



# Evaluation of Phthalic Acid Esters in Fish Samples Using Gas Chromatography Tandem Mass Spectrometry with Simplified QuEChERS Technique

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## Abstract

Phthalic acid esters (PAEs) have become an important food safety concern due to their lipophilic properties and propensity to accumulate in adipose tissue in edible fish. In this study, a simple, sensitive, and accurate analytical method was successfully established for simultaneous determination of 19 PAEs in fish samples using gas chromatography coupled with tandem mass spectrometry (GC–MS/MS). A simplified Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) procedure was applied for sample preparation, and the Plackett-Burman factorial design was utilized for optimizing extraction parameters. The calibration curves were linear in the range 0.01–0.5 mg/kg for all of the analyzed PAEs, and the limits of quantification (LOQs) were 0.05–20 µg/kg which are much lower than those in previous reports. The average spiked recoveries ranged from 71.2 to 116.3%, with relative standard deviations (RSDs) of 3.9 to 16.2% ( $n = 6$ ). Finally, the method was applied to analyze 60 real fish samples taken from Shanghai Municipality, China, and the diisobutyl phthalate (DIBP), dibutyl phthalate (DBP), and bis(2-ethoxyethyl)phthalate (DEHP) were found in almost all fish samples tested in this study. The present study demonstrated that the established method was suitable for market surveillance of 19 PAE residues in fish samples.

**Keywords** Phthalic acid esters · Fish samples · Simultaneous determination · Simplified QuEChERS technique

## Introduction

Phthalic acid esters (PAEs) are a group of compounds produced in large volume that have been prevalently used as important additives in industrial processes and consumer products. PAEs can leach, migrate, or evaporate into various environmental systems through industrial effluent, rainfall, and overland runoff (Xu et al. 2008). Accordingly, a large amount of PAEs are ubiquitous and are increasingly detected in environmental samples including soils (Niu et al. 2014; Sun et al. 2016), aquatic systems (Liu et al. 2008; Mackintosh et al. 2006; Sun et al. 2013), and organisms (Ros et al. 2016). Due to their lipophilic properties and propensity to accumulate in adipose tissue, PAEs

can be accumulated via diffusion across the gills and skin in edible fish and then enter into the food chain.

Humans are exposed to all types of PAEs through food intake over their lifetime, especially for Asian citizens who consume large quantities of fish in their daily diet (Cheung et al. 2008). Several studies based on the contribution of different food types to phthalate exposure have indicated that food is by far the major source of phthalate exposure for the general population (Colacino et al. 2010; Rozati et al. 2002). Some phthalates are reproductive and developmental toxicants to laboratory animals and wild-life (Carbone et al. 2013), and a great deal of concern has been raised regarding their potential endocrine-disrupting effects even at very low concentrations (Mills and Chichester 2005). What is more, PAEs have been indicated to promote the progression of various types of cancers (Soto and Sonnenschein 2010). Therefore, a simple, sensitive, and accurate method for detecting PAEs in fish samples is critically needed to ensure food safety.

Analytical methods reported in previous papers for the determination of PAEs mainly consist of high-performance liquid chromatography-variable wavelength detection (HPLC-

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VWD) (Zhang et al. 2011), liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Garrido Frenich et al. 2009), and gas chromatography-mass spectrometry (GC-MS) (Bradley et al. 2013; Zhu et al. 2014). Method of wavelength detection has been increasingly substituted with MS-based detectors due to its limited identification ability. MS allows low detection limits even if the extraction process could be influenced by matrix effects caused by co-extracts. MS/MS was better at increasing the sensitivity by drastically reducing the background noise without losing its selectivity in PAE identification (Sun et al. 2012). Due to the relatively low polarity and the thermal stability of PAEs, GC combined with MS or MS/MS has become the main tool for the determination of PAEs in food samples. The final identification and quantification of PAEs in this study were performed using GC-MS/MS.

In previous reports, the extraction steps in the sample preparation procedure prior to chromatographic separation included dispersive liquid-liquid extraction (LLE) (Fan et al. 2014; Yan et al. 2011; Zhu et al. 2014), Soxhlet extraction (Mortazavi et al. 2013), stir bar sorptive extraction (SBSE), or solid phase extraction (Carrillo et al. 2008; Moreira et al. 2015). Clean-up steps include gel permeation chromatography (GPC) (Niu et al. 2011) and solid phase extraction (SPE) (Sun et al. 2012). However, the methods mentioned above are complicated and time-consuming and require additional equipment. Similar to detecting other environmental pollutants, a simple preparation procedure for the analysis of PAEs in foods is always the priority. The QuEChERS (quick, easy, cheap, effective, rugged, safe) sample preparation method employs dispersive solid phase extraction (d-SPE). The method was originally developed to improve the analytical quality and laboratory efficiency of conventional methods for extraction of pesticide residues in fruits and vegetables. It is now widely used in multi-matrices including sediments (Czech et al. 2016), tea (Liu et al. 2016), liver (Molina-Ruiz et al. 2014), and fish (Chatterjee et al. 2016; Munaretto et al. 2013). However, the QuEChERS-related application on analysis of PAEs in fish samples is very limited (Bradley et al. 2013).

The purpose of this study was to develop a simple, rapid, and reliable method for the analysis of 19 PAEs in fish samples that combines the advantage of the QuEChERS sample preparation method and the simplicity of ultrasonic-associated extraction based on GC-MS/MS. The extraction and clean-up procedures are the core content of the QuEChERS method, which is more credible when optimized using the experimental design method. The previous reported method has been rarely combined with the experimental design method to optimize and determine a sample preparation procedure. The Plackett-Burman factorial design method was introduced in this study to obtain the optimal parameters.

## Materials and Methods

### Reagents and Chemicals

A standard stock solution containing 1000 mg/L dimethyl phthalate (DMP), diethyl phthalate (DEP), diisopropyl phthalate (DIPrP), diallyl phthalate (DAP), dipropyl phthalate (DPrP), diisobutyl phthalate (DIBP), dibutyl phthalate (DBP), bis(2-methoxyethyl)phthalate (DMEP), bis(4-methyl-2-pentyl) phthalate (BMPP), bis(2-ethoxyethyl)phthalate (DEEP), dipentyl phthalate (DPP), dihexyl phthalate (DHXP), benzyl butyl phthalate (BBP), bis(2-butoxyethyl) phthalate (DBEP), dicyclohexyl phthalate (DCHP), bis(2-ethoxyethyl)phthalate (DEHP), diphenyl phthalate (DPhP), di-*n*-octyl phthalate (DNOP), and dinonyl phthalate (DNP) dissolved in acetonitrile was purchased from Alta Scientific Co., Ltd. (Tianjin China). HPLC-grade acetonitrile, *n*-hexane, and dichloromethane were purchased from Fisher Scientific (Waltham, USA). The d-SPE materials, such as sorbents composed of primary secondary amine (*N*-(*n*-propyl)ethylenediamine (PSA), 40–63  $\mu\text{m}$ ) and octadecylsilane (C18, 40–63  $\mu\text{m}$ ), were purchased from Agilent Technologies (California, USA). Anhydrous magnesium sulfate and sodium chloride (Analytical Reagent grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Ningbo, China).

