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RESEARCH PAPER

Simultaneous determination of 24 personal care products in fish muscle and liver tissues using QuEChERS extraction coupled with ultra pressure liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometer analyses

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Abstract A sensitive and selective quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction combined with dispersive solid-phase extraction (d-SPE) cleanup method was developed to simultaneously extract a wide range of personal care products (16 biocides, 4 synthetic musks, and 4 benzotriazoles) in fish muscle and liver tissues. In order to get satisfactory recoveries, different extraction parameters were optimized, including extraction salts and d-SPE materials, extraction solvents and acetic acid contents in organic phase, and the ratios of solvent and water. Ultra pressure liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry were used to analyze the target compounds in the extracts. Among the 24 personal care products, the recoveries in the range of 70–120 % were obtained for 20, 19, and 12 analytes in fish muscle at the spiking concentrations of 10, 5, and 1 ng/g ww, respectively, and for 13, 12, and 11 analytes in liver at the spiking concentrations of 40, 20, and 4 ng/g ww, respectively. Method quantification limits (MQLs) of all analytes were 0.02–2.12 ng/g ww for fish muscle and 0.22–12.2 ng/g ww for fish liver tissues. The method was successfully applied to wild fish samples collected from Dongjiang River, south China. Twenty-one and 17 of the analytes were found in fish muscle and liver samples, respectively, in at least one site of the river with the concentrations between below MQLs and 119 ng/g ww, respectively.

Keywords Personal care products · Fish muscle and liver · QuEChERS · Matrix effect · UPLC-MS/MS

Introduction

Over the past two decades, contaminants of emerging concern (CECs) have attracted extensive attention from scientists and the general public due to their adverse effects, such as endocrine disruption and reproduction toxicity in humans and aquatic organisms [1]. One important class of CECs is the active ingredients in personal care products (PCPs), including biocides, synthetic musks, and benzotriazoles [2–4]. Biocides are widely included in toothpaste, shampoo, and soaps with the functions of deterring, preventing the action of, or killing the harmful organisms [5]. Synthetic musks are used in a wide variety of cosmetics and perfumes [6]. Benzotriazoles are widely used as a corrosion inhibitor in cooling fluids, hydraulic fluids, and dishwasher detergents; an ultraviolet stabilizer in plastics; and an antifogging agent in photography and airplane defogging fluids [3].

After use, the PCP ingredients usually enter into sewers and then are treated in wastewater treatment plants (WWTPs). Due to the incomplete removal in WWTPs [7], these chemicals will eventually reach the receiving environment. Previous studies have reported detection of PCP ingredients in WWTP effluents [8–10], surface water [3, 11, 12], and sediments [13, 14]. Although biocides, synthetic musks,

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and benzotriazoles show weak acute toxicity to aquatic organisms, their chronic toxicity and endocrine-disrupting effects cannot be neglected [15, 16], such as pathological damage to tissues [17], estrogenic activities [18], and anti-estrogenic effect [19].

The toxicity of chemicals to aquatic organisms usually correlates with their uptake and accumulation in tissues [20]. Hence, it is important to study the accumulation of chemicals in different biological tissues. Analysis of PCP ingredients in biological samples is always a challenge due to the complexity of biological tissues. Ruedel and Boehmer extracted triclosan (TCS) and its potential transformation product methyl-triclosan in fish muscles by using pressurized liquid extraction incorporated with gel permeation chromatography and silica gel column purification [21]. Nakata and Sasaki determined four synthetic musks in fish samples following Soxhlet extraction assisted by gel permeation chromatography and silica gel column cleanup [22]. However, these methods are often expensive and time consuming as well as solvent consuming [23, 24]. In addition, the above methods focused on the analysis of one specific class of chemicals. Considering the co-occurrence of various PCPs in aquatic environments, it is still necessary to develop an effective method to simultaneously extract and detect a wide range of PCPs in biological samples.

The quick, easy, cheap, effective, rugged, and safe (QuEChERS) method is an extraction and cleanup technique originally developed for the determination of pesticide residues in fruits and vegetables [25]. Comparing with some traditional extraction methods, the QuEChERS method has significant advantages of labor and solvent saving, short extraction period, and flexible procedure [26, 27]. Due to its prominent merits, the QuEChERS extraction method is now rapidly developed and used in the analysis of various pharmaceuticals and persistent organic pollutants in soil, sediment, and water matrices [28, 29]. QuEChERS procedures also were successfully applied to the analysis of non-polar and semi-polar chemicals in fatty food matrices salmon, seafood, milk, and fish muscle using GC-MS/MS or LC-MS/MS [30–32].

The objective of this study was to develop a rapid, robust, and sensitive method based on QuEChERS extraction followed by ultra pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometer (GC-MS) analyses for simultaneous determination of a variety of PCP ingredients in fish muscle and liver tissues. The selected PCP ingredients include 16 biocides, 4 synthetic musks, and 4 benzotriazoles, with different physicochemical properties. In order to achieve satisfactory recoveries for these analytes in fish tissues, QuEChERS procedures including extraction salts, dispersive solid-phase extraction (d-SPE) materials, extraction solvents, acetic acid (AA) contents, and ratios of solvent and water were optimized. The optimized method was then applied to analyze the selected analytes in wild fish muscle and liver tissues which were collected from Dongjiang River, south China.

Materials and methods

Chemicals and materials

Twenty-four authentic standards (including 16 biocides, 4 synthetic musks, and 4 benzotriazoles) were supplied by various international suppliers (purity ≥95 % for all chemicals), with detailed information and their physicochemical properties being listed in Table S1 (see Electronic Supplementary Material, ESM). Twelve isotopically labeled internal standards (purity ≥95 % for all chemicals) were also obtained from a variety of international suppliers: fluconazole-D_4, thiabendazole-D_6, imazalil-D_5, AHTN-D_3, and musk xylene-D_{15} were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany); clotrimazole-D_5 was obtained from Toronto Research Chemicals (North York, Canada); ketoconazole-D_8, miconazole-D_5, and methylparaben-D_4 were supplied by Campro Scientific (Berlin, Germany); propylparaben-D_4 was supplied by CDN Isotopes (Pointe-Claire, Canada); and $^{13}C_{12}$-TCS and TCC-D_7 were obtained from Cambridge Isotope Laboratories (Andover, USA).

Reagents of HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were purchased from Merck (Darmstadt, Germany), *n*-hexane was supplied by Burdick Jackson (NJ, USA). Dichloromethane (DCM), formic acid, and ammonium acetate were purchased from CNW Technologies (Dusseldorf, Germany). Acetic acid (AA) was supplied by Kermel (Tianjin, China). All materials for QuEChERS extraction were commercially available. Anhydrous magnesium sulfate ($MgSO_4$) and anhydrous sodium sulfate (Na_2SO_4) were obtained from Alfa Aesar (MA, USA), while anhydrous sodium acetate (NaAc), trisodium citrate dihydrate ($Na_3Cit{\cdot}2H_2O$), disodium hydrogencitrate sesquihydrate ($Na_2HCit{\cdot}1.5H_2O$), and Z-Sep tube were supplied by Sigma-Aldrich (Saint Louis, USA). Anhydrous sodium chloride (NaCl), primary-secondary amine (PSA), ceramic homogenizer, and C18 bulk sorbent were purchased from Agilent (Santa Clara, USA), whereas graphitized carbon black (GCB) was obtained from Agela (Beijing, China). Prior to use, powders of $MgSO_4$ and Na_2SO_4 were baked for 2 h at 400 °C in a muffle furnace to remove phthalates and residual water. A Milli-Q water purification system (Millipore, Watford) was used to prepare ultrapure water. Polypropylene centrifuge tubes of 50 and 15 mL were supplied by Anpel (Shanghai, China). All glassware was successively hand-washed with detergent, tap water, and Milli-Q water, and then baked at 400 °C for 4 h before use.

Stock solutions of each analyte, internal standard, and quality control (QC) standard were prepared in MeOH or DCM

and stored in amber glass vials at −20 °C. Working solutions were prepared by appropriate dilution from the stock solutions.

Fish sampling

Wild fish samples were collected in December 2012 from two sites in the mainstream Dongjiang River in south China, and two sites in its tributary Danshui River which received the discharge of effluents from WWTPs (see ESM Fig. S1). In total, 21 fish were collected, and they belonged to 4 different species, i.e., tilapia, crucian carp, common carp, and snakehead fish. The basic information of sampling sites and fish samples is given in Fig. S1 and Table S2 in the ESM.

Fish were kept alive in aerated containers with river water and transported to laboratory immediately, then anesthetized by tricaine methanesulfonate and sacrificed by rapid dissection. Muscle and liver tissues for each fish were collected and stored at −20 °C. Before extraction, fish muscle samples were cut up with stainless scissors. In contrast, liver samples were employed directly due to their slurry status. The lipid content in muscle tissue was measured by following a previous method [33]. Muscle tissues of three tilapia fish, which were collected from site S1 that was located in the upper stream and less affected by human activities, were mixed and used in the method development.

