Research article

Occurrence of benzotriazole UV stabilizers in coastal fishes

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ABSTRACT

Chemicals added in personal care products are of emerging concern because their fate and their effect on the environment is not completely known. Benzotriazole UV stabilizers (BUVSs) are compounds used in different cosmetic products, which may reach the marine environment through marine discharge from treated waters or directly from bathing areas. Once released into the aquatic ecosystem, BUVSs can be bioaccumulated by aquatic organisms. To identify the human exposure risk, it is important to have suitable analytical methods to determine the presence of BUVSs in these organisms. Because of the complexity of such a biological matrix, selective extraction and detection techniques are required to isolate and quantify these kinds of pollutants at trace levels. In the present work, we optimized a method based on microwave-assisted extraction combined with ultra-high performance liquid chromatography and mass spectrometry detection to determine six benzotriazole compounds in fish samples. The absolute extraction yields provided by the proposed method were higher than 40% for most compounds, with intra-day and inter-day relative standard deviations ranging from 0.27 to 6.06 and 1.12–21.3%, respectively. The limits of quantification were in the range of 1.13–9.66 ng g^{-1} (dry weight). The method was applied to the study of three species of fish (*Boops boops, Sphyraena viridensis, Sphoeroides marmoratus*) that were collected close to three marine outfalls of treated waters on the Gran Canaria Island (Spain) for two years. Four of the six studied compounds, UV-326, UV-328, UV-329 and UV-360, were found with concentrations ranging from 1.34 ng g^{-1} to 45.6 ng g^{-1} (dry weight).

1. Introduction

The evolution of industry has brought important benefits to society. However, the increased quality of life is connected to the introduction of chemical substances in the environment. The European Union states that, from a list of more than 2700 potential marine contaminants, the majority of contaminants are emerging pollutants whose fate and potential effects are poorly understood. Although a majority of these compounds is currently not included in control procedures, the European Union could incorporate them in a "Watch List", a vigilance programme for pollutants that contains candidates to be considered in future regulation (Loos et al., 2018). Among these compounds, the group of personal care products (PCPs) is one of the most diverse. Cos-metics and products related with the sun have gained special interest because of their increasing use due to the growing concern of the population regarding skin protection (Benedé et al., 2014).

Benzotriazole ultraviolet stabilizers (BUVSs) are ingredients commonly added to sunscreens, makeup, lipsticks or hair dyes. Once used, they can reach the environment directly during swimming or other aquatic activities or indirectly by the discharge of sewage, because these compounds pass through wastewater treatment plants without being efficiently removed (Mao et al., 2020; Molins-Delgado et al., 2015). As a consequence, different BUVSs have been found in several samples including wastewater, seawater, sediments and sludges (Montesdeoca-Esponda et al., 2013a,b). On the other hand, plastic debris including microplastics are also a potential source of BUVSs due to their use as an antiaging additive (Rani et al., 2015, 2017).

The occurrence of BUVSs in marine organisms has also been

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demonstrated, with measured concentrations ranging from thousandths to tens of ng/g in fish (Kim et al., 2011a, 2011b; Peng et al., 2015), molluscs (Nakata et al., 2012), crustaceans (Peng et al., 2015), and even mammals (Nakata et al., 2010). BUVSs have also been found in the blood plasma of fish, turtles, birds and dolphins (Lu et al., 2016, 2019).

The entry of this kind of pollutant could be especially dangerous in coastal tourist areas (Tovar-Sa□nchez et al., 2019) such as the Canary Islands, with tourists visiting throughout the year looking for sun and rest at the beach/pool. Therefore, the presence of very crowded touristic beaches and marine outfalls could act as substantial inputs of BUVSs for organisms living close to the coast, so it is essential to know the occurrence of BUVSs in different marine species, especially in fish because they represent food for humans and bigger animals. Due to the lipophilic character of BUVSs (with log K_{ow} coefficients of up to 12), they are more likely to be stored rather rapidly than if they are metabolized or excreted (Gago-Ferrero et al., 2012) and they can be bioaccumulated and biomagnified.

Regarding the worldwide occurrence of BUVSs in fish, few data are available. However, different approaches have been developed to determine BUVSs in freshwater fishes. They have been measured in rivers located in Germany (up to 65 ng g^{-1}) (Wick et al., 2016), India (up to 79 ng g^{-1}) (Vimalkumar et al., 2018), China (up to 3.3 ng g^{-1}) (Peng et al., 2015) or Canada (up to 3.9 ng g^{-1}) (Lu et al., 2016).