### Preparation of Standard Solutions and Samples

A standard stock solution containing 19 PAEs was stored in amber-colored glass vials at  $-20\text{ }^{\circ}\text{C}$ . The working standard solutions for the calibration were diluted with acetonitrile to the final concentration of 10 to 500  $\mu\text{g/L}$ , respectively.

Fresh fish samples were collected from local markets in Shanghai. Approximately 5 g ( $\pm 0.01$ ) of a homogenized fish sample was weighed and transferred to a 50-mL glass centrifuge tube with a conical bottom, after which 5 mL of acetonitrile (1% acetic acid) as the extracting solvent was added. The sample was mixed by vortexing for 1 min to disperse the sample. After addition of 2 g NaCl, the tube was shaken for 1 min and bathed in ultrasonic (KQ-200DE CNC ultrasonic cleaner, Kunshan, China) water for 30 min ( $40\text{ }^{\circ}\text{C}$ , 60 W) and centrifuged at  $1788.8\times g$  for 5 min. Afterwards, 3 mL of supernatant was transferred to a 15 mL d-SPE purge tube containing 150 mg of PSA and 450 mg of anhydrous  $\text{MgSO}_4$ . The tube was vortexed for 1 min, followed by centrifugation at  $1788.8\times g$  for 5 min. Subsequently, 1 mL of the supernatant was filtered through a 0.22- $\mu\text{m}$  membrane filter and was placed in vials for GC-MS/MS analysis.

A Plackett-Burman factorial design experiment for 12 runs was designed to find the significant parameters affecting extraction efficiency. Parameters such as extraction solvent

volume (*V*), weight of salt (*m*), ultrasonic time (*t*), ultrasonic temperature (*T*), and ultrasonic intensity (*E*) were investigated based on variance analysis, and one dummy factor (*D*) was introduced to analyze systematic error or unknown variables affecting extraction efficiency (Morado Pineiro et al. 2013). The chosen low-level (− 1) values and high-level (1) values of extraction solvent volume (*V*), salt weight (*m*), ultrasonic time (*t*), ultrasonic temperature (*T*), and ultrasonic intensity (*E*) were (5, 10 mL), (1, 2 g), (10, 30 min), (20, 40 °C), and (60, 80 W), respectively. The PB design experimental of 19 PAEs is shown in Table 1.

### GC-MS/MS Analysis

The analyses of 19 PAEs were performed with a GC attached to a triple-quadrupole mass spectrometer (GC 7010, MS 7890B, Agilent Technologies, USA) using an HP-5MS (30 m × 0.25 mm, 0.25 μm, Agilent Technologies, USA) capillary column. Samples (1 μL) were injected in the splitless mode. The GC temperature program was as follows: The primary oven temperature was set 60 °C for 1 min, ramped to 220 °C at 20 °C/min, followed by an increase to 280 °C at 5 °C/min, and held at this temperature for 4 min. The carrier gas was helium (the purity is no less than 99.999%), and the flow rate was set constantly at 1.0 mL/min. The injector temperature and ion source temperature were optimized at 285 and 320 °C, respectively. Mass spectrometry was performed in electron impact (EI) mode, with an electron energy 70 eV and a solvent delay time of 5 min. The collision gas was nitrogen (the purity is no less than 99.999%).

The central composite design method was performed to investigate the injector temperature and ion source temperature, to obtain optimal response and sensitivity. The low level, central point, and high level of the variable were set as follows: injector temperature (250, 275, 300 °C) and ion source temperature (265, 295, 325 °C). Three levels of each variable required 11 tests following three duplicate tests in the center. After the designed experiment was performed, regression analysis was performed using SAS software to evaluate the peak areas of the 19 PAEs.

Preliminary experiments were performed using standard solutions, and the parent ions of 19 PAEs were identified by screening mass spectra from 45 to 500 in full-scan mode. The variables of collision energy and MS transitions were optimized by combining the product ion scan chromatograms of the matrix blank and standard solutions. The characteristic ion pairs for the 19 PAEs were confirmed under MRM in positive ion mode. The optimized parameters for the 19 PAEs are summarized (Table 2). Quantitation of PAEs was performed in multiple reaction monitoring (MRM) mode using a matrix-matched standard method.

**Table 1** The Plackett-Burman design experimental and the recoveries of 19 PAEs

No.	Factor	Recoveries (%)																							
		<i>V</i>	<i>m</i>	<i>t</i>	<i>T</i>	<i>E</i>	<i>D</i>	DMP	DEP	DIPtP	DAP	DPrP	DIBP	DBP	DMEP	BMPP	DEEP	DPP	DHXP	BBP	DBEP	DCHP	DEHP	DPhP	DNOP
1	1	1	1	1	1	1	63.54	59.87	79.28	86.51	72.64	65.58	64.38	67.57	66.08	67.45	58.35	65.39	64.16	61.3	63.45	55.8	57.22	67.74	69.39
2	1	−1	−1	−1	1	1	61.41	53.98	77.06	80.71	72.33	69.26	62.79	63.81	70.38	58.77	61.45	71.62	67.39	67.63	67.31	57.61	56.1	69.5	71.6
3	−1	1	1	−1	1	1	75.9	71.25	91.04	95.14	74.43	62.07	53.44	66.15	81.88	58.58	63.23	67.88	76.52	72	74.43	37	64.43	76.4	77.26
4	1	1	−1	−1	−1	1	43.69	40	55.91	61.52	56.61	39.27	35.73	53.72	54.29	40.64	45.7	58.61	54.61	56.21	55.77	36.52	41.32	56.29	58.64
5	1	−1	1	1	−1	1	57.67	63.03	67.86	69.82	63.06	41.42	32.54	54.65	60.28	47.18	44.1	46.84	50.04	46.4	45.68	36.95	37.52	47.43	42.65
6	1	1	1	−1	−1	1	54.49	46.68	58.68	58.68	61.58	42.66	38.3	45.98	59.16	45.01	48.58	56.88	55.88	54.97	54.05	26.95	47.62	56.3	57.38
7	−1	1	−1	1	1	1	106.2	101.34	111.07	99.67	104.35	94.21	83.71	96.16	118.01	89.64	94.35	93.67	92.9	94.03	97.2	52.87	76.12	92.04	90.03
8	−1	1	1	1	−1	1	90.42	104.08	97.86	102.19	99.75	85.1	80.38	100.03	110.81	87.64	102.46	98.76	101.2	99.64	103.58	61.64	88.15	103.55	101.44
9	−1	−1	1	−1	1	1	85.94	92.95	90.06	84.48	90.07	75.32	74.45	73.78	101.13	73.4	85.42	85.69	85.1	77.34	87.08	55.46	66.26	83.71	81.66
10	1	−1	1	1	1	1	64.09	60.97	67.12	69.45	70.72	61.54	62.09	61.94	78.79	63.82	67.66	71.98	67.5	67.52	69.68	46.76	62.53	70.09	69.32
11	−1	−1	−1	1	−1	1	90.31	90.86	94.51	85.63	88.71	66.17	64.14	76.26	92.61	78.29	81.17	77.41	79.5	73.96	79.73	37.11	70.39	75.41	73.59
12	−1	−1	−1	−1	−1	1	86.5	88.73	88.24	78.34	81.77	66.04	69.06	75.66	81.6	61.54	79.5	81.66	84.16	75.67	83.29	52.36	64.59	80.29	77.78