QuEChERS extraction

Two grams of fish muscle or 0.5 g fish liver sample was weighted into a 50-mL polypropylene centrifuge tube, spiked with 100 μL (0.2 μg/mL) internal standards into each tube, and the tube was vortexed for 30 s. Then the sample was equilibrated at 4 °C for 30 min. Five milliliters of Milli-Q water and two ceramic homogenizers were dispensed to each sample; the tube was then vortexed for 1 min to homogenize the sample sufficiently. After that, 10 mL of MeCN containing 1 % AA was added. Subsequently, a salt set containing 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous NaAc was added, and the tube was immediately hand shaken for 1 min to extract the target compounds. The tube was then centrifuged at 2364*g* for 10 min. Seven milliliters of the supernatant (MeCN phase) was transferred to a 15 mL d-SPE tube containing 900 mg anhydrous $MgSO_4$, 150 mg PSA, and 150 mg C18; the tube was vortexed for 1 min and centrifuged at 2364*g* for 10 min. Then 5 mL supernatant was transferred into a 10-mL glass tube, and MeCN was evaporated under a gentle stream of nitrogen. Finally, 200 μL MeOH was added into the glass tube to redissolve the extracts, and the solution was evenly separated into two aliquots in 150-μL centrifuge tubes. The solutions of the two aliquots were dried again under nitrogen stream; one was reconstituted with 100 μL MeOH/H_2O (*v*/*v* = 50/50) for UPLC-MS/MS analysis, while the other reconstituted with 100 μL DCM for GC-MS analysis.

Instrumental conditions

The instruments used for the analysis of target compounds were an Agilent Ultra Pressure Liquid Chromatography 1200 series coupled to an Agilent 6460 triple quadrupole mass spectrometer (UPLC-MS/MS) with electrospray ionization (ESI) source and multiple reaction monitoring (MRM) mode, and an Agilent Gas Chromatography 6890N series integrated with an Agilent 5975B mass spectrometer (GC-MS) with electron impact (EI) source and selective ion monitoring (SIM) mode.

Three instrumental methods were developed based on our previous studies [34–36], which used ESI+ or ESI− modes of UPLC-MS/MS and EI mode of GC-MS to analyze target compounds with different physicochemical properties. The first method was for 10 biocides (fluconazole, carbendazim, thiabendazole, DEET, icaridin, climbazole, ketoconazole, clotrimazole, miconazole, and itraconazole) and 4 benzotriazoles (benzotriazole, 5-TT, CBT, and XT) by UPLC-MS/MS in positive ESI mode. The second method was for 6 biocides (methylparaben, ethylparaben, propylparaben, butylparaben, triclosan, and triclocarban) by UPLC-MS/MS in negative ESI mode. The third method was for 4 synthetic musks (AHTN, HHCB, musk xylene, and musk ketone) by GC-MS in EI mode.

For UPLC-MS/MS with positive ESI mode, a Zorbax SB-C18 (100 mm × 3 mm, 1.8 μm particle size) column with its corresponding precolumn filter (2.1 mm, 0.2 μm) from Agilent Technologies was used for chromatographic separation. The column was kept at 40 °C and the injection volume was 5.0 μL. The mobile phases used were (A) Milli-Q water (containing 5 mmol/L ammonium acetate and 0.05 % formic acid (*v*/*v*)) and (B) methanol. The gradient program was as follows: 50 % B at 0 min, increased to 80 % B in 5 min, stepped to 90 % B in 0.5 min, and held for 5.5 min at a flow rate of 0.30 mL/min; a post run time was set at 4.5 min for column equilibration before the next injection. The whole analysis time for each sample was 15.5 min. For UPLC-MS/MS with negative ESI mode, the column brand, column temperature, and the injection volume were the same as those in positive ESI mode. The mobile phase consisted of Milli-Q water (A) and methanol (B). The gradient elution program was set as follows: 50 % B at 0 min, increased to 56 % B in 3 min, stepped to 90 % B in 1 min, and held for 4.5 min at a flow rate of 0.35 mL/min; a post run time was set at 5 min for column equilibration before the next injection. The whole analysis time for each sample was 13.5 min. The operating conditions (fragmentor voltage, collision energy (CE), precursor ion and product ions for each compound) for mass spectrometry were optimized by Optimizer (Agilent, USA), to maximize the response and increase detection sensitivity (Table 1). Quantitative analysis of the target compounds was performed in multiple

Table 1 Ion transitions and retention times for target compounds in UPLC-MS/MS

Compound	RT (min)	Precursor ions (*m*/*z*)	Product ions (*m*/*z*)	Fragmentor (eV)	CE (eV)	Corresponding IS
ESI+						
Fluconazole	2.885	307.1	70.1	45	44	Fluconazole-D_4
			220.1		16	
Carbendazim	3.112	192.1	132.1	115	36	Thiabendazole-D_6
			160.1		16	
Thiabendazole	3.994	202.0	*131.1*	150	36	Thiabendazole-D_6
			175.0		28	
DEET	6.566	192.1	91.1	45	36	Imazalil-D_5
			119.1		16	
Icaridin	7.019	230.2	67.1	45	40	Imazalil-D_5
			130.1		12	
Ketoconazole	7.922	531.2	*81.1*	220	88	Ketoconazole-D_8
			135.1		44	
Climbazole	7.705	293.1	41.1	115	44	Imazalil-D_5
			69.1		20	
Clotrimazole	9.235	345.1	165.1	45	40	Clotrimazole-D_5
			277.1		4	
Miconazole	10.754	415.0	123.0	170	80	Miconazole-D_5
			159.0		36	
Itraconazole	9.896	705.2	159.0	225	96	Ketoconazole-D_8
			392.2		40	
BT	2.682	120.1	*65*	135	44	Thiabendazole-D_6
			39		20	
5-TT	3.709	134.1	*77*	120	28	Thiabendazole-D_6
			51		48	
CBT	4.343	154	*98.9*	140	24	Thiabendazole-D_6
			72.9		40	
XT	4.725	148.1	*77*	130	28	Thiabendazole-D_6
			51		56	
Clotrimazole-D_5 (IS)[a]	9.190	350.2	169.7	45	32	–
			282.1		4	
Ketoconazole-D_8 (IS)	7.844	539.2	*82.1*	45	56	–
			119.1		52	
Fluconazole-D_4 (IS)	2.873	311.1	172.1	120	24	–
			223.1		16	
Thiabendazole-D_6 (IS)	3.848	208.1	*136.1*	160	40	–
			180.1		28	
Imazalil-D_5 (IS)	7.349	302.1	42.1	145	100	–
			46.1		36	
Miconazole-D_5 (IS)	10.920	420	127.0	175	60	–
			164.0		28	
ESI−						
Methylparaben	2.3	151.0	*92.1*	110	16	Methylparaben-D_4
			136.0		8	
Ethylparaben	3.476	165.1	*92.1*	105	16	Methylparaben-D_4
			137.1		8	
Propylparaben	5.786	179.1	*92.1*	115	20	Propylparaben-D_4
			136.0		8	
Butylparaben	6.4	193.1	*92.1*	120	20	Propylparaben-D_4
			136.0		8	
Triclocarban	7.358	313	126.1	86	13	Triclocarban-D_7
			160.0		5	
Triclosan	7.486	286.9	*35.1*	65	1	$^{13}C_{12}$-Triclosan
Methylparaben-D_4 (IS)	2.29	155.1	*96.1*	100	16	–
			140.1		8	
Propylparaben-D_4 (IS)	5.739	183.1	96.1	125	20	–
			140.1		12	
$^{13}C_{12}$-Triclosan (IS)	7.504	299.0	*35.1*	65	1	–
Triclocarban-D_7 (IS)	7.34	320.0	130.1	107	13	–
			163.0		5	

Numbers in italics are the quantification ions

RT retention time, *CE* collision energy, *IS* internal standard

Table 2 Characteristic ions and retention times for target compounds in GC-MS

Compound	MW	RT	Ions			IS
AHTN-D3	261	15.580	*246.2*	261.2	190.1	–
AHTN	258	15.652	*243.2*	258.2	187.1	AHTN-d3
HHCB	258	15.391	*243.2*	258.2	213.2	AHTN-d3
Musk xylene-D15	312	15.013	*294.2*	293.2		–
Musk xylene	297	15.478	*282*	297		Musk xylene-d15
Musk ketone	294	19.996	*279.1*	294.1	280.1	Musk xylene-d15

Numbers in italics are the quantification ions

MW molecular weight, *RT* retention time, *IS* internal standard

reaction monitoring (MRM) mode. Agilent Mass Hunter V 02.01 software was used for data acquisition.

For GC-MS, synthetic musks were separated on an Agilent DB-5MS column (30 m × 250 mm, 0.25 mm thickness) with helium as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed as follows: from 80 to 170 °C at a rate of 15 °C/min and then to 185 °C at 1 °C/min, finally programmed at a rate of 20 °C/min to 300 °C (held 5 min). The system equilibrium time was 5 min before the next injection, and the whole analysis time for each sample was 36.75 min. The injection port, ionization source, mass analyzer, and transfer line temperatures were set at 280, 250, 150, and 280 °C, respectively. The injection volume was 2.0 μL with splitless mode. The MS was operated in electron impact (EI) mode at 70 eV and in the selected ion monitoring (SIM) mode for quantification purposes. Retention times and ions monitored for each compound are summarized in Table 2.

