Multi-target determination of organic ultraviolet absorbents in organism tissues by ultrasonic assisted extraction and ultra-high performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

A sensitive and reliable method was developed for multi-target determination of 13 most widely used organic ultraviolet (UV) absorbents (including UV filters and UV stabilizers) in aquatic organism tissues. The organic UV absorbents were extracted using ultrasonic-assisted extraction, purified via gel permeation chromatography coupled with silica gel column chromatography, and determined by ultra-high performance liquid chromatography–tandem mass spectrometry. Recoveries of the UV absorbents from organism tissues mostly ranged from 70% to 120% from fish filet with satisfactory reproducibility. Method quantification limits were 0.003–1.0 ng g^{-1} dry weight (dw) except for 2-ethylhexyl 4-methoxycinnamate. This method has been applied to analysis of the UV absorbents in wild and farmed aquatic organisms collected from the Pearl River Estuary, South China. 2-Hydroxy-4-methoxybenzophenone and UV-P were frequently detected in both wild and farmed marine organisms at low ng g^{-1} dw. 3-(4-Methylbenzylidene)camphor and most of the benzotriazole UV stabilizers were also frequently detected in maricultured fish. Octocrylene and 2-ethylhexyl 4-methoxycinnamate were not detected in any sample. This work lays basis for in-depth study about bioaccumulation and biomagnification of the UV absorbents in marine environment.

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1. Introduction

Organic ultraviolet (UV) absorbents, including UV filters and UV stabilizers are widely used in commodities such as cosmetics, sunscreens, and lotions to reduce harmful effects on skin and hair caused by solar radiation [1–3]. In addition, UV stabilizers are also applied in a variety of industrial products such as plastics, textile, building materials, and automobile components to prevent yellowing and degradation of the products due to their capability to effectively absorb UV-A and UV-B radiations [4]. Some of the organic UV absorbents are categorized as high production volume chemicals with great productions worldwide [1]. As a result, they have been widely found in human urine, water, dust, sediment, and organism tissues [5–21]. Some UV filters were quantified up to 3992 ng g^{-1} dry weight (dw) in mussels collected from beaches of south Portugal [19]. Presence of organic UV absorbents in the environment has drawn increasing concerns due to their ecological

potential, such as endocrine disrupting properties and acute toxicity [22–25]. Some organic UV absorbents were suspected to be responsible for coral bleaching by promoting viral infections [26]. Exposure to elevated benzophenone-3 levels was speculated to be associated with endometriosis of women [27]. However, compared to legacy contaminants, such as traditional persistent organic pollutants, environmental occurrence, behavior, and ecological impacts of the organic UV absorbents are far from well-studied.

Reliable and feasible analytical methods are prerequisite for comprehend research of occurrence, fate, and consequently ecological impacts of organic UV absorbents in the environment. So far, there have been some reports about analyzing UV filters or UV stabilizers in various environmental matrices [4,9,14,19,28–50]. The UV absorbents, including various UV filters or UV stabilizers were usually extracted from solid environmental matrices such as sediment, sewage sludge, and organism tissues using ultrasonic-assisted extraction [10], mechanical shaking [3,39], accelerated solvent extraction [9,14,36], soxhlet extraction [4], and microwave-assisted extraction [12,13,41]. The compounds were determined by gas chromatography–mass spectrometry (GC–MS) [39], GC–MS/MS [13,19], high performance

liquid chromatography–tandem mass spectrometry [3], and ultra-performance liquid chromatography–tandem mass spectrometry [9,36]. However, few efforts have been made to simultaneously analyze different groups of UV filters and stabilizers in environmental samples. It is known that UV absorbents can enter the environment indirectly via wastewater and directly via washoff through recreational activities as swimming and leaching from polymers [13]. It is therefore very likely that various UV absorbents accumulate concurrently in the environment, especially in coastal waters where are usually served for agricultural (e.g., fishing and mariculture), industrial (e.g., shipping and offloading) as well as recreational purposes. In addition, most of the available research focused on water and sediment [30,31,33–41,45–47] whereas methods for determination of the UV filters and stabilizers in organism tissues are still limited [4,34,43]. However, many organic UV absorbents have potential for bioaccumulation and biomagnification through trophic web given their strong lipophilicity [14]. Thus, it is valuable to develop a sensitive method for simultaneously measuring commonly used organic UV filters and stabilizers in organism tissues in order to better illustrate their occurrence and consequently ecological potentials in the environment.

In China, more than 20 organic UV absorbents, including cinnamate, dibenzoylmethane derivatives, benzophenone derivatives, salicylate, camphor derivatives, cyanoacrylate, benzoate, benzotriazole derivatives, are being allowed to be added in cosmetics with contents of 4–10%, with the top three being 2-ethylhexyl 4-methoxycinnamate, avobenzone, and benzophenone-3 [44,51,52]). In addition, benzotriazole derivatives (i.e. tinuvins) are one group of UV stabilizers with the highest production and most widespread use in plastics, especially in automobile surface coatings [10]. Consumption of UV stabilizer has reached 7000 t in 2010 in China [52]. Available researches have revealed the presence of some UV filters and UV benzotriazole stabilizers in wastewater [53], sewage sludge [54], sediment [39], and human blood and urine in China [55,56].