*V* solvent volume, *m* salt weight, *t* ultrasonic time, *T* ultrasonic temperature, *E* ultrasonic intensity, *D* one dummy factor

**Table 2** MRM parameters for analysis of 19 PAEs

Number	PAEs	Retention time (min)	Qualitative ions (m/z)	CE (eV)	Quantitative ions (m/z)	CE (eV)
1	DMP	7.683	163 > 77	20	163 > 135	20
2	DEP	8.544	149 > 65	20	149 > 93	20
3	DIPrP	8.918	149 > 65	28	167 > 149	20
4	DAP	9.444	149 > 65	20	149 > 93	20
5	DPrP	9.613	149 > 65	28	149 > 93	20
6	DIBP	10.248	149 > 65	28	149 > 93	20
7	DBP	10.973	149 > 65	28	149 > 93	20
8	DMEP	11.296	149 > 93	20	104 > 76	20
9	BMPP	12.031	149 > 65	28	167 > 149	20
10	DEEP	12.342	149 > 65	20	149 > 93	20
11	DPP	12.704	149 > 93	28	149 > 121	20
12	DHXP	14.802	149 > 65	28	149 > 93	20
13	BBP	14.943	149 > 65	28	91 > 65	20
14	DBEP	16.378	149 > 65	28	149 > 93	20
15	DCHP	17.027	149 > 65	28	149 > 93	20
16	DEHP	17.275	149 > 65	28	167 > 149	20
17	DPhP	17.388	225 > 77	20	225 > 141	20
18	DNOP	18.643	149 > 65	28	149 > 93	20
19	DNP	19.643	149 > 65	28	149 > 93	20

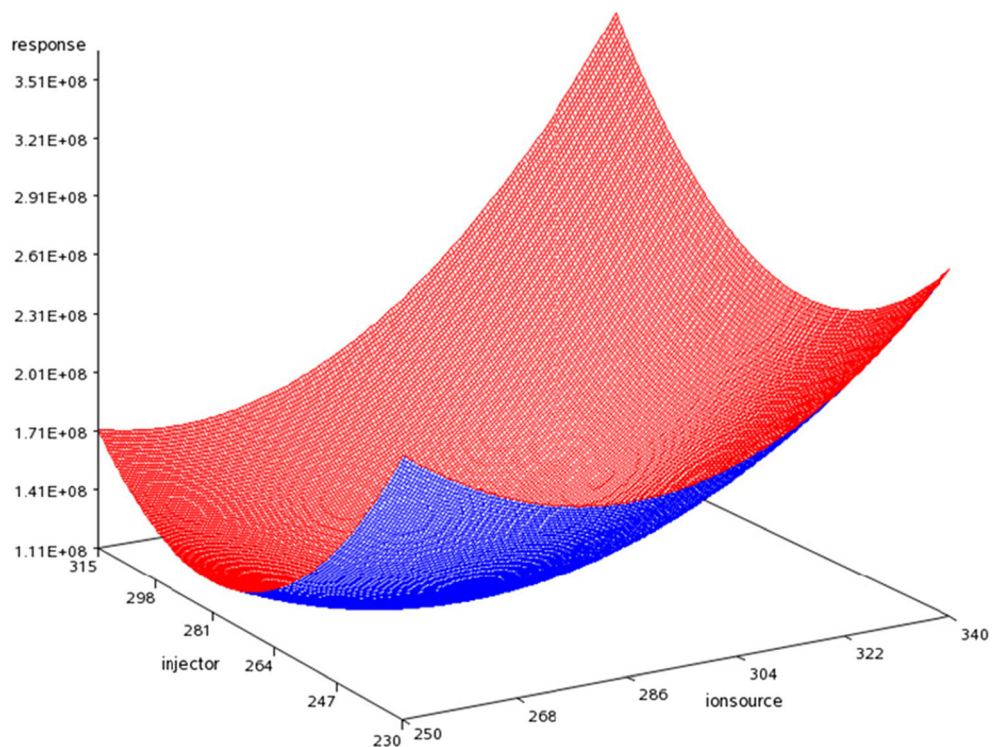
## Results and Discussion

### Optimization of the GC–MS/MS Temperature

GC–MS/MS temperature plays an important role in PAE analysis (Zhou et al. 2016), and a suitable temperature can remove the extraction solvent more quickly without loss of the target chemicals. Response surface methodology models for 19

PAEs were generated in relation to the injector temperature and ion source temperature, to obtain optimal response and sensitivity (Fig. 1). The injector temperature and ion source temperature affected the peak area significantly. As temperature increased from a central point to a high level, the peak area exhibited a rapid increase. The best results were obtained at an injector temperature of 285 °C, an ion source temperature of 320 °C, respectively.

**Fig. 1** Response surface of 19 PAEs at various temperatures of GC-MS/MS

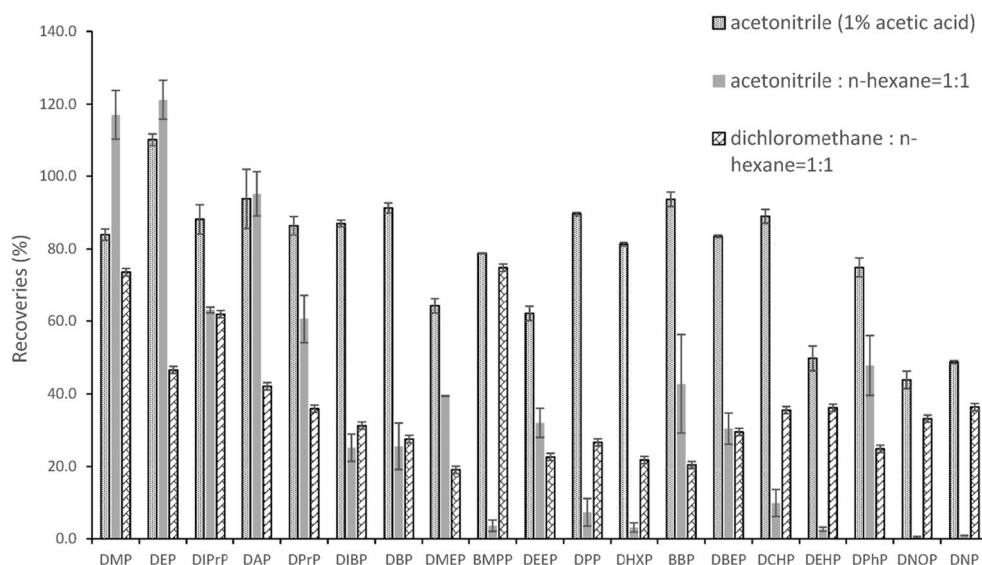


## Optimization of the Extraction Step

The correct choice of solvent is essential for obtaining optimal extraction effects. The solubility of the compound in the solvent and the intensity of the solute/matrix interactions are the two main factors affecting the extraction effects of analyte. The single factor experiment was done to find the optimal extraction solvent. Based on previous study results (Liu et al. 2013; Yang et al. 2015), the extraction effects of three common extracting solvents (acetonitrile (1% acetic acid), acetonitrile: *n*-hexane = 1:1, dichloromethane: *n*-hexane = 1:1) were investigated for the recoveries of 19 PAEs in a blank Sole fish sample spiked with 100 µg/kg for three parallel experiments under the same sample preparation. As shown in Fig. 2, the mean percent recoveries obtained with acetonitrile (1% acetic acid) were significantly better than with other extracting solvents. What is more, the background value of acetonitrile (1% acetic acid) was significantly lower than for other solvents. Hence, acetonitrile (1% acetic acid) was chosen as the extraction solvent.