Quantification and method validation

The internal standard method was used to quantify the concentration of target compounds. For the calibration of UPLC-MS/MS, mixtures of 20 standard compounds with a series of concentrations (0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, and 200 μg/L) and mixtures of 10 internal standards with concentration of 50 μg/L were prepared in MeOH/H_2O (50:50, *v*/*v*) into 2-mL amber glass vials. For the matrix calibration of GC-MS, the mixture of standard chemicals with concentrations of 5, 10, 20, 50, 100, and 200 μg/L and the mixtures of internal standards with concentration of 50 μg/L were prepared in blank muscle or liver matrices.

All instrument analyses were subject to strict quality control procedures. For each group of samples, a solvent blank (the initial mobile phase for LC-MS/MS or DCM for GC-MS), procedure blank (extract of matrices with internal standard and without target analytes), and an independent check standard (50 μg/L standard solution) were run in sequence to check carryover, background contamination, and system performance. Independent check standard (QC sample) was injected approximately every ten injections, and the calculated concentration was required to be within 20 % of the expected value.

Method detection limits (MDLs) and quantification limits (MQLs) of the QuEChERS method were determined as the minimum detectable amount of an analyte from fish muscle or liver

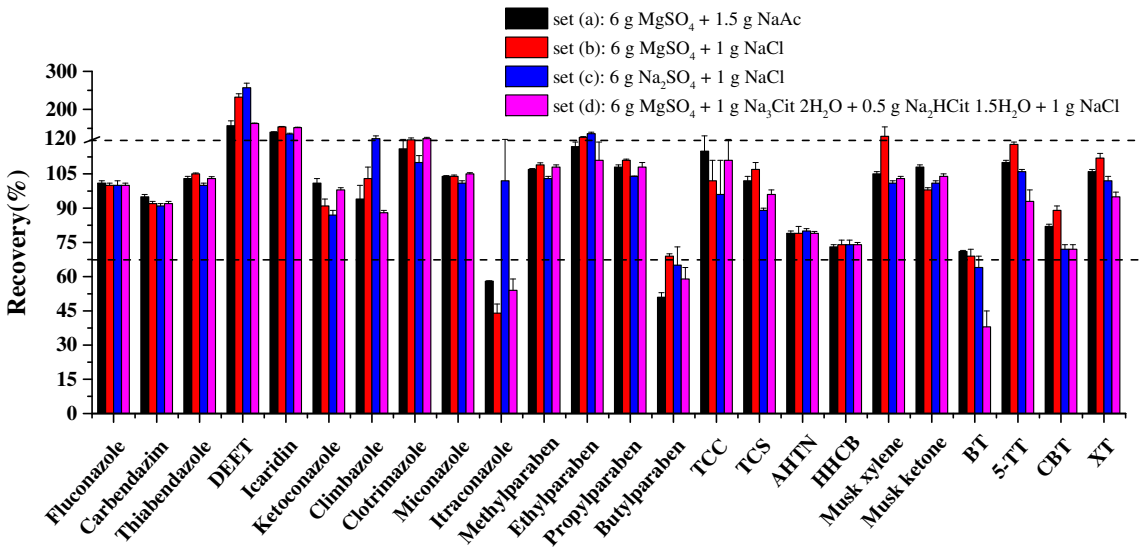


Fig. 1 The recoveries (mean (%) ± standard deviation (%), $n = 3$) of target compounds in fish muscle tissue with different salts used

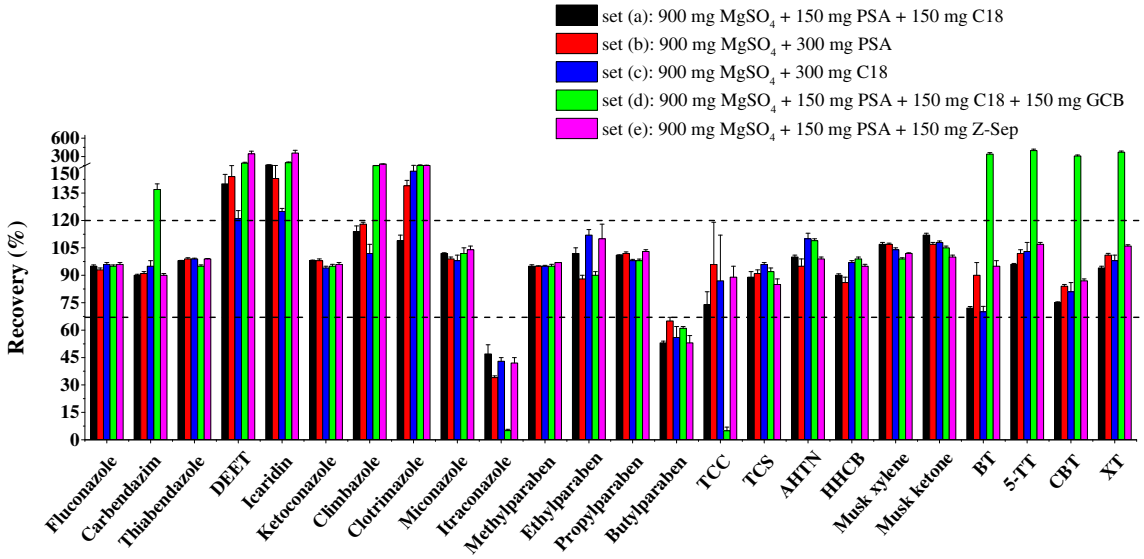


Fig. 2 The recoveries (mean (%) ± standard deviation (%), $n = 3$) of target compounds in fish muscle tissue with different dispersive solid-phase extraction materials used

extracts with 3 and 10 times of signal-to-noise ratios, respectively. The recoveries for each target analyte were obtained at 3 spiking levels (1, 5, and 10 ng/g ww for fish muscle and 4, 20, and 40 ng/g ww for liver) with 3 replicates; the calculation of recoveries are provided in the ESM. The reproducibility was tested at 10 ng/g ww for fish muscle tissue with 7 replicates.

Table 3 The relative and absolute matrix effects (%) of biocides, synthetic musks, and benzotriazoles in fish muscle and liver tissues with spiking concentrations of 10 and 40 ng/g wet weight, respectively

Analyte	Muscle		Liver	
	Relative matrix effect (%)	Absolute matrix effect (%)	Relative matrix effect (%)	Absolute matrix effect (%)
Biocides				
Fluconazole	−2	−1	1	5
Carbendazim	−5	−12	−2	−1
Thiabendazole	−8	−22	−2	−3
DEET	*26*	36	8	8
Icaridin	*47*	16	14	12
Ketoconazole	−6	*−52*	−4	−1
Climbazole	*−37*	*−50*	−9	−5
Clotrimazole	−5	*−76*	n.a.	n.a.
Miconazole	−3	*−82*	−3	−12
Itraconazole	−8	*−50*	*150*	*151*
Methylparaben	−1	−24	−7	*−52*
Ethylparaben	*24*	−1	*26*	−29
Propylparaben	−1	−27	−5	−29
Butylparaben	*−44*	*−68*	*−36*	*−63*
TCC	−8	*−89*	−6	−14
TCS	−7	*−65*	−5	−11
Musks				
AHTN	1	*109*	1	*54*
HHCB	1	*106*	3	40
Musk xylene	8	*168*	1	*97*
Musk ketone	7	*178*	2	*98*
Benzotriazoles				
BT	18	1	9	9
5-TT	19	−1	4	−1
CBT	−1	−18	−15	−16
XT	4	−9	1	−2

Numbers in italics represent those relative matrix effects outside the range of ±20 % or the absolute matrix effect outside the range of ±40 %

n.a. not available

Table 4 The recoveries (mean (%) ± standard deviation (%), $n = 3$), method detection limits (MDLs), and method quantitation limits (MQLs) of biocides, synthetic musks, and benzotriazoles in fish muscle and liver tissues