This work aimed to develop a feasible and sensitive method for multi-target determination of trace amount of most commonly commodity and industrially applied organic UV absorbents in aquatic organism tissues. The targets included six UV filters and seven benzotriazole UV stabilizers belonging to several groups as shown in Table 1. In addition to their widespread use and high production, the investigated UV absorbents are moderately to highly hydrophobic indicated by their log K_{OW} values (Table 1), implying their moderate to high potential for bioaccumulation. The UV absorbents were extracted by ultrasonic-assisted extraction, purified by gel permeation chromatography (GPC) coupled with silica gel column fractionation, and finally determined by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS). The method was validated and applied to detect the UV absorbents in wild and farmed aquatic organisms collected from the Pearl River Delta and Estuary, South China. This work laid basis for comprehensive research about bioaccumulation/biomagnification of the UV absorbents in the environment. To the best of our knowledge, this is the first work about bioaccumulation of the UV absorbents in aquatic organisms in China.

2. Experimental

2.1. Chemicals and reagents

The UV absorbent standards and deuterated standard benzophenone-d$_{10}$ (BP-d$_{10}$) were purchased from Sigma–Aldrich (St. Louis, MO, USA) with purity of 97% or higher. Deuterated standard (±)3-(4-methylbenzylidene)camphor-d$_4$ (4-MBC-d$_4$) (98%) was purchased from C/D/N isotopes (Pointe-Claire, Quebec,

Canada). The structures and physicochemical properties of the UV absorbents are summarized in Table 1. HPLC methanol, acetonitrile, dichloromethane, hexane, formic acid, and ammonium acetate were purchased from Merck (Darmstadt, Germany). Ultra-pure water was generated by a Milli-Q ultra-pure water system (Millipore, Billerica, MA, USA). Analytical grade ethyl acetate and cyclohexane were bought from Fuyu Chemical (Tianjing, China) and were redistilled before use.

Stock standard solutions were prepared in methanol individually at 10 mg L^{-1}. A working standard solution containing all the analytes was subsequently prepared in methanol. All the standard solutions were stored in amber glass vials and kept at −20 °C.

2.2. Samples and preparation

2.2.1. Samples

Red snappers (about 2800 g) were collected from a mariculture farm located in the Pearl River Estuary, South China. Several wild aquatic organisms (e.g., hairtail, squid, goby, pomfret, and squilla) were collected from the Pearl River Estuary using trawling in April 2013. The organism samples were stored at −20 °C until treatment.

2.2.2. Extraction

Fishes, hairtails, and squids were skinned while squillas were deshelled before being dissected carefully with a stainless steel scalpel. Only carcasses of the organisms were used in this work whereas the heads and internal organs were put away. For the big red snappers, the filet and belly were collected separately, while for the wild species, the whole body was used due to their small sizes.

Samples were freeze-dried, ground, and homogenized. About 4 g of each sample (2 g for the belly) was weighed into a 50-mL polytetrafluoroethylene centrifugal tube (Kimble, Vineland, NJ, USA) and spiked with internal standards BP-d$_{10}$ and 4-MBC-d$_4$ at 100 ng g^{-1} dw (dw). The sample was then added with 20 mL of methanol and shaken on a vortex mixer (XW-80A Mixer, Shanghai, China) for 2 min prior to being subjected to ultrasonic-assisted extraction on a YJ-5200D ultrasonic water bath (40 kHz, 300 W) for 15 min. The sample was then centrifuged at 4000 rpm for 10 min at 4 °C (Avanti^{TM}30 centrifuge, Beckman, CA, USA). The clear supernatant was collected in a glass tube. The above extraction procedure was repeated three times and the extracts were combined (about 60 mL in total). One tenth of the extract was split for lipid content measurement and the rest was concentrated on a Syncore® Polyvap R-12 evaporator (Buchi, Flawil, Switzerland) to just dryness.

2.2.3. Cleanup and fractionation

The extract was re-dissolved in 1 mL of ethyl acetate/cyclohexane (50/50, v/v) prior to being subjected to a glass GPC column (1 cm × 40 cm) packed with Biobeads S-X3 (200–400 mesh, Bio-Rad Laboratories, Hercules, CA, USA) for lipids removal. The analytes were eluted with ethyl acetate/cyclohexane (50/50, v/v). The first 15 mL of eluate was discarded and the following 16 mL were collected. The collected eluate was concentrated and the solvent was exchanged to hexane prior to further purification with silica gel column (0.7 cm × 15 cm) fractionation. The UV absorbents were eluted with 15 mL of dichlomethane/ethyl acetate (50/50, v/v) from the silica gel column. The sample was brought to dryness under a gentle stream of nitrogen and then reconstituted in 1 mL of methanol prior to UHPLC–MS/MS analysis.

The lipid content was determined by gravimetric analysis. One-tenth of the ultrasonication extract was transferred into a pre-weighed glass vial and was evaporated to complete dryness. The content of lipids was calculated by the difference of the weight of the vial before and after addition of the extract.

