastics products, but remain present as a freely mobile and leachable phase, they can be lost from soft plastic over time and released to the environment and food during the production or use of the products in which they are present (4). The toxicity of phthalates has been, and still is, the subject of discussion and public concern. Ubiquitous exposure to PAEs may be critical because toxicological studies have demonstrated the considerable adverse effects of phthalates and their metabolites to human health (5). The potential for negative health impacts from these compounds have been studied extensively, and concerns such as the potential for reproductive (6) and developmental harm (7), teratogenicity, embryotoxicity, spermiotoxicity, carcinogenity (3), cardiotoxicity, hepatotoxicity and nephrotoxicity (8) and endocrine disruption (9) have been documented and discussed (10). PAEs have wide distribution, lengthy environmental persistence and a potential tendency for bioaccumulation, so they are regarded as environmental priority pollutants by the Environmental Protection Agency (EPA) (11). Public and scientific concern has increased in recent years about the potential health risks associated with PAEs. DBP, BBP, DEHP, DINP and DIDP were controlled to strictly be additives in plastic materials and articles intended to come into contact with food in EU Directive 2007/19/EC; these cannot exceed the specific migration limit (SML) values of 1.5, 30, 0.3, 9g and 9 mg/kg, respectively. DEHP, BBP and DBP have been restricted under Annex XVII of EU REACH in toys and child care articles (incorporating the old directive regarding phthalates, 2005/84/EC), which states that they shall not be used as substances or as constituents of preparations at concentrations of greater than 0.1% by mass of the plasticized material. Decision 2455/200l/EC of the EU parliament stipulated that DMP, DEP, BBP, DBP, DEHP and dioctyl phthalate (DOP) are as priority toxic pollutants. The US EPA has listed six phthalic acid esters (DMP, DEP, DBP, DEHP, DOP and BBP) among the primary risk pollutants. The Federal Institute for Risk Assessment (BfR) of Germany in 2007 recommended restrictions on the migration of diisobutyl phthalate (DiBP) from packaging for foods: so-called specific migration guidance values of 1 mg DiBP per kilogram of food and 0.5 mg for baby and infant formula. In China, DMP, DBP and DEHP are regarded as priority toxic pollutants (12); phthalate esters included in food may be considered illegal as non-food substances and easy to abuse food additives.

In recent years, studies on PAEs have focused on soil (13, 14), atmosphere (15, 16), water (17, 18), plastic products (19–22), cosmetic (23) and personal care products (24) and food (25–27), but the migration of PAEs from packaging materials has been given little attention, especially in food paper packaging materials. Paper packaging materials usually contain plastic film, printing ink and adhesives, to which are added different kinds of PAEs. When these packaging materials have direct contact with

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food under normal conditions of use, small amounts of the plasticizers are expected to migrate into the food. Therefore, when a kind of paper packaging material is intended to be used as food packaging, a scientific estimation should be provided about the migration of PAEs from the paper packaging material (28).

To provide a scientific and accurate analysis of the migration of PAEs from paper packaging materials, a simulated migration test needs to be conducted. According to National Standards of China (GB/T 23296.1-2009), four food simulants include: distilled water (Simulant A), which substitutes for aqueous foods; 3% acetic acid in water (Simulant B), which substitutes for acidic aqueous foods; 10% aqueous ethanol (Simulant C), which substitutes for alcoholic products; and olive oil (Simulant D), which substitutes for fatty foods. The Simulant D substitutes ethanol and isooctane have higher migration rates than olive oil, which was compensated for by a reduction of contact times and temperatures. However, the rules required that olive oil be used whenever technically possible (without further specification, because feasibility is obviously arguable). (29)