A Plackett-Burman factorial design experiment for 12 runs was performed to find the significant parameters affecting extraction efficiency. Parameters related to the recoveries are studied with the variance analysis method, and the study shows that the significant parameter ( $P < 0.05$ ) affecting the extraction efficiency of 19 PAEs from fish samples was extraction solvent volume, while other parameters had no significant correlation with extraction efficiencies. The dummy factor was not significant, which indicated that no system error or unknown parameters (Ma et al. 2016) affected the experimental results. The non-significant variables of salt weight, ultrasonic time, ultrasonic temperature, and ultrasonic intensity were set as 2 g, 30 min, 40 °C, and 60 W by integration analysis of recoveries of 19 PAEs.

**Fig. 2** Effect of different extraction solvents on the extraction efficiency of 19 PAEs



After that, the single factor experiment was performed to find the optimal extraction volume. As shown in the Plackett-Burman factorial design experiment, a low volume of extraction solvent yielded better recoveries. However, due to volume loss during the extraction and clean up, at least 5 mL of acetonitrile (1% acetic acid) was needed to guarantee the following GC-MS/MS analysis. Extraction solvent volumes were set to 5, 6, 7, 8, and 9 mL to give the best recoveries (as shown in Table 3). The best recoveries ranged from 77 to 106% and were obtained when 5 mL of acetonitrile (1% acetic acid) was added. Recoveries of five types of PAEs listed in the Blacklist of the US Environmental Protection Agency (EPA) are shown in Fig. 3.

## Optimization of Clean-up Steps

Because crude extracts of fish samples contain a large amount of matrix co-extracts, effective removal is crucial for the accurate analysis of trace PAEs. For clean-up optimization, the control blank fish sample (5 g) was extracted with acetonitrile (1% acetic acid), and the extract was spiked at 100 µg/kg level of the target mixture. Clean-up was performed using different combinations of PSA, C18, PSA + C18, and Neutral Al<sub>2</sub>O<sub>3</sub> with 450 mg MgSO<sub>4</sub>, and recoveries of the 19 PAEs in two replicates from different treatments were compared to confirm the optimized sorbents. The clean-up achieved using PSA followed by 450 mg MgSO<sub>4</sub> showed the best recoveries (as shown in Fig. 4). PSA, which has both primary and secondary amines, could effectively remove fatty acid and nonpolar lipid co-extractives (Molina-Ruiz et al. 2014). This occurs because PSA forms hydrogen bonds with compounds containing hydroxyl or carboxy group (Anastassiades et al. 2003). PSA + MgSO<sub>4</sub> was chosen for the clean-up procedure.

**Table 3** Recoveries of 19 PAEs for different extraction solvent volume

V (ml)	Recoveries (%)	DMP	DEP	DIPrP	DAP	DPrP	DIBP	DBP	DMEP	BMPP	DEEP	DPP	DHXP	BBP	DBEP	DCHP	DEHP	DPhP	DNOP	DNP
5	89.43	103.51	106.27	106.13	104.92	87.36	82.78	76.93	103.11	89.14	100.45	104.03	105.9	97.58	106.07	93.59	91.11	96.29	87.6	
6	31.25	74.96	92.55	113.44	112.11	106.79	101.54	105.31	132.16	77.39	127.15	124.6	125.86	115.73	124.4	84.51	109.94	129.07	131.08	
7	64.13	99.66	107.35	110.56	111.15	98.48	88.37	91.25	117.56	69.74	110.45	115.64	115.88	103.04	115.56	87.52	96.77	119.84	122.26	
8	40.57	74.65	86.17	94.22	96.38	86.83	84.05	86.2	106.11	55.33	105.06	106.53	106.11	94.29	105.74	81.2	92.08	104.29	107.78	
9	73.89	62.7	70.03	78.39	79.25	68.46	71.67	73.08	92.26	39.23	91.12	92.39	90.99	87.16	93.1	72.08	81.74	98.5	96.27	

The amounts of sorbents used were carefully validated. A comparison of percentage recoveries of 19 PAEs with 25, 50, 75, and 100 mg/mL of PSA is presented in Fig. 5. Fifty milligrams per milliliter of PSA displayed better clean-up efficiency, with recoveries of PAEs ranging from 61 to 115%, which were higher than the other tested levels. Finally, a modified QuEChERS preparation method for the simultaneous determination of 19 PAEs was established.

### Calibration and Method Validation

A six-point calibration curve using a matrix-matched standard solution was obtained through weighted linear regression ( $1/x$ ) and showed good linearity over the selected concentration range (10, 20, 50, 100, 200, 500  $\mu\text{g/L}$ ), with determination coefficients ( $r^2$ ) exceeding 0.99 for 19 PAEs. Matrix effects due to the co-elution of matrix components can suppress or enhance analyte signals, which would lead to false quantitative results. In this study, to evaluate the matrix effects, the slopes obtained in the matrix-matched calibration were compared to those obtained in the standard solution calibration, and the slope ratios of the matrix/solvent were calculated for each of the PAEs. The MEs were calculated with the following equation:

$$MEs(\%) = (K_{matrix}/K_{solvent} - 1) \times 100\%$$

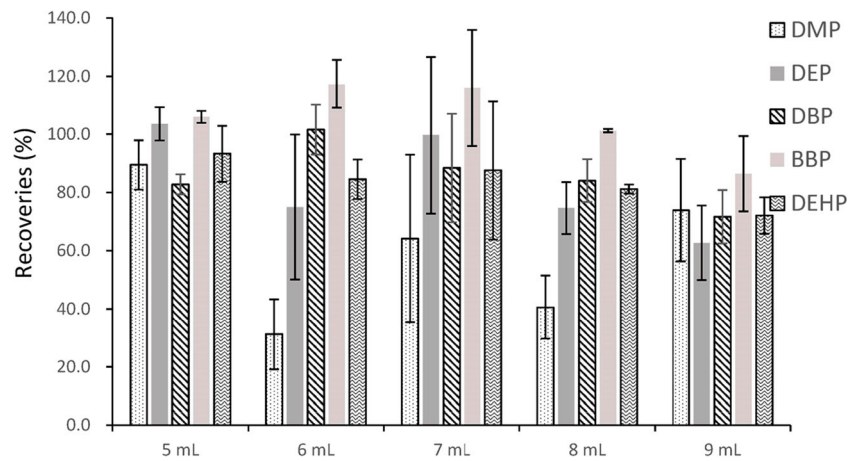
where  $K$  corresponds to the slope of the calibration curve. The preparation steps were the same as mentioned above.