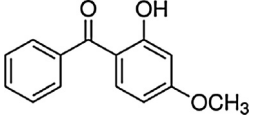**Table 1**
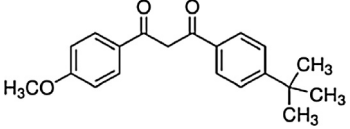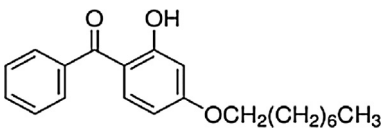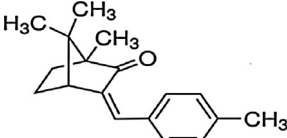The investigated UV absorbents and their structures.
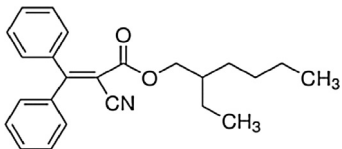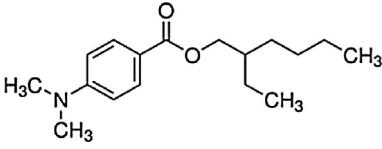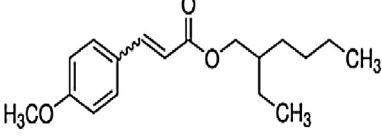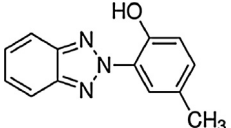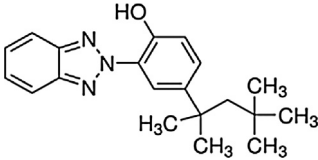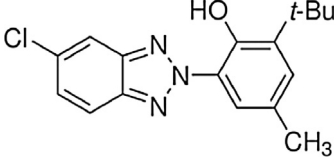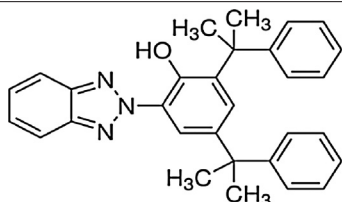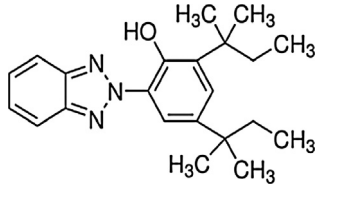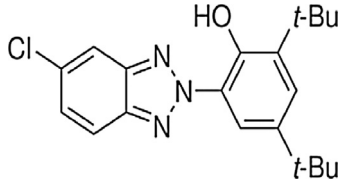
Group	Compound (abbreviation)	CAS. No.	Log K_{OW}	Structure
Benzophenones	2-Hydroxy-4-methoxybenzophenone (BP-3)	131-57-7	3.52[a]	
	Avobenzone (AVO)	70356-09-1	2.41[b]	
	UV-531	1843-05-6	6.42[c]	
Camphor	3-(4-Methylbenzylidene)camphor (4-MBC)	36861-47-9	5.47[a]	
Cyanoacrylates	Octocrylene (OCR)	6197-30-4	6.88[a]	
Benzoates	Octyldimethylparaaminobenzoate (ODPABA)	21245-02-3	5.77[b]	
Cinnamates	2-Ethylhexyl 4-methoxycinnamate (EHMC)	5466-77-3	5.80[b]	
Benzotriazoles	UV-P	2440-22-4	3.00[a]	
	UV-329	3147-75-9	6.21[a]	
	UV-326	3896-11-5	5.52[a]	

Table 1 (*Continued*)

Group	Compound (abbreviation)	CAS. No.	Log K_{OW}	Structure
	UV-234	70321-86-7	7.67[a]	
	UV-328	25973-55-1	7.22[d]	
	UV-327	3864-99-1	6.75[d]	

[a] [10].
[b] [2].
[c] http://www.sigmaaldrich.com/.
[d] [39].

2.3. UHPLC–MS/MS identification and quantification

The analytes were determined on a Thermo ACCELA UHPLC system coupled with a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA) with atmospheric pressure chemical ionization (APCI) interface operated in positive mode.

Separation was achieved on a 2.1 mm × 50 mm ACQUITY BEH C18 column (1.7 μm of particle size, Waters, Milliford, MA, USA) pre-connected with a 4-mm guard column packed with the same material (Phenomenex, CA, USA). The mobile phase was comprised of ultrapure water with 0.2% formic acid and 5 mM ammonium acetate (mobile phase A) and methanol (mobile phase B). The flow gradient started with 60% B and linearly increased to 75% B in 6 min and hold for 6 min, then to 100% B and hold for 6 min, and finally back to 60% B. A post-time of 10 min was set after each run for column equilibration. The injection volume was 5 μL. The solvent flow rate was 0.40 mL min^{-1} and the column temperature was kept at 40 °C.

The capillary voltage was 4000 V and temperature was 350 °C. Nitrogen was used as both the sheath gas and aux gas at 13.3 and 10 L min^{-1}, respectively. A precursor ion and two product ions were chosen for each analyte in full scan mode and product ion scan mode, respectively. S-lens and collision energy were optimized in selected ion reaction mode (SRM). The working parameters for each analyte are detailed in Table 2. Data acquisition was performed in SRM mode. Identification of the analytes in the environmental samples was achieved by comparing the retention time and the ratio of the two selected ion transitions with those of the standards. The relative standard deviations (RSDs) of the retention times and ion transition ratios should be within 2% and 20%, respectively.