Solid-phase extraction (SPE) is a widely used sample preparation technique for the isolation of selected analyses. The principal goals of SPE are to enrich and purify samples and transfer them from the sample matrix to a different solvent or to the gas phase. There is no doubt that SPE is a popular sample preparation technique in many areas, including environmental, pharmaceutical, clinical, food and industrial chemistry (42). SPE has been used to expertly enrich and purify PAEs from water (30–32) and food (12, 33). There are many methods for the determination of PAEs, such as gas chromatography (GC) (22, 27, 34), gas chromatography–tandem mass spectrometry (GC–MS-MS) (13, 35, 36), high-performance liquid chromatography (HPLC) (23) and liquid chromatography (LC)–MS-MS (10); among these methods, GC and GC–MS-MS are the primary determination technologies. HPLC can be used as an alternative analytical technique and is particularly useful for the analysis of isomeric mixtures and metabolites of phthalates because this method does not require prior derivatization of the sample (12). HPLC with diode array detection (DAD) can provide a full spectrum scan for a chemical and produce the maximum absorbance wavelength. Li *et al.* created a method for the analysis of DEP, di-*n*-propyl-phthalate (DnPP), di-*n*-butyl-phthalate (DnBP), di-cyclohexyl-phthalate (DcHP) and DEHP via HPLC–ultraviolet (UV) detection in environmental water samples (37). Jara *et al.* developed a method for the determination of BBP and DEHP in water samples by HPLC–UV (38). De Orsi *et al.* created a simple and rapid analytical method for the determination of DMP, DEP, dipropyl phthalate (DPP), diisobutyl phthalate (DiBP), BBP (V), DBP and DEHP in nail cosmetics by HPLC–photodiode array (PDA) detection (39). These methods only studied PAEs in a matrix, and the descriptions of the UV absorbance of different PAEs were inconsistent.

The present study followed three purposes. The first was to design a simulated migration test to provide a scientific and accurate estimation of the migration of PAEs from paper packaging materials. The second was to develop a fast and reliable method, applicable for HPLC, for the enrichment and purification of 10 phthalic acid esters in four food simulants. The third purpose was to develop a fast HPLC procedure for the separation and quantitation of the 10 phthalic acid esters released from food paper packaging materials.

Experimental

Reagents and solvents

The standards (purity > 96%) ofDMP (CAS: 131-11-3), DEP (CAS: 84-66-2),DPP (CAS: 131-16-8),BBP (CAS: 85-68-7), DiBP (CAS: 84-69-5), DcHP (CAS: 84-61-7), diamyl phthalate (DAP; CAS: 131-18-0), di-*n*-hexyl phthalate (DHP; CAS: 84-75-3), di-*n*-octyl phthalate (DNOP; CAS: 117-84-0) and DEHP (CAS: 117-84-0) were provided by Sigma (St. Louis, MO). Their chemical structures are shown in Supplementary Figure 1. Chromatographic grade acetonitrile and methanol were manufactured by SK Chemicals (Korea). Analytical grade ethanol and acetic acid were purchased from Tianjin Chemical (Tianjin, China). Purified water was obtained from a Millipore purification system (Billerica, MA) operating at a resistivity of 18.2 MΩ/cm.

Individual phthalic acid ester stock solutions (1,000 μg/mL) were prepared in methanol and stored at −18°C. A mixed stock solution (100 μg/mL) of the 10 PAEs was prepared by diluting individual stock solutions with methanol and storing at 4°C in brown glass bottles. The mixed solution was replaced every two weeks to prevent the possible decomposition and reaction of phthalic acid esters.

Apparatus

A liquid chromatograph was used (Agilent 1200 Series, Germany), which consisted of a quaternary pump, an online degasser, a thermostatic column compartment, an automatic sampler and DAD. The column was an Eclipse XDB-C18 (150 × 4.6 mm i.d., 5 μm; Agilent, Palo Alto, CA). The calorstat (IKA KS