The lack of information about the presence of these compounds is especially warning for marine species (Table 1). BUVSs have been found in marine fish species collected in the Manila Bay (Philippines) (Kim et al., 2011a, 2011b), with the highest detection frequencies for UV-P, UV-320 and UV-328 and greater concentrations detected for demersal species instead pelagic species. In addition, adult samples show higher BUVS levels compared to juvenile samples. UV-P was also measured in demersal fish in the Pearl River delta (China) with a significant detection frequency (Peng et al., 2015). UV-320, UV-326, UV-327 and UV-328 were found in tidal flat and shallow water species from the Ariake Sea (Japan) (Nakata et al., 2009). In Europe, UV-328 has been measured in fish from Norway (Langford et al., 2015).

Due to the complexity of the matrix, the analysis of biota samples requires selective extraction techniques and sensitive determination. Moreover, different pretreatment procedures are required to obtain the desirable portion, such as skin and bone removal, peeling, dissection or deshelling (Cadena-Aizaga et al., 2020). Depending on the specie and the size of the fish, different parts of the fish are typically analysed: separate muscle (Kim et al., 2011a, 2011b) and liver (Nakata el al. 2009), belly (Peng et al., 2015) soft tissue (homogenates of muscle, stomach, intestines and liver) (Langford et al., 2015) or the whole organism (Nakata el al. 2009).

Several extraction techniques such as Soxhlet (Nakata et al., 2010), ultrasound assisted extraction (USE) (Peng et al., 2015; Lu et al., 2018)), high-speed solvent extraction (HSSE) (Kim et al., 2011a, 2011b), pressurized liquid extraction (PLE) (Langford et al., 2015; Wick et al., 2016) or QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) methodology (Picot Groz et al., 2014) have been applied in the analysis of BUVSs in aquatic organisms achieving good recoveries.

In this work, a method based on microwave-assisted extraction (MAE) coupled to ultra-high performance liquid chromatography with tandem mass spectrometry detection (UHPLC-MS/MS) has been optimized to determine six BUVSs (UV-P, UV-326, UV-327, UV-238, UV-329, UV-360) in different kinds of fish. This extraction technique has been already applied in the determination of BUVSs in sediments and sludges (Montesdeoca-Esponda et al., 2013) and also in seaweeds (Pacheco-Jua□rez et al., 2019). However, to the best of our knowledge, MAE has not been previously employed in the analysis of BUVSs in fish samples.

MAE represents an important alternative for the rapid extraction of samples as it reduces the extraction time and allows for the preparation of multiple samples in one single step. In addition to efficiency, MAE allows the use of lower volumes of organic solvents than other methodologies (Esteve-Turrillas et al., 2004).

Once the method was optimized, it was applied to the study of three species of fish collected close to three marine outfalls of treated waters in the Gran Canaria Island (Spain) for two years. In a previous work, BUVSs have already been measured in sewage, coastal seawater and sediment samples taken from these marine outfall areas, with detected concentrations in the range of 13.12−1933 ng L^{-1}, 67.01−2419 ng L^{-1} and 4.42−2162 ng kg^{-1} dry weight, respectively (Montesdeoca-Esponda et al., 2019). One of the target compounds has also been found in algae samples from the Gran Canaria Island (Pacheco-Jua□rez et al., 2019).

Three different fish species (*Boops boops, Sphyraena viridensis, Sphoeroides marmoratus*), which have different eating habits and roles in the ecosystem, were studied. *Boops boops* is an omnivorous species that feeds by scraping algae, and it is eaten by carnivore big fish, as, for example, the also studied *Sphyraena viridensis,* while *Sphoeroides marmoratus* feeds on molluscs and small invertebrates. Since *Boops boops* and *Sphyraena viridensis* are consumed very much in the Canary Islands, they could act as a vector of pollution for humans. Therefore, from both ecological environment and human health points of view, it is important to determine and evaluate the presence of BUVSs in fish.

2. Experimental

2.1. Sample collection and preparation

The fish samples (*Boops boops*, *Sphyraena viridensis* and *Sphoeroides marmoratus)* were manually caught in coastal areas close to three different marine outfalls in the Gran Canaria Island (Spain) by a pro-fessional fisherman. The selected marine outfalls correspond to three important wastewater treatment plants on the Gran Canaria Island, located in Las Palmas de Gran Canaria (LPGC), Arinaga (ARI) and San Agustín (SAN) (Fig. 1) and they were monitored every season for two years. Specifically, the samples were collected on July 2016, October 2016, January 2017, April 2017, July 2017, October 2017, January 2018 and April 2018.