Instrument control and data acquisition were managed with Thermal XCalibur (Thermo Scientific, San Jose, CA, USA). Quantification was performed by internal standard method using deuterated compounds (BP-d$_{10}$ and 4-MBC-d$_{4}$) as the internal standards (Table 2). A 10-point calibration curve from 0.01 to 200 μg L^{-1} was established for each analyte.

2.4. Method validation

Recovery tests were performed by fortifying the filet of the red snappers with the mixture standard solution at 5, 50, and 100 ng g^{-1} dw and fortifying the belly samples at 100 ng g^{-1} dw, respectively. The fortified samples were kept at 4 °C overnight prior to treatment in the same way as the corresponding non-fortified samples. The recovery was calculated by the following equation:

$$\text{Recovery}(\%) = \frac{C_{SO} - C_O}{C_S} \times 100 \tag{1}$$

C_{SO} and C_O is the measured concentration in the fortified and corresponding non-fortified samples, respectively, and C_S is the spiking concentration.

Matrix effect (ME) was evaluated by the protocol described previously [57]. The standard solutions (100 ng mL^{-1}) were added into sample extracts and analyzed with UHPLC–MS/MS. The matrix effects were then calculated as the ratio of signal response of the analyte in matrix extract and in standard solutions deducting 1. ME of 0 demonstrated no matrix effect while ME > 0 or ME < 0 represented signal enhancement or suppression, respectively. The bigger the absolute value is, the stronger the matrix effect tends to be.

A standard solution containing 100 μg L^{-1} of each analyte was subjected to ultrasonic treatment for 45 min to monitored stability of the UV absorbents during ultrasonication.

A procedural blank and a duplicate sample were set in each batch of 6–8 samples to evaluate the procedural contamination and method reproducibility, respectively. A standard solution containing all the analytes and a solvent blank were injected along with every 10 samples to assess the instrument performance and potential contamination.

Table 2
The optimum UHPLC–MS/MS parameters for the analytes and method performance.

Analyte	Ion transition[a]	S-lens (V)	Collision energy (eV)	RSD[b] (%)	LOQ[c] (ng g^{-1} dw)	Internal standard
BP-3	**229 → 151**	54	19	4.3	0.08	BP-d$_{10}$
	229 → 105	54	19			
4-MBC	**255 → 212**	73	19	2.9	0.2	4-MBC-d$_4$
	255 → 171	73	19			
OCR	**362 → 232**	51	33	4.6	0.1	4-MBC-d$_4$
	362 → 204	51	20			
ODPABA	**278 → 166**	75	29	10.3	0.005	4-MBC-d$_4$
	278 → 151	75	19			
AVO	**311 → 161**	58	22	8.8	1	BP-d$_{10}$
	311 → 135	58	21			
EHMC	**291 → 161**	39	27	3.5	10	4-MBC-d$_4$
	291 → 133	39	17			
UV-P	**226 → 120**	60	19	5.9	0.01	4-MBC-d$_4$
	226 → 107	60	18			
UV-531	**327 → 215**	54	26	17.9	0.005	4-MBC-d$_4$
	327 → 137	54	18			
UV-329	**324 → 212**	116	26	18.2	0.003	4-MBC-d$_4$
	324 → 57	116	23			
UV-326	**316 → 260**	113	26	6.3	0.01	4-MBC-d$_4$
	316 → **107**	113	19			
UV-234	**448 → 370**	120	33	7.1	0.004	4-MBC-d$_4$
	448 → 119	120	19			
UV-328	**352 → 282**	74	29	8.7	0.6	4-MBC-d$_4$
	352 → 212	74	22			
UV-327	**358 → 302**	124	26	8.9	0.6	4-MBC-d$_4$
	358 → 246	124	21			
BP-d$_{10}$	**193 → 110**	45	32			
	193 → 82	45	16			
4-MBC-d$_4$	**259 → 216**	68	16			
	259 → 160	68	18			
	259 → 174	68	17			

[a] The product ion in bold is for quantification.
[b] Relative standard deviation of replicate analysis of standard solution at 50 ng mL^{-1} (n = 6).
[c] Method quantification limit based on 4 g of sample.

Considering widespread usage of the UV absorbents, great precautions were taken in the whole experiment to minimize potential contamination. Personnel conducting the samplings and experiments were not allowed to wear cosmetics and body lotions possibly containing the UV absorbents. Nitrile surgical gloves were used throughout the whole procedure. All glassware was baked at 450 °C for 4 h and the steel tweezers and scalpels were washed successively with HPLC grade methanol and ultrapure water prior to use. Samples were stored in dark and the GPC and silica gel chromatography were performed in fume hood with light off to reduce photodegradation and other potential interferences as much as possible.

3. Results and discussions

3.1. Optimization of UHPLC–MS/MS

Methanol and acetonitrile were evaluated as the organic mobile phase for optimizing the separation and sensitivity. Methanol generated better peak shapes and gave higher intensity for the analytes and therefore was selected as the organic mobile phase. Volatile additives, e.g., ammonium acetate, ammonium formate, and formic acid have usually been chosen as mobile phase additives to improve sensitivity of MS detection for many pharmaceuticals [58]. Hence, ultrapure water added with ammonium acetate (1 and 5 mM) and formic acid (0.1% and 0.2%) was tested as the aqueous mobile phase.