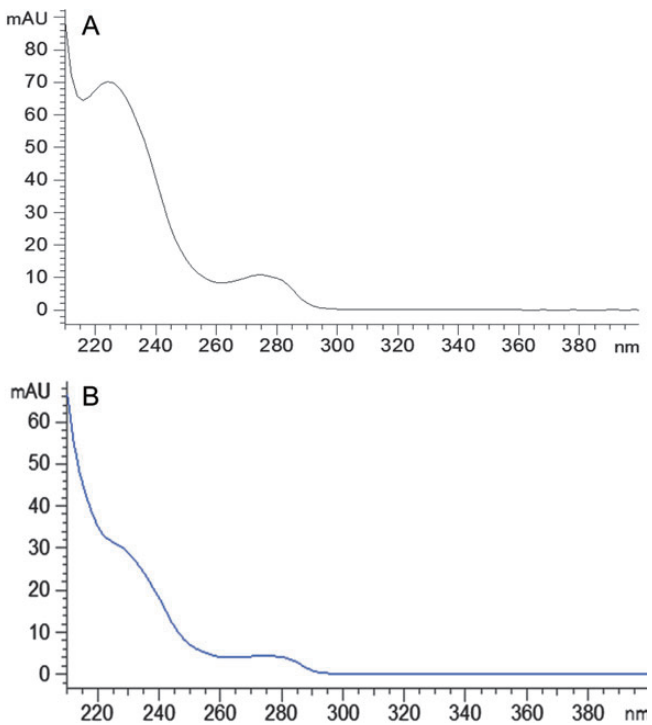

Figure 1. Spectrogram of nine PAEs except BBP (A); spectrogram of BBP (B). Chromatographic conditions: standard concentration (10 μg/mL); acetonitrile as mobile phase A and water as mobile phase B, 60:40; analysis time of 60 min; total flow rate of 1.0 mL/min; column temperature set at 30°C; injection volume of 20 μL.

4000i control) and the vortex oscillator (IKA MS3 basic) were made by IKA; the analytical balance (Mettler Toledo XS 205) was from Mettler Toledo; the MEGA BE-C18 SPE columns (1 GM, 6 mL) were made by Agilent; the pressure blowing concentrator was brought from Caliper Life Sciences Technical Support Center.

Analytical procedure

Simulant migration test

According to National Standards of China (GB/T 23296.1-2009), the four food simulants include: Simulant A, which substitutes for aqueous foods; Simulant B, which substitutes for acidic aqueous foods; Simulant C, which substitutes for alcoholic products; and Simulant D, which substitutes for fatty foods. The test condition of 40°C for 10 days was defined as the most stringent condition. The specific experimental procedures were as follows: food paper packaging materials of 60 cm^2 (surface area) were placed into a 250 mL flask with a glass stopper. In accordance with the provisions of 6 dm2, 1 L and 100 mL of food simulants were added to the flask (29). The flask was placed in the calorstat at 40°C for 10 days. As few plastic apparatuses as possible were used to lower the possibility of background contamination in the analysis of PAEs.

SPE conditions

Simulant D contained a high concentration of ethanol, so it could be concentrated directly by nitrogen blowing. Simulants A, B and C were enriched and purified by a C18 SPE column. The C18 SPE column was pretreated by washing with 5 mL of acetonitrile and 5 mL of purified water before each SPE procedure. Ten milliliters of food simulant that contained PAEs was passed through the pre-conditioned C18 SPE column at the optimum flow rate. After the food simulant passed through, the PAEs retained on the SPE column were eluted with an optimal volume of 10 mL of acetonitrile and the resulting eluent was blown to nearly dry with a gentle nitrogen flow at 30°C. The dried sample was redissolved in 1 mL of methanol. Finally, the extracted sample was filtered through a 0.22 μm filter and analyzed by HPLC with DAD.

HPLC analysis

The detection was performed at a wavelength of 224 nm. A gradient elution solvent was applied that contained acetonitrile as mobile phase A and water as mobile phase B. The mobile phase gradient profile (where t refers to the time in minutes) was as follows: t_0, A = 65%; t_3, A = 65%; t_{20}, A = 95%; t_{30}, A = 95%; t_{31}, A = 65%; t_{35}, A = 65%. The total flow rate was 1.0 mL/min. The column temperature was set at 30°C and the injection volume was 20 μL.

Method validation

The concentrations of the 10 phthalic acid esters in four food simulants were quantified by using linear regression of response against concentration. The areas of the chromatographic peaks were measured. The method was validated for specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy in four food simulants.

Results and Discussion

Optimization of chromatographic conditions

First, four columns were used in an attempt to analyze the 10 PAEs: Agilent 20 RBAX SB-C18 (150 × 2.1 mm i.d., 5 μm) (29), Inertsil ODS-3 (250 × 4.6 mm i.d., 5 μm; Japan) (31), Agilent Hypersil SI (200 × 4.6 mm i.d., 5 μm) and Agilent Eclipse XDB-C18 (150 × 4.6 mm i.d., 5 μm). The results showed that the retention times of PAEs were short in the Agilent Hypersil SI column; the other three columns showed good retention times for the PAEs. Taking into consideration that the XDB-C18 column was suited for a broader range of pH of chemicals, it was chosen column for further research.