Analyte	Muscle						Liver					
	1 (ng/g ww)	5 (ng/g ww)	10 (ng/g ww)	MDLs (ng/g ww)	MQLs (ng/g ww)	Linearity (R^2)	4 (ng/g ww)	20 (ng/g ww)	40 (ng/g ww)	MDLs (ng/g ww)	MQLs (ng/g ww)	Linearity (R^2)
Biocides												
Fluconazole	66 ± 0.3	76 ± 2	101 ± 1	0.04	0.13	0.9989	71 ± 0.04	80 ± 0.02	88 ± 1	0.18	0.61	0.9994
Carbendazim	74 ± 0.3	84 ± 2	95 ± 1	0.03	0.09	0.9998	*200 ± 5*	*56 ± 2*	66 ± 9	0.07	0.22	0.9997
Thiabendazole	75 ± 0.1	88 ± 1	103 ± 1	0.03	0.11	0.9997	71 ± 0.1	88 ± 0.8	89 ± 0.9	0.11	0.38	0.9995
DEET	*195 ± 0.3*	116 ± 1	*157 ± 13*	0.01	0.04	0.9962	*244 ± 4*	98 ± 1	*123 ± 4*	0.10	0.32	0.9964
Icaridin	124 ± 0.09	*123 ± 2*	94 ± 6	0.01	0.02	0.9993	*286 ± 7*	*158 ± 0.6*	*156 ± 9*	0.08	0.28	0.9992
Ketoconazole	75 ± 0.1	91 ± 0.6	*140 ± 1*	0.06	0.21	0.9971	*46 ± 0.04*	87 ± 5	79 ± 0.6	0.79	2.65	0.9996
Climbazole	*38 ± 0.9*	*49 ± 2*	101 ± 2	0.09	0.30	0.9999	*56 ± 0.04*	*18 ± 0.8*	*19 ± 1*	0.35	1.16	0.9998
Clotrimazole	100 ± 0.8	79 ± 1	116 ± 5	0.18	0.59	0.9976	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Miconazole	104 ± 0.7	89 ± 1	104 ± 0.3	0.11	0.35	0.9998	106 ± 0.4	113 ± 7	91 ± 3	0.69	2.30	0.9998
Itraconazole	*17 ± 0.6*	*26 ± 3*	*58 ± 0.2*	0.02	0.06	0.9982	*34 ± 0.8*	*20 ± 5*	*47 ± 6*	0.24	0.81	0.9991
Methylparaben	72 ± 0.1	76 ± 1	107 ± 0.4	0.03	0.08	0.9981	86 ± 0.6	115 ± 12	85 ± 1	0.17	0.57	0.9986
Ethylparaben	*65 ± 0.2*	94 ± 2	117 ± 2	0.02	0.08	0.9957	113 ± 2	*69 ± 19*	110 ± 8	0.08	0.28	0.9958
Propylparaben	83 ± 0.1	91 ± 1	108 ± 1	0.06	0.20	0.9999	80 ± 0.2	86 ± 1	92 ± 1	0.08	0.26	0.9999
Butylparaben	*52 ± 0.5*	*31 ± 3*	*51 ± 2*	0.03	0.11	0.9998	*26 ± 0.6*	*9.5 ± 1*	*25 ± 7*	0.12	0.41	0.9979
TCC results	*185 ± 3*	94 ± 10	115 ± 15	0.07	0.22	0.9950	95 ± 2	*68 ± 9*	*69 ± 20*	0.35	1.17	0.9989
TCS results	*210 ± 14*	101 ± 2	102 ± 2	0.04	0.14	0.9978	*148 ± 2*	*125 ± 34*	116 ± 6	0.38	1.28	0.9974
Musks												
AHTN	*52 ± 0.6*	86 ± 2	100 ± 1	0.10	0.34	0.9980	113 ± 0.1	115 ± 4	79 ± 0.3	0.46	1.53	0.9990
HHCB	*61 ± 0.4*	74 ± 1	90 ± 0.8	0.23	0.76	0.9980	70 ± 1	118 ± 5	*145 ± 0.04*	0.59	1.96	0.9990
Musk xylene	n.a.	90 ± 1	107 ± 1	0.64	2.12	0.9990	n.a.	112 ± 6	110 ± 0.01	3.66	12.2	0.9980
Musk ketone	n.a.	96 ± 1	112 ± 1	0.48	1.60	0.9980	n.a.	101 ± 0.9	107 ± 0.04	3.59	11.9	0.9990
Benzotriazoles												
BT	120 ± 0.9	73 ± 3	72 ± 0.9	0.12	0.40	0.9995	*42 ± 3*	*190 ± 18*	*170 ± 23*	0.90	3.02	0.9992
5-TT	85 ± 0.3	94 ± 1	96 ± 0.5	0.02	0.07	0.9997	*23 ± 2*	*122 ± 20*	*161 ± 30*	0.74	2.48	0.9996
CBT	*51 ± 0.2*	*57 ± 0.5*	75 ± 0.5	0.06	0.21	0.9996	*21 ± 0.6*	*68 ± 3*	81 ± 9	0.30	0.98	0.9995
XT	*61 ± 0.02*	79 ± 1	94 ± 1	0.11	0.36	0.9994	*29 ± 2*	80 ± 11	*125 ± 22*	0.96	3.21	0.9999

Numbers in italics represent those recoveries outside the range of 70–120 %

ww wet weight, *n.a.* not available, R^2 correlation coefficients

The variance significances of matrices removal efficiency between different d-SPE sorbents were analyzed by ANOVA test in SPSS (Statistical Package for Social Sciences, version 13.0). The value of $p \leq 0.05$ means significant difference, while $p > 0.05$ means no significant difference.

Matrix effect for each compound was assessed based on the instrumental responses from matrix without chemical standards, matrix spiked with chemical standards, and mobile phase spiked with chemical standards [28]. The detailed calculation of relative and absolute matrix effect is given in the ESM. The matrix effect values greater or less than 0 % indicate signal enhancement or suppression, respectively.

Results and discussion

Instrumental analysis

The developed instrumental methods achieved good resolution for all target analytes in MRM mode of UPLC-MS/MS and SIM mode of GC-MS. The total ion chromatograms of 24 target compounds at the concentration of 50 μg/L on UPLC-MS/MS and GC-MS are shown in ESM, Fig. S2. The calibration curves for the target compounds on UPLC-MS/MS and GC-MS showed good linearity ($R^2 > 0.995$). Thus, the modified instrumental conditions could be used for the analysis of target analytes at the concentration range of 0.1–200 μg/L by UPLC-MS/MS and at 5–200 μg/L by GC-MS.

Optimization of extraction salts

During QuEChERS extraction process, salts are often added to enhance the separation between water and organic phases and improve the extraction efficiencies of target analytes from matrix to organic phases [25]. In this study, four salt sets were tested, i.e., set (a): 6 g $MgSO_4$ + 1.5 g NaAc; set (b): 6 g $MgSO_4$ + 1 g NaCl; set (c): 6 g Na_2SO_4 + 1 g NaCl, and set (d): 6 g $MgSO_4$ + 1 g $Na_3Cit{\cdot}2H_2O$ + 0.5 g $Na_2HCit{\cdot}1.5H_2O$ + 1 g NaCl. Among them, salt sets (a) and (d) are buffered salts used extensively for pesticide extraction, which have been developed as official methods by AOAC (2007) and UNI EN (2009) [37, 38], respectively. Salt set (b) is the original selection when the QuEChERS method was proposed in 2003. Salt set (c) uses Na_2SO_4 instead of $MgSO_4$, since Na_2SO_4 does not release heat like $MgSO_4$ during hydration process [25].

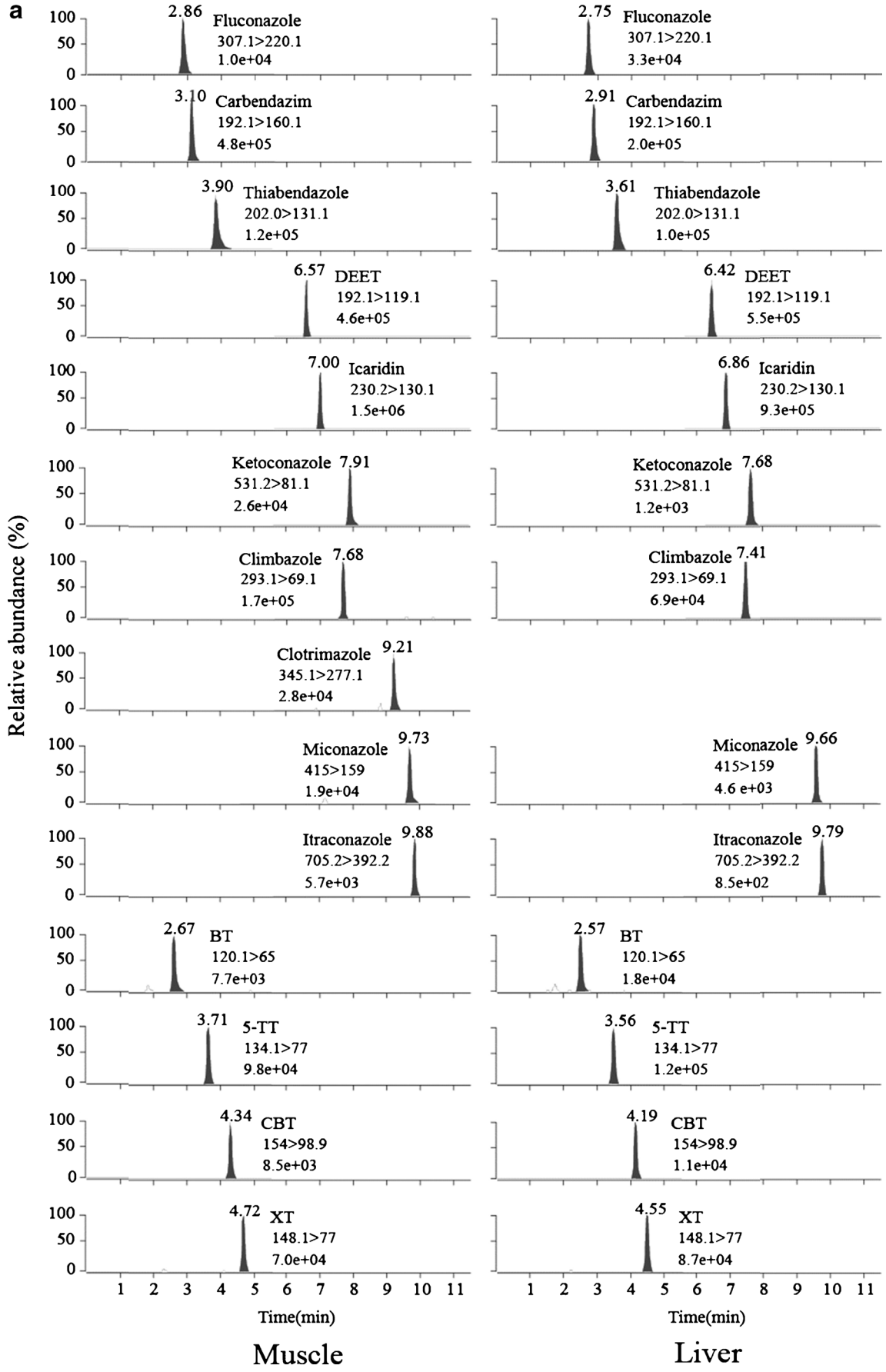
The recoveries of the target compounds with different extraction salts are shown in Fig. 1. It can be seen that most of the compounds achieved satisfactory recoveries when the four salt sets were used. But for several target compounds, the recoveries were higher than 120 % (DEET and icaridin) or lower than 70 % (itraconazole, butylparaben, and BT). These deviations could be explained by different molecular structures to their internal standards. Compounds with a long alkyl chain are prone to enter into the MeCN phase, while polar compounds are difficult to transfer from water to the MeCN phase during salt extraction. If the extraction efficiencies of these compounds are not consistent with their corresponding internal standards, these compounds will show higher or lower recoveries. The phenomena were also reported in other matrices for these chemicals, such as the poor recoveries of DEET and itraconazole in wastewater and sludge samples [35].