Compared to standard analytes in the solvent, signal enhancements were observed in most of the analytes, especially for DBEP. The results showed that the matrix effects led to moderate signal enhancement for 6 of the 19 test analytes (ME > 10 to 50%), whereas for 13 analytes, the signal enhancement was high (ME > 50%) (Chatterjee et al. 2016). To minimize the mistakes caused by matrix effects, the quantitation of 19 PAEs was performed using matrix-matched analytical solutions.

Recoveries were evaluated by spiking the fish samples at three concentration levels of 20, 50, and 200  $\mu\text{g/kg}$ , with six replicates. The mean recoveries of 19 PAEs ranged from 71 to 116%, and the relative standard deviations (RSD) ranged from 4 to 16%, as presented in Table 4. The LODs and LOQs of the 19 PAEs ranged from 0.01 to 10 and 0.05 to 20  $\mu\text{g/kg}$ , respectively, and the LODs were determined as three times the signal-to-noise ratio, while the LOQs were determined as ten times the signal-to-noise ratio.

Xu et al. (2014) got LOQs of 23 PAEs at the range of 10–100  $\mu\text{g/kg}$ , whereas the values that ranged from 0.05 to 20  $\mu\text{g/kg}$  were obtained in this study. Most LOQs are much lower than those in previous reports (Bradley et al. 2013; Du et al. 2016; Liu et al. 2013). Thus, based on the above mentioned

**Fig. 3** Effect of extraction solvent volume on the extraction efficiency of five PAEs



experiments, the established method is suitable for the measurement of 19 PAEs in fish samples.

Li Luo et al. (2017) developed a sample preparation procedure for 4-*n*-octylphenol, 4-*n*-nonylphenol, and bisphenol A based on the Plackett-Burman factorial design method. However, the previous reported method has been rarely combined with the experimental design method to optimize and establish PAE determination method for fish samples.

### Precautions for Minimizing Background Contamination

To eliminate cross-contamination and background interference of those PAEs that are ubiquitously present, special precautions were taken before sample preparation procedure: Screening all solvents for 19 PAEs prior to analysis and using the “cleanest” ones throughout the study, all glassware was soaked in acetonitrile for 2 h and dried at 200 °C for 2 h. After cooling, the glassware was wrapped in aluminum foil to prevent the adsorption of PAEs from the air prior to use. MgSO<sub>4</sub>

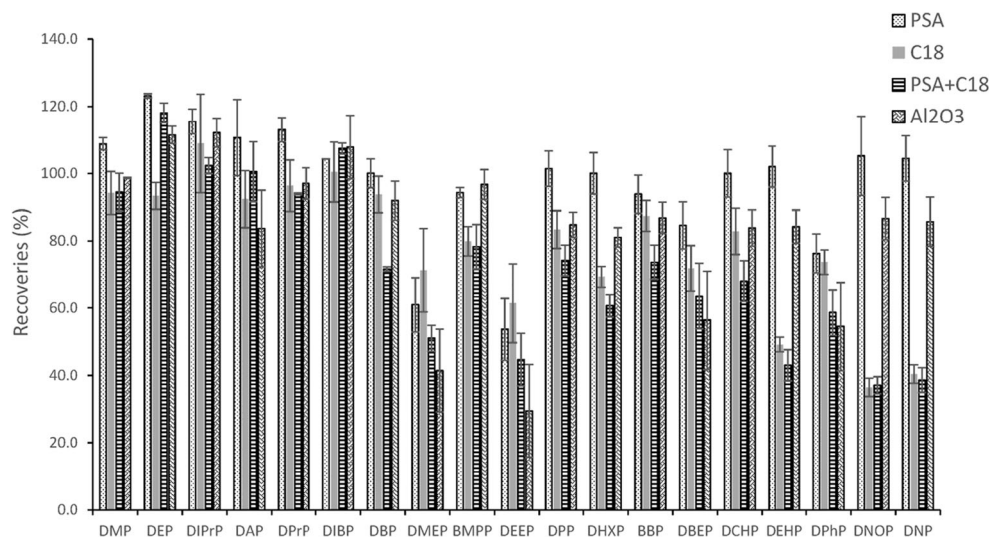
and NaCl were both heated at 650 °C for 4 h in a muffle furnace and stored in sealed glass vials after cooling. Plastic materials were removed from the laboratory as much as possible.

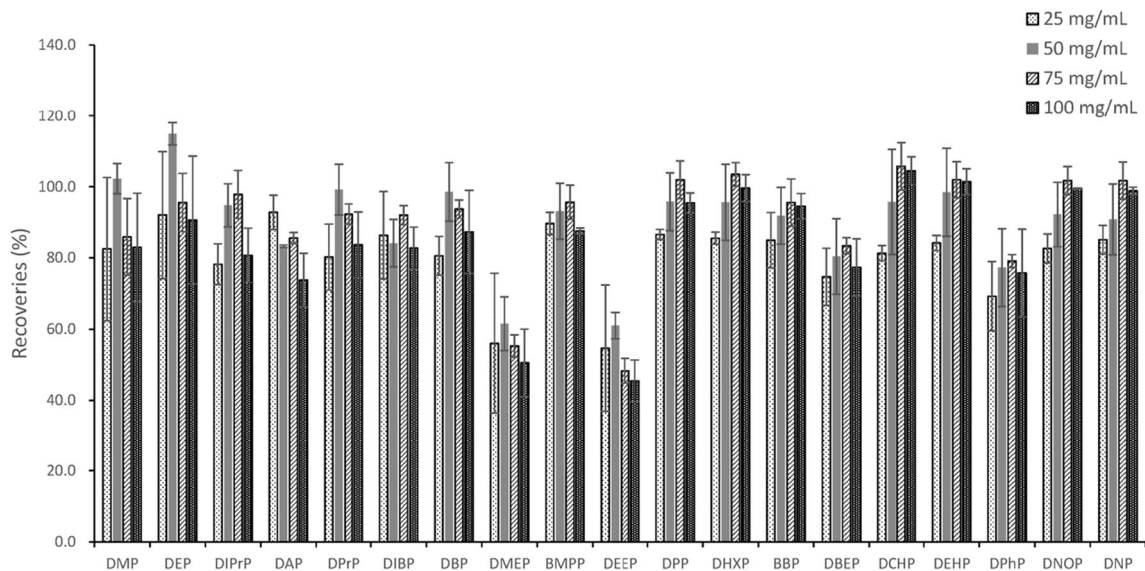
Additionally, contamination was initially quantified by performing blank injections. The results showed that varied levels of DMP, DEP, DIBP, DBP, and DEHP were detected. DBP and DEHP were the main contaminations, which were detected at 6.71 and 13.42 µg/kg, and the values were lower than those reported in earlier study (Guo and Kannan 2012; Zhou et al. 2016). Thus, it is important to take necessary measures to minimize the contamination from background.