The organisms were dissected, and muscle and viscera were prepared to analyse them separately. A pool of samples was prepared for each specie, location and date, and they were subject to freeze drying at −55 ªC (using a LyoQuest model, Telstar, Barcelona, Spain), mixed and sifted to a particle size smaller than 300 μm.

To optimize and validate the extraction methodology, samples of *Boops boops* taken offshore were spiked with 250 ng g^{-1} of a mixture of the target analytes.

2.2. Reagents and materials

Target compounds, whose main characteristics are listed in Table 2, were obtained from Sigma-Aldrich (Madrid, Spain). Stock solutions at 250 μg mL^{-1} were prepared in acetone and stored in glass-stoppered bottles at 2−5 ◆C in dark conditions, while daily standards were prepared in methanol.

Solvents and additives were employed as extractants, and mobile phases were purchased from Panreac Quimica (Barcelona, Spain).

Phree Phospholipid Removal SPE cartridges from Phenomenex Espan˜a (Madrid, Spain) and 0.2 μm syringe polyethylene terephthalate (PET) filters from Macherey-Nagel (Germany) were employed to clean the extracts.

2.3. Analytical determination

The determination of the pollutants was carried out by an ACQUITY UHPLC (Waters Chromatography, Barcelona, Spain) equipped with a Binary Solvent Manager (BSM), a 2777 autosampler, a column manager and a triple quadrupole mass spectrometry detector (TQD) with an electrospray interface (ESI). All components were controlled with MassLynx Mass Spectrometry software. An ACQUITY UHPLC Waters

Table 1
BUVSs determined in marine fish samples by different techniques.

Studied fish	Target compounds	Extraction procedure	Determination technique	Recoveries (%)	LODs	Found concentration ($ng \cdot g^{-1}$)	Reference
20 fish species from the families	UV-P UV-9	HSSE, hexane:acetone (1:1, v/v) at 10 mL/min, 30 min and 30 ❖C Deactivated silica gel	UHPLC-MS/MS	71–112	0.2–9 ($pg \cdot g^{-1}$) (lw)	0.03–0.41 (ww) -	Kim et al., (2011a)
- Carangidae,	UV-234					0.02–0.66 (ww)	
- Mugilidae,	UV-320					0.001–0.41 (ww)	
- Clupeidae,	UV-326					0.02–0.49 (ww)	
- Gerreidae,	UV-327					0.004–0.54 (ww)	
- Teraponidae,	UV-328					0.03–0.56 (ww)	
- Engraulidae,	UV-329					0.005–0.52 (ww)	
- Scombridae,							
- Polynemidae,							
- Sillaginidae,							
- Mullidae,							
- Scatophagidae,							
- Labridae,							
- Serranidae							
- Valamugil buchanani - Epinephelus corallicola	UV-P	HSSE, hexane:acetone (1:1, v/v) at 10 mL/min, 30 min and 30 ❖C Deactivated silica gel	UHPLC-MS/MS	71	8 ($pg \cdot g^{-1}$) (lw)	0.06–0.21 (ww)	Kim et al., (2011b)
- Mugil cephalus	UV-9			103	0.2 ($pg \cdot g^{-1}$) (lw)	-	
	UV-234			111	2 ($pg \cdot g^{-1}$) (lw)	0.02–0.34 (ww)	
	UV-320			96	0.3 ($pg \cdot g^{-1}$) (lw)	0.001–0.02 (ww)	
	UV-326			102	8 ($pg \cdot g^{-1}$) (lw)	0.14–0.48 (ww)	
	UV-327			104	8 ($pg \cdot g^{-1}$) (lw)	0.006–0.13 (ww)	
	UV-328			112	9 ($pg \cdot g^{-1}$) (lw)	0.001–0.03 (ww)	
	UV-329			108	2 ($pg \cdot g^{-1}$) (lw)	0.04–0.06 (ww)	
- *Gadus morhua*	UV-234	PLE, dichloromethane: hexane (1:1, v/v), 3 ◆ 5 min cycles at 100 ❖C GPC and Envirogel column	LC-HRMS and GC-HRMS	46	10 ($ng \cdot g^{-1}$) (LOQ) (ww)	-	Langford et al., (2015)
	UV-327			72	50 ($ng \cdot g^{-1}$) (LOQ) (ww)	-	
	UV-328			68	10 ($ng \cdot g^{-1}$) (LOQ) (ww)	<10–19.5	
	UV-329			72	25 ($ng \cdot g^{-1}$) (LOQ) (ww)	-	
- Herbivorous and omnivorous mudskippers	UV-320	Soxhlet, DCM:hexane (8:1, v/v), 5 h GPC and silica gel column	GC/MS	114	0.05 ($ng \cdot g^{-1}$) (ww)	<0.05–7 ((ww)	Nakata et al., (2009)
- Teleost fish (flathead, solefish, right eye flounder, sandperch, sweetlips, mullet, sea bass, hairtail)	UV-326			122	0.1 ($ng \cdot g^{-1}$) (ww)	<0.1–5.6 (ww)	
	UV-327			114	0.12 ($ng \cdot g^{-1}$) (ww)	<0.12–13 (ww)	
- Cartilaginous fish (eagle ray and hammerhead shark)	UV-328			110	0.15 ($ng \cdot g^{-1}$) (ww)	<0.15–55 (ww)	
- Small wild species (whole) - Farmer Red snapper (filet and belly)	UV-P	UAE, 3 x 20 mL MeOH, 15 min GPC column	UHPLC–MS/MS	79-98 (filet, 3 levels) 71 (belly)	0.003–1 ($ng \cdot g^{-1}$) (LOQs) (dw)	0.03–3.33 (wild species) (dw) 0.40 (filet) (dw) 0.67 (belly) (dw)	Peng et al., (2015)
	UV-329			55-102 (filet, 3 levels) 91 (belly)		0.11 *(Goby)* (dw)	
	UV-326			74-96 (filet, 3 levels) 42 (belly)		7.95 (filet) (dw) 11.4 (belly) (dw)	
	UV-324			79-91 (filet, 3 levels) 65 (belly)		0.20 (filet) (dw) 0.26 (belly) (dw)	
	UV-327			70-85 (filet, 3 levels) 56 (belly)		1.0 (filet) (dw) 1.8 (belly) (dw)	
	UV-328			78-90 (filet, 3 levels) 58 (belly)		0.8 (belly) (dw)	
	UV-531			89-120 (filet, 3 levels) 74 (belly)		7.90 (fillet) (dw) 12.14 (belly) (dw)	