Extraction sets with or without buffered salts also displayed obvious influence on the extraction efficiencies of target analytes [39]. Overall, the sets with buffered salts (sets (a) and (d)) showed better recoveries and lower deviations than the sets with non-buffered salts (set (b) and (c)), especially for DEET and itraconazole (Fig. 1). The recoveries of DEET with non-buffered salt sets (b) and (c) were 232 and 257 %, which were much higher than those with buffered salts sets (a) and (d). For itraconazole, although the recovery with non-buffered salt set (c) reached to 102 %, which was better than the other two buffered salt sets, the standard deviation with salt set (c) was also high to 19 %. Briefly, for 24 target analytes, the recoveries of totally 20, 17, 18, and 18 kinds of compounds were in the range of 70–120 % when salt sets (a), (b), (c), and (d) were used, respectively. Hence, salt set (a) was selected as the optimal extraction salt due to the best recoveries and smallest deviations for most target analytes.

Optimization of d-SPE cleanup

Extracts usually contain various matrix substances such as humic acid and fats [40]. Especially for biota tissues, lipids, proteins, amino acids, and other biomolecules could also have been extracted out simultaneously with target chemicals during the salt extraction process. Hence, a further cleanup step is essential before instrumental analysis. In the conventional QuEChERS method, $MgSO_4$ is usually employed to reduce the residual water in the extract, while other d-SPE sorbents are used to retain the matrix co-extracts [25, 28]. So the selection of d-SPE sorbents is important in removing the interfering substances. PSA sorbent is a weak anion exchanger which can effectively remove various polar matrix components, such as

Fig. 3 **a** LC-MS/MS MRM chromatograms (positive ESI source) of 10 biocides and 4 benzotriazoles at the spiking concentration of 10 ng/g wet weight in fish muscle and 40 ng/g wet weight in liver for each analyte. Retention time, precursor ion and production ion, and abundance for each analyte are indicated in the chromatogram. **b** LC-MS/MS MRM chromatograms (negative ESI source) of 6 biocides at the spiking concentration of 10 ng/g wet weight in fish muscle and 40 ng/g wet weight in liver for each analyte. Retention time, precursor ion and production ion, and abundance for each analyte are indicated in the chromatogram


a
Relative abundance (%)
2.86 Fluconazole 307.1>220.1 1.0e+04
3.10 Carbendazim 192.1>160.1 4.8e+05
3.90 Thiabendazole 202.0>131.1 1.2e+05
6.57 DEET 192.1>119.1 4.6e+05
7.00 Icaridin 230.2>130.1 1.5e+06
Ketoconazole 7.91 531.2>81.1 2.6e+04
Climbazole 7.68 293.1>69.1 1.7e+05
Clotrimazole 9.21 345.1>277.1 2.8e+04
Miconazole 9.73 415>159 1.9e+04
Itraconazole 9.88 705.2>392.2 5.7e+03
2.67 BT 120.1>65 7.7e+03
3.71 5-TT 134.1>77 9.8e+04
4.34 CBT 154>98.9 8.5e+03
4.72 XT 148.1>77 7.0e+04
2.75 Fluconazole 307.1>220.1 3.3e+04
2.91 Carbendazim 192.1>160.1 2.0e+05
3.61 Thiabendazole 202.0>131.1 1.0e+05
6.42 DEET 192.1>119.1 5.5e+05
6.86 Icaridin 230.2>130.1 9.3e+05
Ketoconazole 7.68 531.2>81.1 1.2e+03
Climbazole 7.41 293.1>69.1 6.9e+04
Miconazole 9.66 415>159 4.6 e+03
Itraconazole 9.79 705.2>392.2 8.5e+02
2.57 BT 120.1>65 1.8e+04
3.56 5-TT 134.1>77 1.2e+05
4.19 CBT 154>98.9 1.1e+04
4.55 XT 148.1>77 8.7e+04
100
50
0
1 2 3 4 5 6 7 8 9 10 11
Time(min)
Muscle
Liver

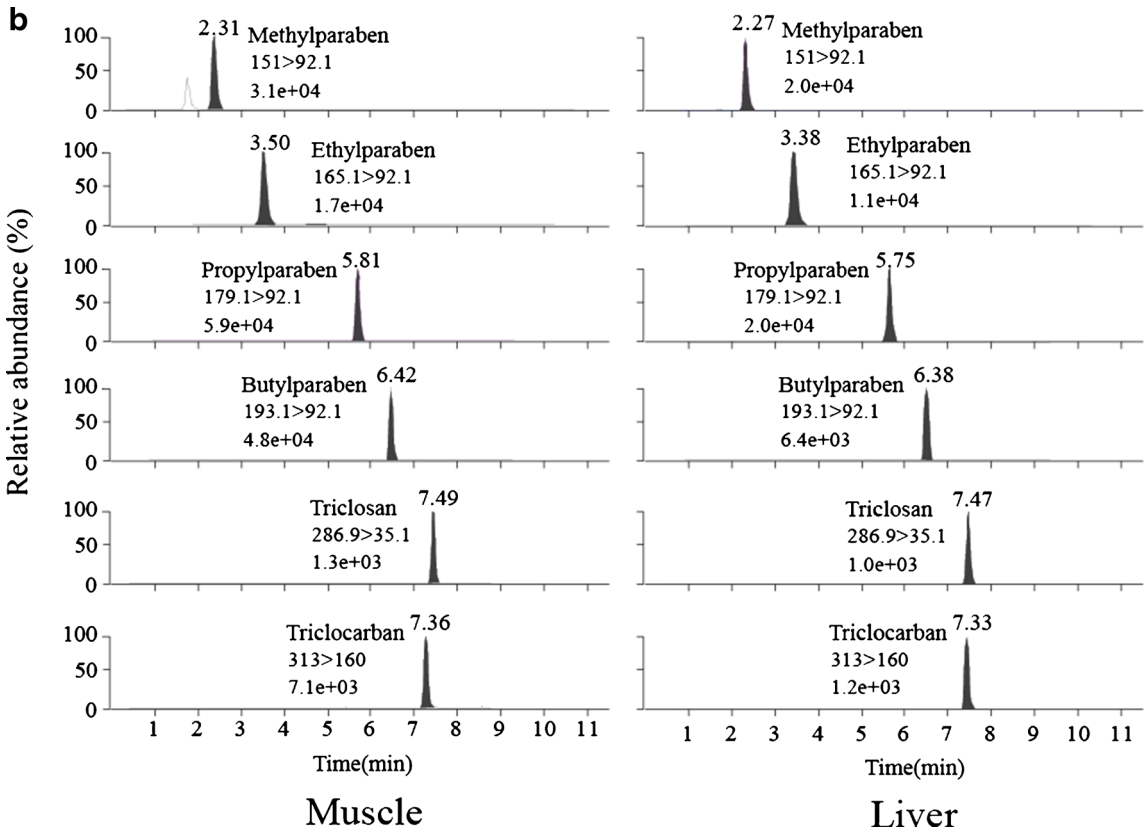


Fig. 3 (continued)

organic acids, certain polar pigments, and sugars [25]. C18 is used to remove long chain fatty compounds and other non-polar interferences [41]. GCB is known to remove hydrophobic interaction-based compounds such as pigments, and it is usually used in the purification of fruits and vegetables [42, 43]. The adsorption function of Z-Sep sorbent is based on the interaction of a zirconium (Zr) atom with phosphate groups in phospholipids, by a Lewis acid-base mechanism [44]. In this study, five d-SPE sets containing single or mixtures of above sorbents were tested, i.e., set (a): 900 mg $MgSO_4$ + 150 mg PSA + 150 mg C18; set (b): 900 mg $MgSO_4$ + 300 mg PSA; set (c): 900 mg $MgSO_4$ + 300 mg C18; set (d): 900 mg $MgSO_4$ + 150 mg PSA + 150 mg C18 + 150 mg GCB; and set (e): 900 mg $MgSO_4$ + 150 mg PSA + 150 mg Z-Sep.