Second, the maximum absorbance wavelengths of the 10 PAEs were studied. DEHP and DNOP had long retention times on the XDB-C18 column, so acetonitrile and water were used as mobile phases. Spectral scanning of a single standard found that all 10 PAES had the same spectrogram. In Supplementary Figure 2, three-dimensional (3D) spectrograms are shown of all 10 PAEs. Figure 1A shows the spectrogram of nine PAEs except BBP and Figure 1B shows the spectrogram of BBP. As shown in Figure 1A, all 10 PAES had the same spectrogram; as shown in Figures 1A and 1B, each of the PAEs had maximum absorbance peaks at approximately 224 and 274 nm, and the absorbance at 224 nm was approximately seven times greater than at 274 nm. As a result, the absorbance parameter of the DAD was set at approximately 224 nm.

Finally, the mobile phase gradient was studied and the final chromatographic method was established. The retention times of DMP and DEP were shorter than the other PAEs; thus, if the proportion of acetonitrile in the mobile phase was too high, DMP and DEP could not be well separated and the retention times of BBP, DiBP, DAP and DCHP were not significantly different. When the proportion of acetonitrile was set at 65% in the first 3 min, DMP and DEP showed good separation. From the third to the 20th minute, the proportion of acetonitrile rose from 65 to 95% and DPP, BBP, DiBP, DAP, DCHP and DHP were well separated. Because DEHP and DNOP were strongly reserved in the column, the proportion of acetonitrile was kept unchanged at 95% from the 20th to the 30th minute so that DEHP and DNOP were washed out from the column at the end. After this, the proportion of acetonitrile was returned to 65% from 30th to the 31th minute, which was maintained until the 35th minute. As shown in Figure 2, the retention times of the 10 PAEs were: 2.12 min (DMP), 2.97min (DEP), 2.12 min (DMP), 5.06 min (DPP), 7.98 min (BBP), 8.61 min (DiBP), 13.42 min (DAP), 14.06 min (DCHP), 24.23 min (DEHP) and 25.64 min (DNOP). At the same concentration (10 μg/mL), the absorbance values of DMP, DEP and DiBP were clearly higher than the others.

Optimization of extraction conditions

SPE elution volume optimization

To completely elute the PAEs retained on the column, the optimal elution volume of acetonitrile was studied. The C18 SPE column was pretreated by washing with 5 mL of acetonitrile and 5 mL of purified water, and 10 mL of food simulant (A, B, C and D) that contained PAEs (10 μg/mL) was passed through the preconditioned C18 SPE column and the outflows from the

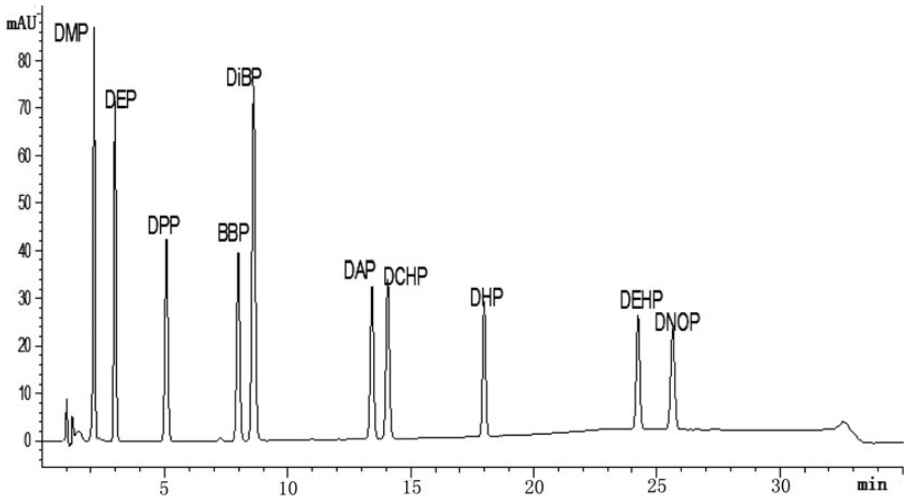

Figure 2. Chromatogram of the 10 PAE standards. Chromatographic conditions: standard concentration (10 μg/mL); acetonitrile as mobile phase A and water as mobile phase B; mobile phase gradient profile (where *t* refers to the time in minutes) of t_0, A = 65%, t_3, A = 65%, t_{20}, A = 95%, t_{30}, A = 95%, t_{31}, A = 65%, t_{35}, A = 65%; total flow rate of 1.0 mL/min; column temperature of 30°C; injection volume of 20 μL.