The result showed that ultrapure water with 0.2% formic acid and 5 mM ammonium acetate (pH 2.9) gave the best sensitivity for the analytes.

Montesdeoca-Esponda et al. [45] observed co-elution of some benzotriazole UV stabilizers (e.g., UV-326, UV-327 and UV-328) on an octadecyl silica-based RP UHPLC column. Although complete separation is not indispensable in SRM mode, sufficient separation helps to improve detectability and to reduce ion suppression [58], whereas co-elution may impair proper quantification of the analytes due to competitive ionization [34]. Elution gradient was therefore optimized to ensure sufficient separation of the analytes. Baseline separation and good peak shapes were achieved for the analytes as shown in Fig. 1 using the gradient described above.

APCI and electronic spray ionization (ESI) have been widely used in determination of various UV absorbents [12,32,34,35,37,40–43,45–47,49,50]. ESI was reported to provide better sensitivity for compounds of medium to high polarity and APCI is favorable for ionization of compounds with low polarity [47,59]. Considering low to medium polarity of the analytes, both APCI and ESI modes were tested to optimize the MS conditions. Most analytes showed obviously stronger signal responses in APCI mode except EHMC and ODPAPB that showed slightly higher sensitivity in ESI mode. In addition, ESI gave especially poor sensitivity to AVO that could hardly for quantification. Thus, APCI was finally adopted for determination of the analytes. The optimum MS parameters are documented in Table 2.
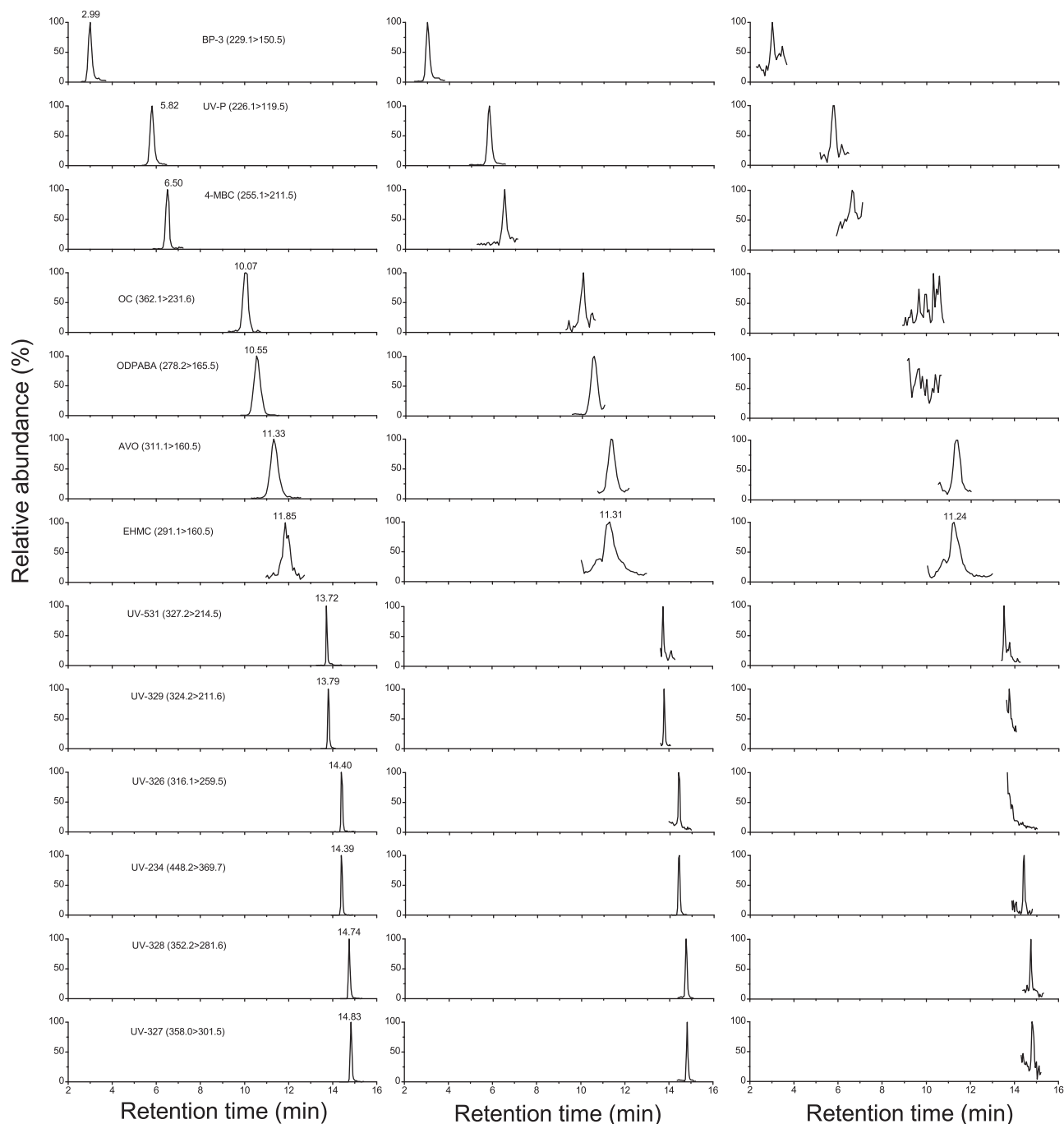
Fig. 1. Selected ion transition chromatograms the organic UV absorbents in standard solution at 5 μg L^{-1} (left), spiked fish filet sample at 5 μg L^{-1} (middle), and the unspiked fish filet sample (right). Figures on top of the peaks are retention times.