The recoveries for the target compounds using different d-SPE sets are displayed in Fig. 2. Among the 24 analytes, the recoveries of total 20, 19, 19, 12, and 18 kinds of compounds were in the range of 70–120 % when d-SPE sets (a), (b), (c), (d), and (e) were used, respectively. Actually, it can be seen that the majority of analytes displayed low standard deviation values (<10 %) when the five d-SPE sets were used. And the recoveries for the majority of analytes were between 70 and 120 %, when using sets (a), (b), and (c). But when GCB was used (set (d)), the recoveries for carbendazim, DEET, icaridin, climbazole, clotrimazole, and four benzotriazoles (BT, 5-TT, CBT, XT) were higher than 120 % and up to 398 %, while those for itraconazole and TCC were below 5 %. Obviously, differences of absorption abilities between analytes and their internal standards with GCB resulted in the recovery deviations. The phenomenon is due to the inconsistency of chromatographic response for the internal standard and analyte, which will result in the overestimated final concentration of target compounds, especially for those analytes without corresponding isotopically labeled internal standards. The results demonstrated that GCB was not effective in d-SPE because the removal of some target compounds occurred, which is consistent with the previous study [42, 45]. GCB shows strong absorption ability to planar compounds due to its highly polar groups on the surface, such as thiabendazole-D_6 that acts as the internal standard of four benzotriazoles (BT, 5-TT, CBT, and XT) [40, 46]; the recoveries of four benzotriazoles were high to 398 %. Besides, for the samples purified with the d-SPE set containing Z-Sep sorbent (set (e)), the recoveries for most of the analytes did not show any improvement compared with the samples cleaned with sets (a), (b), and (c). On the contrary, DEET and icaridin displayed unusually high recoveries up to 344 and 356 %, respectively, when Z-Sep was added. This is probably due to the strong adsorption of Z-Sep to their internal standard imazalil-D_5. Imazalil-D_5 is a weak base (pK_a = 6.54) with end-functionalized alkylene [47]; this special structure may lead to the electron attachment between itself and Z-Sep (the Zr atom containing an unoccupied orbital acts as a Lewis acid) [44].

In order to further evaluate the matrix removal efficiencies of different d-SPE sets, we also compared the weight loss of the same extract fish matrices after being purified with various d-SPE materials. As exhibited in Fig. S3 (see ESM), the matrix removal capacity (explained as average removal weight) had the following order: set (d) > set (e) > sets (c), (b), and (a). The d-SPE set containing GCB sorbent shows the most efficient substrate removal capacity, with almost double the matrix weight removed than the other d-SPE sorbents. The statistical analysis showed significant differences in substrate removal efficiency between the d-SPE set containing GCB (set (d)) and the other three sets without GCB (sets (c), (b), and (a)) ($p < 0.05$), suggesting that GCB had strong substrate removal capacity compared with the other d-SPE sets. But GCB also showed strong absorption for most target compounds as discussed above. As reported by other scientists, Z-Sep sorbent can provide cleaner extracts than PSA/C18 sorbent for fish muscle [31]. In our study, Z-Sep can slightly enhance the co-extractive removal efficiency, but it also induces a low recovery of imazalil-D_5. So the d-SPE sets with GCB and Z-Sep were not chosen as the final d-SPE sorbents. For d-SPE sets (c), (b), and (a), there were no significant differences among them in substrate removal efficiencies ($p > 0.05$). Although single sorbents of PSA or C18 could remove equivalent matrix, due to the complexity of fish tissues, the combination of PSA and C18 would probably have better matrix removal performance for trace interferences in fish. Hence, d-SPE set (a) containing sorbents of 900 mg $MgSO_4$, 150 mg C18, and 150 mg PSA was selected as the optimal purification material.

Selection of solvent, AA content, and MeCN/water ratio

Influence of extraction solvent

The extraction efficiency of target analytes in samples is closely related to the selection of organic solvents, sample nature, and physicochemical properties of the analytes. According to the definition of the U.S. of Food and Drug Administration for fatty foods (>2 % fat composition) [48], tilapia (0.98 % ± 0.26 % in muscle tissue) belong to low fatty food. In this study, four solvents (all of the solvents acidified with 1 % AA), including (a) MeCN + 5 % DCM; (b) MeCN + 10 % DCM; (c) MeCN + 5 % DCM + 5 % *n*-hexane; and (d) MeCN were tested.

The recoveries obtained for most target compounds with the four solvents were in the range of 70–120 %, except for DEET and icaridin with recoveries >120 % (see ESM, Table S3). DCM and *n*-hexane are good solvents for lipid, suggesting good extraction for compounds with high lipid solubility [39]. However, the addition of DCM alone or both DCM and *n*-hexane did not increase the recoveries of those target analytes with a long alkyl chain, such as butylparaben (see ESM, Table S3). For solvent (d) with MeCN only, the deviations of recoveries for most compounds were smaller than those with other solvents. Therefore, solvent (d) of MeCN with 1 % AA was chosen as the final extraction solvent.

Influence of extraction AA content

In fruits and vegetables, the initial acetic-based buffer salt was achieved by adding 1 % AA in MeCN to improve stability of base-sensitive pesticides [39]. Acidification could also help disrupt the compound-protein binding in biological samples; thus, it has been widely used in the extraction of various compounds [49]. In this study, different percentages (0, 1, and 5 %) of AA in MeCN were tested in order to acquire satisfactory recoveries.

The use of buffering during the extraction yields pH <4 in the MeCN extract and pH >5 in the water phase, which ensure good recoveries of either acid- or base-sensitive analytes [43]. Our results showed that the recoveries for most target compounds were 70–120 % with 1 % AA in MeCN as extraction solvent (see ESM, Table S4). However, the recoveries of DEET and icaridin were higher than 120 %, while the recoveries of itraconazole and propylparaben were lower than 70 % as they were difficult to be extracted as discussed above. Obviously, the recoveries of BT and butylparaben decreased with the increasing of AA content in MeCN. This is probably due to the fact that the increase of acidity accelerates the hydrolysis or cationization of BT [50] and butylparaben, which leads to their poor partition in MeCN phase [43]. Since 17, 19, and 15 compounds had their recoveries in the range of 70–120 % when various amounts of AA (0, 1, and 5 %) were added into MeCN, respectively, the solvent of 1 % AA in MeCN was considered as the best choice.

Influence of the MeCN/water ratio

Both fish muscle and liver are solid biological samples, while the QuEChERS technique is based on the separation of water and organic layers which is induced by a combination of salts. Thus, the existence of water is important to make the samples more accessible to the extraction solvent (MeCN) [51]. The influence of the MeCN/water ratio used in the first step of QuEChERS extraction was tested. It was found that almost all of the analytes were not sensitive to the three V_{MeCN}/V_{water} ratios (4:1, 2:1, and 1:1), whereas butylparaben was poorly extracted with ratios 4:1 and 1:1 (see ESM, Table S5). Since the V_{MeCN}/V_{water} ratio of 2:1 showed the best results for majority of the compounds, it was selected as the optimal condition.

Matrix effects

Matrix effects are common in the analysis of environmental and biological samples by UPLC-MS/MS and GC-MS, with signal suppression/enhancement effects due to the presence of interfering matrices [28]. In this study, the absolute and relative matrix effects in fish muscle and liver tissues were evaluated, with the results given in Table 3. The absolute matrix effects of 12 analytes (five azole antifungals, butylparaben, TCC, TCS, and four synthetic musks) in muscle were outside the range of −40 to +40 %, and six compounds (itraconazole, methylparaben, butylparaben, and three musks) in liver exceeded the range of −40 to +40 %. But the relative matrix effects for most of the analytes were between −20 and +20 %, except for some compounds without isotopically labeled internal standards in muscle and liver samples.