columns were determined by HPLC. It was found that the PAEs in Simulants A, B and C were completely retained in the columns when 10 mL of food stimulant was passed through the SPE column, but DMP and DEP in Simulant D were not completely retained in the columns. This was because DMP and DEP had short retention times in the column and Simulant D contained a high percentage of ethanol, which could be concentrated directly by nitrogen blowing. The PAEs retained on the SPE column were eluted with an optimal volume of acetonitrile. The resulting eluent was blown to nearly dry with a gentle flow of nitrogen at 30°C. The dried sample was redissolved in 1 mL of methanol. Finally, the extracted sample was filtered through a 0.22 μm filter and analyzed by HPLC with DAD. As shown in Figure 3, it was found that the 10 PAEs could be completely eluted from the column when the elution volume of acetonitrile was 10 mL. When the elution volume of acetonitrile was 5 mL, DMP, DEP and DPP were completely eluted from the column; at 6 mL, BBP and DiBP were completely eluted; at 7 mL, DCHP and DHP were completely eluted; at 8 mL, DHP was completely eluted; at 10 mL, DEHP and DNOP were completely eluted.

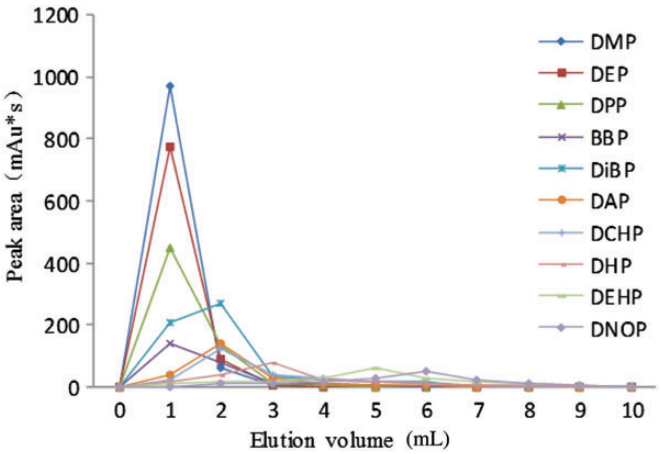

Figure 3. Study on the optimal elution volume of acetonitrile.

Analysis of the effectiveness of the concentration method

An analysis was conducted regarding the effectiveness of the concentration method. The food simulants (10 mL of A, B and C) that contained PAEs (100 ng/mL) were concentrated by SPE and Simulant D (10 mL) that contained PAEs (100 ng/mL) was concentrated directly by nitrogen blowing instead of SPE. All operations were repeated three times and the recovery values were obtained. As shown in Figure 4, the recovery values of all 10 PAEs in the simulants were greater than 70%. As a result, the method was suitable for the concentration of the 10 PAEs.

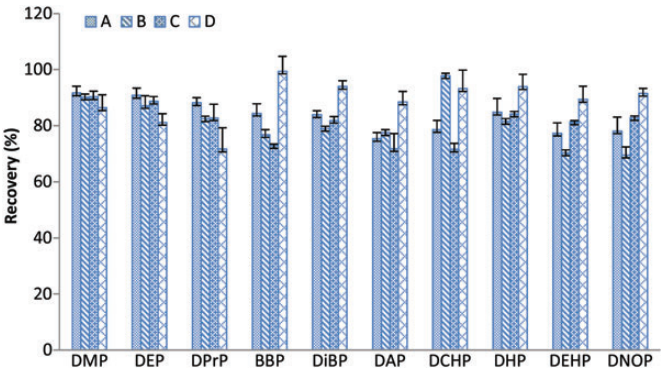

Figure 4. The effectiveness analysis of the concentration method.

Validation of the method

Linearity range

Standard solutions of different concentrations of the 10 PAEs were prepared by diluting the mixed stock solution (100 μg/mL) of the 10 PAEs with methanol, which was determined by HPLC. The calibration equations, correlation coefficients and linearity ranges of the 10 PAEs were obtained. As shown in Table I, the

linearity range varied between 50 and 1,000 ng/mL for DMP, DEP, DPP, BBP and DiBP and 100 and 1,000 ng/mL for DAP, DEHP, DNOP, DCHP and DHP. It was observed that the linearity range for each compound was wide, which ensured the acquisition of reliable data for food simulants with low and high contents of PAEs (40).

The correlation coefficient (r) was greater than 0.9999 in all cases.