### Application of the Developed Method to Fish Samples

The developed method was applied to the simultaneous determination of 19 PAEs in 60 random fish samples obtained from local markets in Shanghai Municipality, the levels of 19 PAEs are presented in Fig. 6. Analyses were performed in duplicate, and average levels in the blanks were subtracted. Samples

**Fig. 4** Recoveries of four selected sorbents (PSA, C18, PSA + C18, and neutral Al<sub>2</sub>O<sub>3</sub>) for 19 PAEs in fish samples





**Fig. 5** Effect of the amount of PSA on the extraction efficiency of 19 PAEs from fish samples

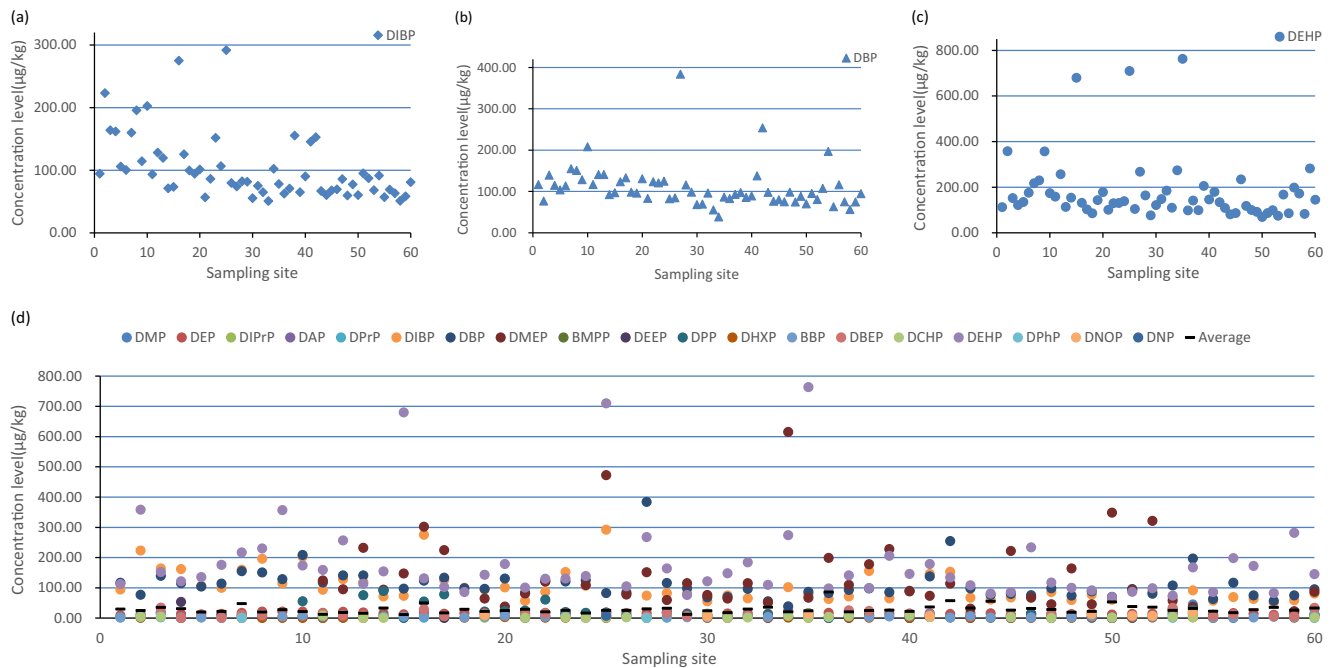
were mainly saltwater fish and were types consumed by most local residents. Detection frequencies of 19 PAEs in these fish samples ranged from 0 to 100%, with concentration ranges of not detected (n.d.) to 763.22  $\mu\text{g}/\text{kg}$ . From the PAE figure (Fig. 6d), the mean concentrations of 19 PAEs in fish samples mostly ranged from 11.06 to 85.57  $\mu\text{g}/\text{kg}$ , similar to concentrations reported in a previous study (Gu et al. 2014).

DEHP, DBP, and DIBP showed the most frequently detected and highest concentrations compared to other PAEs. DEHP concentrations ranged from 65.89 to 763.22  $\mu\text{g}/\text{kg}$ , which were well below the maximum residue limit (MRL) value of 1500  $\mu\text{g}/\text{kg}$  recommended by the National Health and Family Planning Commission of the People's Republic of China (NHFPC). DBP concentrations ranged from 38.47 to

**Table 4** Linear equations, correlation coefficients, linearity range, recoveries, RSD, LOD, LOQ, and matrix effect of 19 PAEs

Analyte	Linear regression equation	( $r^2$ )	Linearity range ( $\mu\text{g}/\text{L}$ )	Recoveries (%)	RSD (%)	LOD ( $\mu\text{g}/\text{kg}$ )	LOQ ( $\mu\text{g}/\text{kg}$ )	Matrix effects (%)
DMP	$Y = 1.75 \times 10^5 X + 1.97 \times 10^6$	0.9957	10–500	89.7–94.9	5.2–7.9	10	20	40
DEP	$Y = 2.10 \times 10^6 X + 2.87 \times 10^7$	0.9956	10–500	97.4–103.7	5.2–6.7	0.3	1	45
DIPrP	$Y = 2.36 \times 10^6 X + 3.51 \times 10^7$	0.9920	10–500	101.6–106.5	3.9–6.9	1	5	44
DAP	$Y = 9.83 \times 10^5 X + 1.77 \times 10^6$	0.9986	10–500	102.4–110.1	5.7–8.7	5	10	61
DPrP	$Y = 2.88 \times 10^6 X + 4.12 \times 10^7$	0.9964	10–500	100.9–110.1	5.0–6.6	0.5	2	40
DIBP	$Y = 2.77 \times 10^6 X + 8.61 \times 10^7$	0.9979	10–500	77.5–99.0	5.7–8.9	0.03	1	46
DBP	$Y = 3.45 \times 10^6 X + 1.62 \times 10^8$	0.9940	10–500	74.8–91.8	6.5–6.7	0.01	0.05	50
DMEP	$Y = 1.21 \times 10^5 X + 1.16 \times 10^5$	0.9970	10–500	81.5–100.3	10.4–16.0	7	20	63
BMPP	$Y = 1.03 \times 10^6 X + 1.71 \times 10^6$	0.9986	10–500	103.0–116.3	6.3–8.1	5	10	61
DEEP	$Y = 5.28 \times 10^5 X + 1.77 \times 10^6$	0.9972	10–500	79.7–100.2	6.8–14.4	10	20	66
DPP	$Y = 2.02 \times 10^6 X + 1.78 \times 10^7$	0.9965	10–500	101.1–115.2	6.2–7.1	0.5	1	64
DHXP	$Y = 4.79 \times 10^6 X + 7.80 \times 10^7$	0.9952	10–500	100.8–111.2	5.6–6.9	0.3	1	70
BBP	$Y = 2.24 \times 10^6 X + 1.08 \times 10^7$	0.9987	10–500	100.3–114.3	5.7–7.0	1	2	72
DBEP	$Y = 1.03 \times 10^6 X + 4.77 \times 10^6$	0.9986	10–500	87.1–103.4	6.0–13.2	5	10	80
DCHP	$Y = 3.89 \times 10^6 X + 3.31 \times 10^7$	0.9978	10–500	103.6–112.8	5.4–6.9	1	5	73
DEHP	$Y = 2.96 \times 10^6 X + 2.18 \times 10^8$	0.9957	10–500	71.2–88.7	7.3–15.9	0.01	0.05	71
DPhP	$Y = 3.82 \times 10^3 X + 3.96 \times 10^2$	0.9967	10–500	83.3–102.6	4.4–13.8	10	20	73
DNOP	$Y = 1.43 \times 10^6 X + 2.67 \times 10^6$	0.9979	10–500	99.1–106.2	5.5–7.0	5	10	79
DNP	$Y = 4.12 \times 10^6 X + 1.12 \times 10^7$	0.9978	10–500	100.0–107.0	5.7–7.4	3	5	76





**Fig. 6** Distribution of the concentration level of DIBP, DBP, DEHP, and 19 PAEs in 60 fish samples

383.85  $\mu\text{g}/\text{kg}$ , and one sample exceeded the maximum residue limit (MRL) value of 300  $\mu\text{g}/\text{kg}$  recommended by the NHFPC. The higher levels of DIBP, DBP, and DEHP were related to the widespread and persistent presence of plasticizer use in industry.