For most compounds, fish muscle and liver samples exhibited the same trend of relative and absolute matrix effects (whether enhancement or suppression), and the matrix effects in liver samples were weaker than those in muscle samples, except for clotrimazole and itraconazole. The analytes determined by LC-MS/MS were more likely to be suppressed rather than enhanced by matrices in this study [31], since co-eluting compounds tend to compete for either the total available charge or the available surface area of the droplet in the surface of the MS detector and then result in the ion suppression of the analyte [52]. For DEET and icaridin, obvious matrix enhancement effects were observed, which is often the case with biological and environmental samples due to the complicated interaction between analytes and matrices [28, 53]. As demonstrated by some analytes (e.g., propylparaben, TCC, and TCS) in Table 1, use of an isotopically labeled analogue of an analyte as the internal standard could correct the matrix suppression or enhancement effect since these analogues normally co-elute exactly at the same retention time and experience the same suppression/enhancement effects as the analytes [54, 55].

For synthetic musks analyzed by GC-MS, only matrix enhancement effects were observed, with the intensity of enhancement in muscle higher than that in liver samples. Matrix-induced response enhancement is common in GC-MS analysis [31]. The components in matrices can partially deactivate the active sites and reduce the thermal stress, which improves mass transfer of the analyte to the detector [56]. Since it is difficult to obtain extracts without co-extracted components, matrix-matched calibration was used to compensate significant matrix effects and to improve the linearity, reliability, and accuracy of analytical results in GC-MS [56, 57]. As the results exhibited in Table 3, after revised by matrix-matched calibration, the relative matrix effects of four synthetic musks were below 10 %.

Quantification and method evaluation

The developed method was evaluated by recoveries, MDLs, MQLs, intra-day repeatability, and inter-day reproducibility with the optimized experimental procedures. Procedural blanks were tested during each batch of experiment; target compounds were not detected in the procedural blank samples, which excluded the background interference.

The recoveries of all target compounds, spiked at levels of 1, 5, and 10 ng/g ww for muscle samples, and 4, 20, and 40 ng/g ww for liver samples, are displayed in Table 4. The recoveries of most compounds were in the range of 50–150 % with relative standard deviations ≤5 %. Compared with those compounds with isotope-labeled internal standards, poor recoveries and higher derivations were usually found at the lowest spiked levels both in muscle and liver tissues for some compounds without isotopically labeled internal standards such as DEET, icaridin, itraconazole, and butylparaben. In particular, the quantitation of clotrimazole in liver was intensively disturbed by a strong adjacent interfering peak. As a potent antifungal agent, clotrimazole has a high binding affinity for heme to form a stable and soluble complex [58], which may result in its failure of extraction from liver samples. Figures 3 and 4 exhibit the LC-MS/MS and GC-MS chromatograms of the analytes in fish samples for the recovery experiment, with spiking concentrations of 10 and 40 ng/g

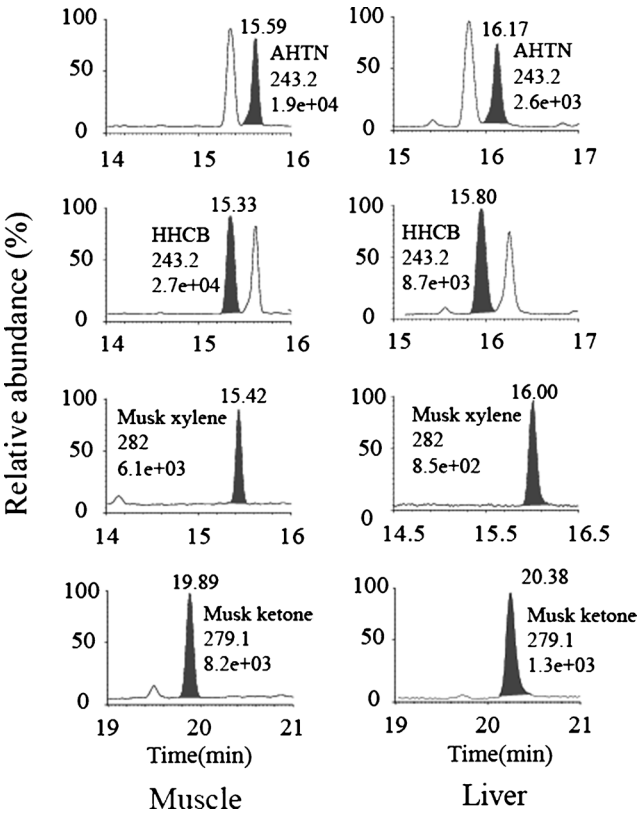


Fig. 4 GC-MS SIM chromatograms (EI source) of four synthetic musks spiked in fish samples before extraction at the concentration of 10 ng/g in wet weight muscle and 40 ng/g wet weight in liver for each analyte

Table 5 Concentration (ng/g wet weight) of target compounds in fish muscles collected from Dongjing River

Compound	Site S2			Site S3			Site S4		
	Detection	Range	Median	Detection	Range	Median	Detection	Range	Median
Biocides									
Fluconazole	6/9	<MQL–0.18	<0.13	6/6	<MQL	<0.13	3/3	<MQL	<0.13
Carbendazim	5/9	<MQL–0.97	nd	0/6	nd	nd	0/3	nd	nd
Thiabendazole	9/9	<MQL	<0.11	6/6	<MQL	<0.11	3/3	<MQL	<0.11
DEET	9/9	0.46–1.76	0.99	6/6	0.71–1.07	0.89	3/3	0.88–1.23	0.98
Icaridin	9/9	0.03–0.07	0.03	6/6	0.02–0.03	0.03	3/3	0.03	0.03
Ketoconazole	6/9	<MQL–0.43	<0.21	3/6	<MQL	<0.21	3/3	<MQL	<0.21
Climbazole	3/9	<MQL–2.3	nd	5/6	<MQL–2.17	<0.3	3/3	<MQL	<0.3
Clotrimazole	9/9	<MQL	<0.59	6/6	<MQL	<0.59	3/3	<MQL	<0.59
Miconazole	6/9	0.03–1.06	nd	2/6	0.17–0.18	nd	0/3	nd	<0.35
Methylparaben	9/9	0.54–0.9	0.63	6/6	0.42–11.8	0.67	3/3	0.43–0.62	0.47
Ethylparaben	9/9	0.59–0.94	0.65	6/6	0.51–0.65	0.57	3/3	0.52–0.57	0.57
Propylparaben	9/9	<MQL–0.25	<0.2	6/6	<MQL–0.33	<0.2	3/3	<MQL	<0.2
TCC	9/9	<MQL–10.5	0.36	6/6	0.28–3.01	0.93	3/3	1.04–2.74	1.35
TCS	9/9	0.58–5.24	1.45	5/6	0.94–9.23	1.47	3/3	2.94–5.47	3.20
Musks									
AHTN	9/9	<MQL–12.8	4.66	6/6	<MQL–2.63	1.17	3/3	<MQL–4.54	1.15
HHCB	9/9	<MQL–31.9	12.4	6/6	<MQL–38.3	5.11	3/3	<MQL–24.2	13.5
Musk ketone	1/9	9.96	9.96	1/6	2.4	2.40	1/3	10.1	10.1
Benzotriazoles									
BT	9/9	1.48–16.6	2.99	6/6	1.83–3.92	2.28	3/3	1.95–5.04	3.88
5-TT	9/9	0.84–1.68	1.25	6/6	0.46–1.2	0.88	3/3	0.81–1.35	1.01
CBT	9/9	<MQL–0.25	<0.21	6/6	<MQL	<0.21	3/3	<MQL	<0.21
XT	1/9	<MQL	nd	2/6	<MQL	nd	2/3	<MQL	<0.36

<*MQL* lower than the method quantitation limits, *nd* not detected

Table 6 Concentration (ng/g wet weight) of target compounds in fish liver samples collected from Dongjing River

Compound	Site S2			Site S3		Site S4	
	Detection	Range	Median	Detection	Concentrations	Detection	Concentration
Biocides							
Fluconazole	5/7	<MQL–0.72	<0.61	2/2	1.1, 1.91	1/1	<MQL
Thiabendazole	7/7	<MQL	<0.38	2/2	<MQL	1/1	<MQL
DEET	7/7	2.14–4.68	2.76	2/2	1.42, 2.15	1/1	4.22
Ketoconazole	2/7	<MQL	nd	2/2	<MQL	1/1	<MQL
Climbazole	3/7	<MQL–7.45	<1.16	2/2	<MQL, 1.83	1/1	1.00
Miconazole	4/7	1.14–1.15	<2.3	2/2	1.15	1/1	1.15
Methylparaben	7/7	1.85–2.96	2.41	2/2	1.92, 2.65	1//1	2.02
Ethylparaben	7/7	2.3–3.58	2.51	2/2	2.61, 2.56	1/1	2.40
Propylparaben	7/7	<MQL	<0.26	2/2	<MQL	1/1	<MQL
TCC	3/7	1.98–4.59	<1.17	2/2	<MQL, 7.14	1/1	2.07
TCS	7/7	2.43–25.3	10.00	2/2	25.4, 23.6	1/1	25.1
Musks							
AHTN	5/7	5.9–71.9	9.70	1/2	16.4	0/1	nd
HHCB	5/7	<MQL–31.2	<1.96	1/2	119	1/1	25.1
Benzotriazoles							
BT	7/7	8.71–36.2	11.3	2/2	26.9, 54.6	1/1	33.7
5-TT	7/7	<MQL–7.33	3.99	2/2	3.57, 4.24	1/1	10.1
CBT	7/7	<MQL	<0.98	2/2	<MQL	1/1	<MQL