3.2. Optimization of extraction

Impacts of the type and volume of extraction solvent and extraction cycles on the extraction efficiency were investigated. Previous study reported satisfactory extraction efficiency by mixture of dichloromethane and hexane (50/50, v/v), acetone and heptane (1/1, v/v), and hexane and acetone for some UV filters and stabilizers in fish and marine mammals [11,13,34,43]. Nakata et al. [4] used mixture of dichloromethane and hexane (8/1, v/v) to extract benzotriazole UV stabilizers from organisms. Acetonitrile was used to extract EMHC, ODPABA, OCR, UV-P, and UV-326 from marine mussels [19]. Given the wide range of the UV absorbents in this

study with varying lipophilicity (Table 1), in combined consideration of cost effectiveness and environmental friendliness, methanol and dichloromethane/hexane (50/50, v/v) were tested to extract the UV absorbents from fish filet and belly samples. Comparable and satisfactory recoveries were obtained for the UV filters using the two solvents by fortifying the samples with standard solution at 100 ng g^{-1} dw. However, for most of the benzotriazole UV stabilizers, better recoveries were obtained using methanol (Fig. 2). Therefore, methanol was finally used as the extraction solvent.

Regarding the extraction cycles, a fourth extraction was performed after the fortified samples have been extracted three times and the extract was collected and analyzed separately. Only
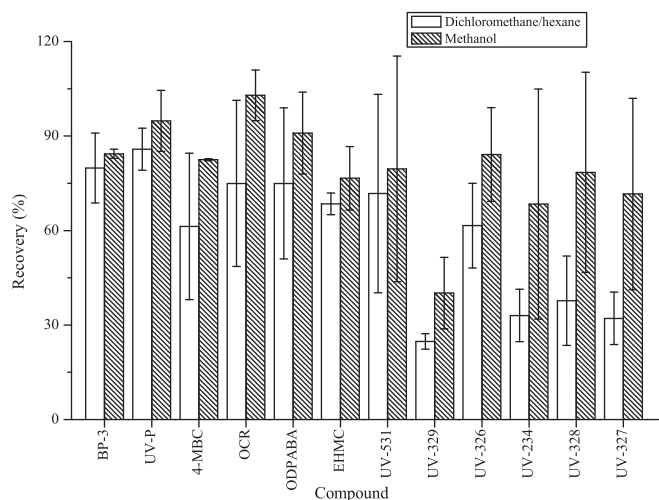
Fig. 2. Recoveries (%) of the organic UV absorbents from fish filet with different extraction solvents (n = 3). Full names of the compounds see in Table 1.

Fig. 3. Recoveries (%) of the UV absorbents from fish filet and matrix effect (ME, %) before and after silica gel column fractionation (n = 3). Full names of the compounds see in Table 1.

negligible quantities of some benzotriazole compounds (e.g., UV 327 and UV 328) were detected in the fourth extract. Therefore, three cycles were finally adopted.

The UV absorbents did not show significant losses after ultrasonication for 45 min, indicating feasibility of ultrasonic assisted extraction for the analytes, which was in line with the result of Moreta and Tena [60].