## Conclusions

PAEs are ubiquitous and unavoidably carry the risk of cross-contamination, which can result in overestimated levels. Therefore, special precautions were taken in the experimental procedure to eliminate background interference. The single factor experiment was used to determine the optimal extraction solvent, and then a two-level Plackett-Burman factorial design experiment for 12 runs was performed to find the significant parameters affecting extraction efficiency, followed by a single factor experiment to find the optimal extraction volume. Acetonitrile (1% acetic acid) as an extractive solvent gave better recoveries compared to acetonitrile-*n*-hexane (1:1) and dichloromethane-*n*-hexane (1:1). For clean-up steps, various types and amounts of sorbents were studied, and the optimal clean-up steps were confirmed. The developed clean-up procedures only use a small amount of sorbent (combination of PSA +  $\text{MgSO}_4$ ). Matrix effects were also considered to ensure accurate quantitation. Good separation and responses for 19 PAEs were achieved within 27 min in a single analysis.

The developed method was applied to determine 19 PAEs in 60 real fish samples taken from Shanghai Municipality, China. The reported outcomes enable their rapid, sensitive,

and effective determination in fish samples at trace levels. Generally, this study offers a simple, sensitive, reliable and practical method for the quantitation and monitoring of trace PAEs in complex matrices such as fish samples, requiring less sorbent for clean-up and less time for analysis.

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## Compliance with Ethical Standards

**Conflict of Interest** Yanyang Xu, Rui Weng, Yushun Lu, Xinlu Wang, Dan Zhang, Yun Li, Jing Qiu, and Yongzhong Qian declare that they have no conflict of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed Consent** Informed consent is not applicable in this study.

## References

- Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. *J AOAC Int* 86:412–431
- Bradley EL, Burden RA, Leon I, Mortimer DN, Speck DR, Castle L (2013) Determination of phthalate diesters in foods. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 30:722–734. <https://doi.org/10.1080/19440049.2013.781683>

- Carbone S, Ponzo OJ, Gobetto N, Samaniego YA, Reynoso R, Scacchi P, Moguilevsky JA, Cutrera R (2013) Antiandrogenic effect of perinatal exposure to the endocrine disruptor di-(2-ethylhexyl) phthalate increases anxiety-like behavior in male rats during sexual maturation. *Horm Behav* 63:692–699. <https://doi.org/10.1016/j.yhbeh.2013.01.006>
- Carrillo JD, Martinez MP, Tena MT (2008) Determination of phthalates in wine by headspace solid-phase microextraction followed by gas chromatography-mass spectrometry. Use of deuterated phthalates as internal standards. *J Chromatogr A* 1181:125–130. <https://doi.org/10.1016/j.chroma.2007.12.053>
- Chatterjee NS, Utture S, Banerjee K, Ahammed Shabeer TP, Kamble N, Mathew S, Ashok Kumar K (2016) Multiresidue analysis of multiclass pesticides and polyaromatic hydrocarbons in fatty fish by gas chromatography tandem mass spectrometry and evaluation of matrix effect. *Food Chem* 196:1–8. <https://doi.org/10.1016/j.foodchem.2015.09.014>
- Cheung KC, Zheng JS, Leung HM, Wong MH (2008) Exposure to polybrominated diphenyl ethers associated with consumption of marine and freshwater fish in Hong Kong. *Chemosphere* 70:1707–1720. <https://doi.org/10.1016/j.chemosphere.2007.07.043>
- Colacino JA, Harris TR, Schecter A (2010) Dietary intake is associated with phthalate body burden in a nationally representative sample. *Environ Health Perspect* 118:998–1003. <https://doi.org/10.1289/ehp.0901712>
- Czech T, Bonilla NB, Gambus F, González RR, Marínsáez J, Vidal JL, French AG (2016) Fast analysis of 4-tert-octylphenol, pentachlorophenol and 4-nonylphenol in river sediments by QuEChERS extraction procedure combined with GC-QqQ-MS/MS. *Sci Total Environ* 557–558:681–687
- Du L, Ma L, Qiao Y, Lu Y, Xiao D (2016) Determination of phthalate esters in teas and tea infusions by gas chromatography-mass spectrometry. *Food Chem* 197(Pt B):1200–1206. <https://doi.org/10.1016/j.foodchem.2015.11.082>
- Fan Y, Liu S, Xie Q (2014) Rapid determination of phthalate esters in alcoholic beverages by conventional ionic liquid dispersive liquid-liquid microextraction coupled with high performance liquid chromatography. *Talanta* 119:291–298. <https://doi.org/10.1016/j.talanta.2013.11.023>
- Garrido French A, de las Nieves Barco Bonilla M, Lopez Martinez JC, Martinez Vidal JL, Romero-Gonzalez R (2009) Determination of di-(2-ethylhexyl)phthalate in environmental samples by liquid chromatography coupled with mass spectrometry. *J Sep Sci* 32:1383–1389. <https://doi.org/10.1002/jssc.200900020>
- Gu YY, Yu XJ, Peng JF, Chen SB, Zhong YY, Yin DQ, Hu XL (2014) Simultaneous solid phase extraction coupled with liquid chromatography tandem mass spectrometry and gas chromatography tandem mass spectrometry for the highly sensitive determination of 15 endocrine disrupting chemicals in seafood. *J Chromatogr B Anal Technol Biomed Life Sci* 965:164–172. <https://doi.org/10.1016/j.jchromb.2014.06.024>
- Guo Y, Kannan K (2012) Challenges encountered in the analysis of phthalate esters in foodstuffs and other biological matrices. *Anal Bioanal Chem* 404:2539–2554. <https://doi.org/10.1007/s00216-012-5999-2>
- Liu P, Chen H, Gao G, Hao Z, Wang C, Ma G, Chai Y, Zhang L, Liu X (2016) Occurrence and residue pattern of phthalate esters in fresh tea leaves and during tea manufacturing and brewing. *J Agric Food Chem* 64:8909–8917. <https://doi.org/10.1021/acs.jafc.6b03864>
- Liu W, Chen J, Hu J, Ling X, Tao S (2008) Multi-residues of organic pollutants in surface sediments from littoral areas of the Yellow Sea, China. *Mar Pollut Bull* 56:1091–1103. <https://doi.org/10.1016/j.marpolbul.2008.03.021>
- Liu Y, Wang S, Wang L (2013) Development of rapid determination of 18 phthalate esters in edible vegetable oils by gas chromatography tandem mass spectrometry. *J Agric Food Chem* 61:1160–1164. <https://doi.org/10.1021/jf3053884>
- Luo L, Yang Y, Wang Q, Li H-p, Z-f L, Z-p Q, Z-g Y (2017) Determination of 4-n-octylphenol, 4-n-nonylphenol and bisphenol A in fish samples from lake and rivers within Hunan Province, China. *Microchem J* 132:100–106. <https://doi.org/10.1016/j.microc.2017.01.012>
- Ma L, Wang L, Tang J, Yang Z (2016) Optimization of arsenic extraction in rice samples by Plackett–Burman design and response surface methodology. *Food Chem* 204:283–288. <https://doi.org/10.1016/j.foodchem.2016.02.126>
- Mackintosh CE, Maldonado JA, Ikononou MG (2006) Sorption of phthalate esters and PCBs in a marine ecosystem. *Environ Sci Technol* 40:3481–3488
- Mills LJ, Chichester C (2005) Review of evidence: are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? *Sci Total Environ* 343:1–34. <https://doi.org/10.1016/j.scitotenv.2004.12.070>
- Molina-Ruiz JM, Cieslik E, Walkowska I (2014) Optimization of the QuEChERS method for determination of pesticide residues in chicken liver samples by gas chromatography-mass spectrometry. *Food Anal Methods* 8:898–906. <https://doi.org/10.1007/s12161-014-9966-8>
- Morado Pineiro A, Moreda-Pineiro J, Alonso-Rodriguez E, Lopez-Mahia P, Muniategui-Lorenzo S, Prada-Rodriguez D (2013) Arsenic species determination in human scalp hair by pressurized hot water extraction and high performance liquid chromatography-inductively coupled plasma-mass spectrometry. *Talanta* 105:422–428. <https://doi.org/10.1016/j.talanta.2012.10.070>
- Moreira MA, Andre LC, Cardeal Zde L (2015) Analysis of plasticiser migration to meat roasted in plastic bags by SPME-GC/MS. *Food Chem* 178:195–200. <https://doi.org/10.1016/j.foodchem.2015.01.078>
- Mortazavi S, Bakhtiari AR, Sari AE, Bahramifar N, Rahbarizadeh F (2013) Occurrence of endocrine disruption chemicals (bisphenol A, 4-nonylphenol, and Octylphenol) in muscle and liver of, Cyprinus Carpio common, from Anzali wetland, Iran. *Bull Environ Contam Toxicol* 90:578–584
- Munaretto JS, Ferronato G, Ribeiro LC, Martins ML, Adaime MB, Zanella R (2013) Development of a multiresidue method for the determination of endocrine disruptors in fish fillet using gas chromatography-triple quadrupole tandem mass spectrometry. *Talanta* 116:827–834. <https://doi.org/10.1016/j.talanta.2013.07.047>
- Niu L, Xu Y, Xu C, Yun L, Liu W (2014) Status of phthalate esters contamination in agricultural soils across China and associated health risks. *Environ Pollut* 195:16–23. <https://doi.org/10.1016/j.envpol.2014.08.014>
- Niu Y, Zhang J, Wu Y, Shao B (2011) Simultaneous determination of bisphenol A and alkylphenol in plant oil by gel permeation chromatography and isotopic dilution liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 1218:5248–5253
- Ros O, Vallejo A, Olivares M, Etxebarria N, Prieto A (2016) Determination of endocrine disrupting compounds in fish liver, brain, and muscle using focused ultrasound solid-liquid extraction and dispersive solid phase extraction as clean-up strategy. *Anal Bioanal Chem* 408:5689–5700. <https://doi.org/10.1007/s00216-016-9697-3>
- Rozati R, Reddy PP, Reddanna P (2002) Role of environmental estrogens in the deterioration of male factor fertility. *Fertil Steril* 78:1187–1194
- Soto AM, Sonnenschein C (2010) Environmental causes of cancer: endocrine disruptors as carcinogens. *Nat Rev Endocrinol* 6:363–370. <https://doi.org/10.1038/nrendo.2010.87>
- Sun H, Yang Y, Li H, Zhang J, Sun N (2012) Development of multiresidue analysis for twenty phthalate esters in edible vegetable oils by microwave-assisted extraction-gel permeation chromatography-solid