<*MQL* lower than the method quantitation limits, *nd* not detected

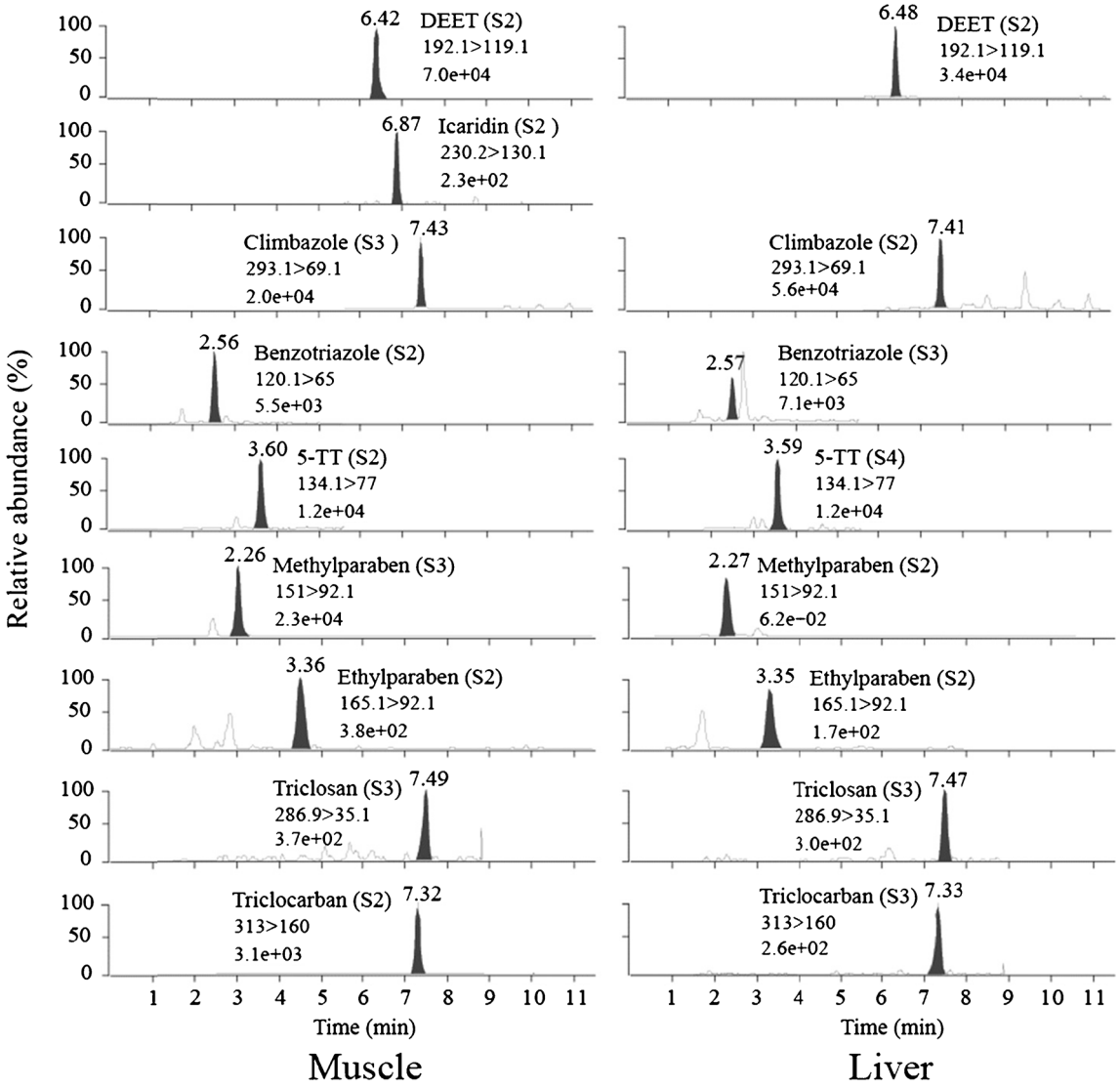


Fig. 5 LC-MS/MS MRM chromatograms of some analytes detected in wild fish muscle and liver samples. Site, retention time, precursor ion and production ion, and abundance for each analyte are indicated in the chromatogram

for muscle and liver samples, respectively. In general, the optimized method provides acceptable recoveries for most target compounds and can be applied to the determination of different PCP ingredients in fish muscle and liver matrices.

The MDLs and MQLs of the analytes in fish muscle and liver tissues are given in Table 4. In general, the complex matrices in liver samples increased the background noise, which resulted in higher MQLs in liver than in muscle tissues. The MQLs in this study are equal to or lower than the MQLs reported by other studies using Soxhlet extraction [22], pressurized liquid extraction [21], or ultrasonic extraction [59]. The MQLs for TCS, methylparaben, ethylparaben, HHCB, and musk ketone in this study are at the same magnitude as those previous reported works [60, 61].

Both intra-day and inter-day precision were also calculated based on the extraction and analysis results of standard mixture spiking in matrices with seven duplicates on the same day and in

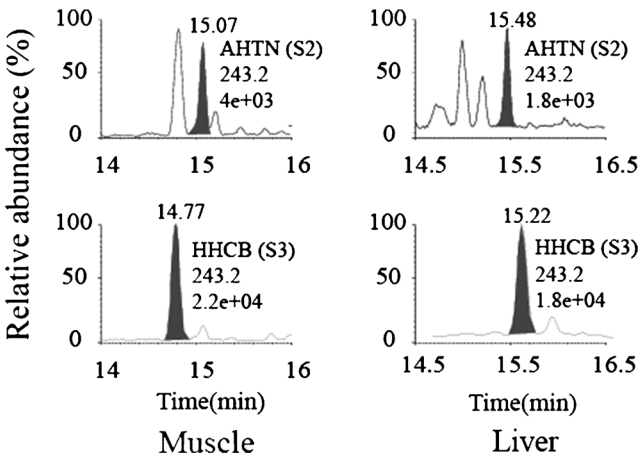


Fig. 6 GC-MS SIM chromatograms (EI source) of two typical musks detected in wild fish muscle and liver samples. Site, retention time, precursor ion and production ion, and abundance for each analyte are indicated in the chromatogram

2 weeks, respectively. As displayed in Table S6 (see ESM), the intra-day repeatabilities for the target compounds vary from 1.9 to 27.9 % and 0.8 to 35 % for muscle and liver samples, respectively, while the reproducibilities range from 2.1 to 27.1 % and 1.4 to 34.1 % for muscle and liver samples, respectively. For most of the compounds, both the intra-day and inter-day precision values were lower than 15 %, indicating that the optimized QuEChERS extraction method exhibited good reproducibility and repeatability.

Application to real samples

The optimized method was applied to analyze PCPs in wild fish samples from Dongjiang River in south China. In total, 18 fish muscle samples and 10 liver samples were obtained from three sites. The detection frequencies and concentrations of target analytes in fish muscle and liver tissues are displayed in Table 5 and Table 6, respectively. Meanwhile, the chromatograms of some analytes with high detection frequencies or concentrations in selected fish samples are also displayed in Figs. 5 and 6.

In total, 21 analytes (except for itraconazole, butylparaben, and musk xylene) and 17 analytes (except for clotrimazole, carbendazim, icaridin, itraconazole, butylparaben, musk xylene, and musk ketone) were detected at least one time in muscle and liver tissues, respectively. Most of the target compounds showed high detection frequencies, indicating bioaccumulation of these chemicals by wild fish. The concentrations for most analytes in liver tissue were higher than those in muscle tissue. The maximum detected concentrations for HHCB and BT were up to 119 and 54.6 ng/g ww in liver samples, respectively. The results of wide detection of DEET, AHTN, and HHCB were inconsistent with those reported by Baduel C et al. and Picot Groz M et al. [31, 61]. The investigated rivers receive discharge of domestic wastewaters from the cities in the region [7]. These have resulted in contamination of wild fish by PCP ingredients. Therefore, measures should be taken to reduce the input of these chemicals like PCPs into the riverine environment in order to protect the ecosystem and human health.

Conclusion

We developed a sensitive and selective sample preparation method based on QuEChERS extraction and d-SPE cleanup for simultaneous extraction of multi-classes of PCPs (18 biocides, 4 synthetic musks, and 4 benzotriazoles) in wild fish muscle and liver tissues, followed by UPLC-MS/MS and GC-MS analyses. High sensitivity, satisfactory recovery, and good quantitative precision are acquired for most of the target analytes. The developed method has been successfully applied to the determination of target compounds in wild fish samples, showing the potential application on the routine monitoring of PCP chemicals in biological samples.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval In this study, wild fish collection and ethical care were performed in strict accordance with the recommendations of Animal Care Quality Assurance in China.

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