3.3. Purification

It is indispensable to remove lipids that are abundant in extracts of organism tissues prior to instrumental analysis because the lipids may cause strong interference and lead to seriously biased results [14]. Several protocols have been applied for lipids removal, such as HPLC fractionation [13,28], sulfuric acid digestion [42,43], freezing-lipid filtration [61], and GPC purification [4]. In this work, hexane–methanol partitioning, sulfuric acid digestion, freezing-lipid filtration, and GPC were tested to purify the ultrasonication extracts. Sulfuric acid digestion caused significantly decomposition of the UV absorbents and thus was not applicable. Hexane–methanol partitioning did not give satisfactory purification for all the UV absorbents, with spiky peaks and low sensitivities. Freezing-lipid filtration showed only good effect on some compounds (e.g., BP-3, 4-MBC, and EHMC). However, most benzotriazole UV stabilizers did not get satisfactory purification after freezing-lipid filtration. In addition, OCR and AVO could hardly be determined due to strong interference after both freezing and hexane–methanol partitioning. In contrast, GPC gave relatively better purification efficiency for all the UV absorbents. However, some analytes still suffered from serious matrix interference after GPC as shown by poor reproducibility of recoveries and matrix effects (Fig. 3a and b) as well as tailing peaks because some low mass weight lipids may co-present in relatively high amounts compared with trace levels of the UV absorbents. Solid phase extraction (SPE) and silica gel column fractionation were therefore tested for further purification considering their wide uses, simplicity, and cost-effectiveness [4,15,43]. The result demonstrated that SPE with HLB absorbent (Waters, Milford, MA, USA) did not significantly improve either the peak shapes or reproducibility. On the other hand, the analytical reproducibility was obviously improved for most analytes following the silica gel column fractionation (Fig. 3a and b), indicating the matrix interference has been further removed. Therefore, GPC coupled with silica gel column fractionation were adopted for sample purification in order
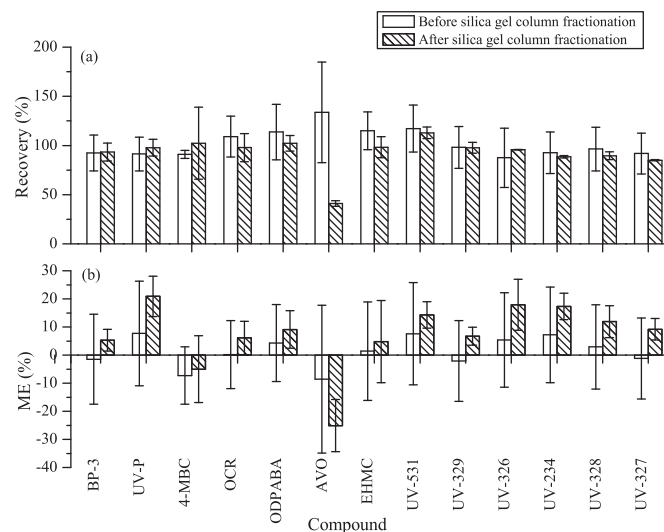
to ensure appropriate determination of the UV absorbents at trace levels in the complex organism tissues.

3.4. Method validation

Recoveries obtained from fortified fish filet were 70–120% with good reproducibility for most analytes at three spiked levels for most analytes (Table 3). Lower recoveries from the fish belly sample (42–94%, Table 3) could possibly be ascribed to the higher lipid content (Table 4) and subsequently stronger matrix effect, especially for those compounds with high lipophilicity, such as UV-324, UV-237, and UV-238. However, recovery could not be calculated for EHMC at low spiked level of 5 ng g^{-1} dw due to strong matrix interference (Fig. 1 and Table 3). Relatively low recoveries were obtained for AVO at the spiked concentration of 50 ng g^{-1} dw from filet sample and UV-326 from belly sample. Besides, reproducibility was not good enough for 4-MBC at the spiked level of 50 ng g^{-1} dw. More work is therefore needed to improve sample treatment, especially purification in the future.

Efficient extraction of some benzotriazole UV stabilizers has been a challenge as reported previously [45]. However, satisfactory recoveries were obtained for the benzotriazole UV stabilizers from fish filet and fish belly samples, comparable with those for

Table 3
Recoveries (%, mean ± SD, n = 3) of the UV absorbents.

Analyte	Spiking level			
	Fish filet			Fish belly
	5	50	100	100
BP-3	88.3 ± 2.1	93.4 ± 9.1	102.0 ± 9.8	93.6 ± 13.2
UV-P	78.9 ± 2.6	97.8 ± 8.5	94.0 ± 11.3	70.6 ± 5.2
4-MBC	86.0 ± 6.2	102.4 ± 36.6	95.8 ± 8.2	80.8 ± 7.2
OCR	115.6 ± 14.2	97.8 ± 14.3	105.0 ± 9.9	87.9 ± 13.0
ODPABA	85.5 ± 2.5	102.3 ± 7.9	92.4 ± 9.6	64.2 ± 6.4
AVO	74.2 ± 12.6	41.1 ± 2.9	82.8 ± 6.7	58.4 ± 5.5
EHMC	Interference[a]	98.3 ± 10.6	109.5 ± 5.4	81.1 ± 5.2
UV-531	119.8 ± 1.5	112.9 ± 5.9	88.9 ± 8.7	74.3 ± 15.5
UV-329	54.8 ± 17.3	97.7 ± 5.6	101.6 ± 11.6	91.4 ± 2.0
UV-326	77.7 ± 0.8	95.8 ± 0.3	74.0 ± 5.4	42.2 ± 10.2
UV-234	79.3 ± 1.4	88.6 ± 1.3	90.5 ± 4.7	65.3 ± 2.6
UV-328	77.7 ± 4.2	89.6 ± 4	78.7 ± 4.6	58.1 ± 6.4
UV-327	70.4 ± 0.4	85 ± 0.5	73.4 ± 6.7	55.7 ± 9.4

[a] Calculation could not be performed due to matrix interference.

Table 4

Occurrence of the UV absorbents in the marine organism tissues of the Pearl River Estuary, South China (ng g^{-1} dw).