Recovery of standards

Recovery studies were conducted by analyzing the four spiked food simulants six times at three different fortified concentrations of 30, 60 and 90 ng/mL after a simulant migration test. Additionally, a blank sample was analyzed, which was prepared in a disposable cup that did not contain the 10 PAEs. The 10 PAEs in the four food simulants were concentrated by SPE and nitrogen blowing, and determined by HPLC. As shown in Table II, the recovery values of DMP, DEP, DPP, BBP, DiBP, DAP, DCHP, DHP, DEHP and DNOP in the four food simulants were in the ranges of 87.75–106.34%, 79.95–101.06%, 73.49–99.85%, 75.61–106.97%, 78.88–96.58%, 74.25–98.32%, 72.36–103.01%, 77.88–99.28%, 71.42–93.70% and 71.27–99.40%, respectively. Precision values were evaluated by measuring the relative standard deviations (RSDs) of the recovery values (41); the RSDs of DMP, DEP, DPP, BBP, DiBP, DAP, DCHP, DHP, DEHP and DNOP in four food simulants were between 0.86 and 4.52%, 1.29 and 4.03%, 1.45 and 8.00%, 1.58 and 4.91%, 1.03 and 4.92%, 1.85 and 7.50%, 0.97 and 5.23%, 1.42 and 6.50%, 0.97 and 4.90 and 1.59 and 6.91%, respectively. The preceding results suggested that the method demonstrated good precision and repeatability. In

Table I
Analytical Parameters of the Proposed Method

Phthalates	Calibration equation	r	Linearity range (ng/mL)	EF	LOD (ng/mL)	LOQ (ng/mL)
DMP	$y = 48.97x - 10.72$	0.99991	50–1,000	10	1.8	6.0
DEP	$y = 42.18x - 7.11$	0.99994	50–1,000	10	2.5	8.4
DPP	$y = 37.26x - 9.52$	0.99992	50–1,000	10	4.1	13.7
BBP	$y = 36.28x - 6.56$	0.99995	50–1,000	10	4.3	14.5
DiBP	$y = 67.80x - 10.34$	0.99996	50–1,000	10	2.4	8.1
DAP	$y = 29.56x - 5.18$	0.99996	100–1,000	10	5.7	19.0
DCHP	$y = 29.81x - 4.86$	0.99996	100–1,000	10	5.8	19.4
DHP	$y = 24.97x - 4.25$	0.99995	100–1,000	10	6.3	21.3
DEHP	$y = 24.60x - 2.41$	0.99994	100–1,000	10	6.8	22.9
DNOP	$y = 24.67x - 4.32$	0.99995	100–1,000	10	7.5	23.8

Table II
Recovery Values of PAEs Determined by Analyzing Four Spiked Food Simulants ($n = 6$)