- phase extraction-gas chromatography-tandem mass spectrometry. *J Agric Food Chem* 60:5532–5539. <https://doi.org/10.1021/jf3009603>
- Sun J, Huang J, Zhang A, Liu W, Cheng W (2013) Occurrence of phthalate esters in sediments in Qiantang River, China and inference with urbanization and river flow regime. *J Hazard Mater* 248–249:142–149. <https://doi.org/10.1016/j.jhazmat.2012.12.057>
- Sun J, Pan L, Zhan Y, Lu H, Tsang DCW, Liu W, Wang X, Li X, Zhu L (2016) Contamination of phthalate esters, organochlorine pesticides and polybrominated diphenyl ethers in agricultural soils from the Yangtze River Delta of China. *Sci Total Environ* 544:670–676. <https://doi.org/10.1016/j.scitotenv.2015.12.012>
- Xu D, Deng X, Fang E, Zheng X, Zhou Y, Lin L, Chen L, Wu M, Huang Z (2014) Determination of 23 phthalic acid esters in food by liquid chromatography tandem mass spectrometry. *J Chromatogr A* 1324:49–56
- Xu G, Li F, Wang Q (2008) Occurrence and degradation characteristics of dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in typical agricultural soils of China. *Sci Total Environ* 393:333–340. <https://doi.org/10.1016/j.scitotenv.2008.01.001>
- Yan H, Cheng X, Liu B (2011) Simultaneous determination of six phthalate esters in bottled milks using ultrasound-assisted dispersive liquid-liquid microextraction coupled with gas chromatography. *J Chromatogr B Anal Technol Biomed Life Sci* 879:2507–2512. <https://doi.org/10.1016/j.jchromb.2011.07.001>
- Yang J, Li Y, Wang Y, Ruan J, Zhang J, Sun C (2015) Recent advances in analysis of phthalate esters in foods. *Trends Anal Chem* 72:10–26. <https://doi.org/10.1016/j.trac.2015.03.018>
- Zhang H, Chen X, Jiang X (2011) Determination of phthalate esters in water samples by ionic liquid cold-induced aggregation dispersive liquid-liquid microextraction coupled with high-performance liquid chromatography. *Anal Chim Acta* 689:137–142. <https://doi.org/10.1016/j.aca.2011.01.024>
- Zhou RZ, Jiang J, Mao T, Zhao YS, Lu Y (2016) Multiresidue analysis of environmental pollutants in edible vegetable oils by gas chromatography-tandem mass spectrometry. *Food Chem* 207:43–50. <https://doi.org/10.1016/j.foodchem.2016.03.071>
- Zhu H, Cui S, Wang W, Miao J, Feng J, Chen J (2014) Determination of phthalate esters in wine using dispersive liquid-liquid microextraction and gas chromatography. *Anal Lett* 47:1874–1887. <https://doi.org/10.1080/00032719.2014.891126>