Species	Lipid (%)	BP-3	UV-P	4-MBC	ODPABA	AVO	UV-531	UV-329	UV326	UV-234	UV-328	UV-327
Wild species												
Pomfret	12.0	ND	1.28	ND	ND	ND	ND	ND	ND	ND	ND	ND
Goby	8.2	0.276	1.05	ND	ND	ND	ND	0.105	ND	ND	ND	ND
Flounder	8.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Osteomugil	8.5	ND	0.03	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hairtail	8.9	0.106	3.33	ND	ND	ND	ND	ND	ND	ND	ND	ND
Anchovy	8.9	ND	1.22	2.3	ND	ND	ND	ND	ND	ND	ND	ND
Arrow fish	2.5	1.068	0.47	ND	ND	ND	ND	ND	ND	ND	ND	ND
Collichthys	11.1	0.797	2.58	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sleeve-fish	19.7	0.408	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Squilla	18.7	1.520	0.07	ND	ND	ND	ND	0.225	ND	ND	ND	ND
Whelk	8.7	ND	ND	0.2	ND	ND	ND	ND	ND	ND	ND	ND
Farmed red snapper												
Filet	9.9	0.59 ± 0.12	0.40 ± 0.02	14.7 ± 4.5	0.239 ± 0.039	33 ± 12	7.898 ± 0.301	ND	7.95 ± 1.01	0.202 ± 0.031	<LOQ	1.0 ± 0.1
Belly	11.6	0.80 ± 0.17	0.67 ± 0.02	41.5 ± 2.7	0.36 ± 0.057	52 ± 14	12.141 ± 0.369	ND	11.38 ± 1.04	0.260 ± 0.029	0.8 ± 0.1	1.8 ± 0.1

ND, not detected; LOQ, method quantification limit. The UV absorbents not detected or non-quantifiable in any sample were not listed.

sediment [10,39] and fish samples [34]. Recoveries for the other UV filters fell in range of those reported previously for sediment [10] and organisms [13]. Overall, the gained recoveries for the UV absorbents in this work were acceptable for multi-target extraction in such a complex environmental matrix as organism tissues.

The instrumental limit of quantification for each analyte was determined as the lowest concentrations giving a ratio of signal to noise (S/N) of 10. The method limits of quantification (LOQ) were thereafter estimated based on the instrumental limits of quantification, recoveries, matrix effects, and concentration factors, which were in the range of 0.003–1.0 ng g^{-1} dw based on 4 g of sample except for EHMC (10 ng g^{-1} dw) due to strong matrix interference at low levels (Table 2). This result was better or comparable with those reported previously [4,13,15,19,33].

The matrix effects ranged from −25 ± 9% to 21 ± 7% (Fig. 3b), which were satisfactory for multi-target determination of trace analytes in complex environmental matrices. In addition, use of the deuterated internal standards helped counteracting the matrix effect. However, isotope-labeled counterparts were not commercially available for all the UV absorbents during the period of this work. More efforts will be made in our future work to find a more appropriate internal standard for each analyte to improve the quantification accuracy.

The instrumental precision was monitored by injection of standard solution at 50 ng mL^{-1} at the beginning, in the middle, and at the end of each analyzing batch, with RSD of 2.9–18.2% (Table 2).

Trace amount of OCR, UV-531, and UV-234 were found in the blanks. Concentrations of these UV absorbents in environmental samples were corrected by subtracting their average concentrations in the blanks.

3.5. Application to environmental samples

The developed method was applied to measure the UV absorbents in farmed and wild marine organisms collected from the Pearl River Estuary, South China. BP-3 and UV-P were widely detected in the wide and farmed organisms at low ng g^{-1} dw. 4-MBC was only occasionally observed in the wild species, whereas it was detected up to 41.5 ng g^{-1} dw in the belly of the maricultured red snapper. OCR and EHMC were not detected in any sample although they were reported to be widespread and as high as 7112 ng g^{-1} in wild mussels from Europe [13,19]. The benzotriazole UV stabilizers were rarely detected in the wild organisms while UV-531, UV-326, UV-P, UV-234, UV-328, and UV-327 were detected above the limits of quantification in both filet and belly of the maricultured snapper. Concentrations of the UV absorbents were higher in the belly than in the filet of

the fish (Table 3). The detection frequencies and concentrations of the UV absorbents in the wild aquatic organisms from the Pearl River Estuary were relatively lower than those reported in other regions [4,5,7–9,12,13,15,19,33,41]. Sources and bioaccumulation/biomagnification of the UV absorbents in the marine organisms warrant further research.

4. Conclusions

A sensitive and feasible method was developed and validated for simultaneous determination of several groups of commonly used UV absorbents in aquatic organism tissues. These UV absorbents are widely applied in commodities and industrial products. The UV absorbents were extracted from organism tissues by ultrasonic-assisted extraction, purified by GPC coupled with silica gel column fractionation, and finally determined by UHPLC–MS/MS. Satisfactory recoveries and analytical reproducibility were obtained for most analytes and the limits of quantification ranged from 0.003 to 1 ng g^{-1} dw. The method was applied to detect the UV absorbents in wild and farmed marine organisms collected from the Pearl River Estuary, South China. BP-3 and UV-P were frequently detected in the organisms while the benzotriazole UV stabilizers were mostly observed in the maricultured fish.

This work developed a method for simultaneous analysis of several groups of UV filters and UV stabilizers that were usually determined separately in environmental matrices in previous researches. Given their likely concurrence in the environment due to the similar sources, this multi-target method can be a useful tool for in depth study of bioaccumulation and biomagnification of the UV absorbents in the environment and consequently impacts on ecological systems.

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