Phthalates	Spiking levels (ng/mL)	Food substitutes											
		Simulant A			Simulant B			Simulant C			Simulant D		
		Mean ($n = 6$)	Recovery (%)	RSD (%)	Mean ($n = 6$)	Recovery (%)	RSD (%)	Mean ($n = 6$)	Recovery (%)	RSD (%)	Mean ($n = 6$)	Recovery (%)	RSD (%)
DMP	30	31.90	106.34	3.40	29.27	97.58	2.03	30.18	100.75	4.52	28.84	96.13	0.86
	60	58.52	97.54	2.91	60.12	100.20	1.28	60.35	100.59	2.14	60.85	101.42	1.76
	90	81.95	91.05	1.87	81.00	90.00	0.87	81.43	90.48	1.51	78.98	87.75	3.90
DEP	30	26.94	89.80	2.24	24.43	81.44	3.66	26.36	87.88	3.70	26.83	89.43	2.76
	60	58.92	98.20	2.86	54.35	90.59	3.74	60.64	101.06	2.26	57.62	96.03	4.03
	90	85.95	90.55	2.02	78.00	86.67	3.17	72.09	89.01	1.29	71.96	79.95	3.27
DPP	30	29.70	99.00	3.06	28.36	94.53	2.17	29.96	99.85	4.50	25.16	83.87	7.60
	60	52.24	87.07	2.27	49.00	81.67	1.45	50.00	83.34	4.74	44.09	73.49	8.00
	90	81.97	91.08	4.03	79.14	87.93	2.77	83.78	93.09	3.21	83.07	92.30	5.76
BBP	30	26.12	87.08	1.89	25.57	85.22	4.02	24.42	81.41	2.73	32.09	106.97	3.74
	60	51.55	85.92	3.33	46.28	77.13	1.58	45.37	75.61	4.91	59.80	99.66	3.58
	90	81.39	90.43	1.98	78.89	87.66	2.99	71.08	78.98	2.56	77.89	86.54	4.11
DiBP	30	26.78	89.28	4.10	25.83	86.09	4.92	25.84	86.13	3.37	26.31	87.69	1.70
	60	56.06	93.44	2.25	55.54	92.57	1.73	53.83	89.71	1.85	54.33	90.55	2.84
	90	75.63	84.03	1.15	70.99	78.88	1.03	73.74	81.93	1.23	86.92	96.58-	3.75
DAP	30	23.23	77.42	1.85	22.28	74.25	3.38	22.28	74.27	2.14	29.50	98.32	4.35
	60	46.36	77.27	3.60	45.89	76.48	4.98	44.71	74.52	7.50	53.71	89.51	3.71
	90	73.20	81.33	4.70	76.46	84.96	3.22	73.90	82.11	5.72	77.26	85.84	2.98
DCHP	30	26.93	89.76	2.06	24.26	80.86	3.19	27.03	90.10	3.54	30.90	103.01	5.23
	60	46.74	77.90	3.03	58.60	97.67	0.97	43.42	72.36	2.24	56.91	94.85	5.00
	90	79.16	87.95	1.83	83.11	92.34	2.44	79.14	87.93	3.77	83.19	92.43	4.67
DHP	30	23.36	77.88	1.47	25.67	85.57	3.91	28.79	95.96	1.42	29.78	99.28	4.67
	60	49.46	82.44	5.02	49.21	82.01	2.90	53.06	88.43	6.50	56.63	94.38	3.38
	90	81.12	90.13	4.66	79.06	87.85	1.90	85.38	94.87	4.43	87.11	96.79	4.39
DEHP	30	24.47	81.56	3.17	25.41	84.69	2.99	24.64	82.12	2.67	27.95	93.61	2.68
	60	45.40	75.67	4.00	42.85	71.42	4.51	25.31	84.37	4.47	26.61	88.69	3.97
	90	71.91	79.90	3.88	77.91	86.57	4.90	81.76	90.84	1.07	84.33	93.70	0.97
DNOP	30	22.89	76.30	2.62	24.83	82.78	4.11	23.08	76.92	3.34	29.82	99.40	2.67
	60	45.77	76.29	4.85	42.76	71.27	6.91	51.92	86.53	4.86	54.50	90.84	2.58
	90	74.89	83.21	1.95	67.10	74.55	5.38	76.35	84.83	5.10	89.01	98.90	1.59

general, excellent recovery percentages were found for the analyzed components, which decreased the chances of losses during extraction and analysis and guaranteed the reliability of the optimized method (40).

Limits of detection and quantification

The LOD was defined as the concentration that produced a signal-to-noise ratio of 3:1 and the LOQ was determined as the concentration that produced a signal-to-noise ratio of 10:1.

The signal was enhanced with an enrichment factor (EF) of 10 by using the SPE procedure; the LODs and LOQs of the proposed method are shown in Table I. The LODs of DMP, DEP, DPP, BBP, DiBP, DAP, DCHP, DHP, DEHP and DNOP were1.8, 2.5, 4.1, 4.3, 2.4, 5.7, 5.8, 6.3, 6.8 and 7.5 ng/mL, respectively; the LOQs of DMP, DEP, DPP, BBP, DiBP, DAP, DCHP, DHP, DEHP and DNOP were 6.0, 8.4, 13.7, 14.5, 8.1, 19.0, 19.4, 21.3, 22.9 and 23.8 ng/mL, respectively. The LOD and LOQ values were low, suggesting that the standard method allowed the detection of small quantities of these PAEs (40).

Application of the method

To demonstrate the applicability of the proposed method, it was used for the determination of the migration of these 10 PAEs from 10 different kinds of paper packaging materials. Two were detected in Simulant D, these migrations were 320 and 112 ng/mL.

Conclusions

A new SPE-HPLC–DAD method was developed for the separation and quantification of DMP, DEP, DPP, BBP, DiBP, DcHP, DAP, DHP, DNOP and DEHP released from food paper packaging materials; the method has been proved to be feasible. The method may be used to forecast the migration of these 10 phthalic acid esters from food paper packaging materials to food.

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