HSSE: high-speed solvent extraction.
PLE: pressurized liquid extraction.
GPC: Gel permeation chromatography.
UAE: ultrasound assisted extraction.

lw: lipid weight.
ww: wet weight.
dw: dry weight.

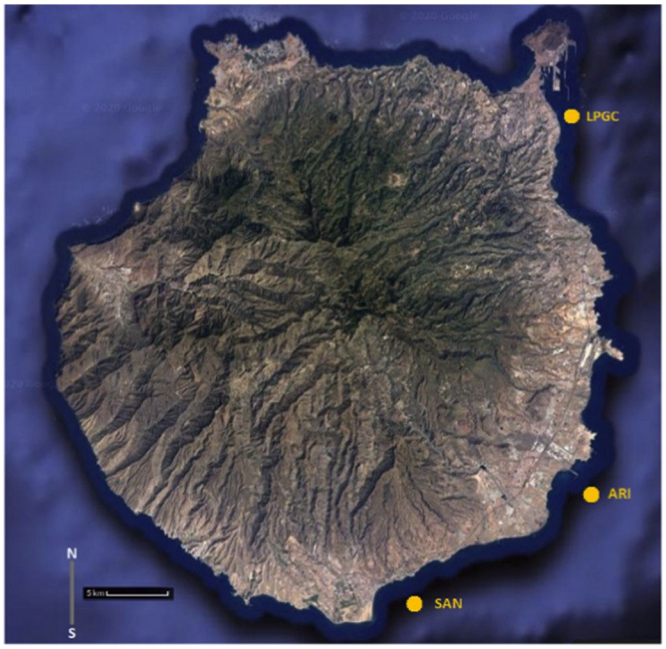


Fig. 1. Location of sampled marine outfalls in Gran Canaria Island (Spain).

BEH C18 column (50 ◆ 2.1 mm and 1.7 μm particle size) was used at 35 ◆C under a flow rate of 0.6 mL/min of methanol with 0.1% of formic acid in isocratic mode. The optimum ESI parameters were as follows: capillary voltage at 4 kV, cone voltage at 30 V, extractor voltage at 2 V, RF lens voltage at 1 V, source and desolvation temperature at 150 ◆C and 450 ◆C, respectively, desolvation gas flow (nitrogen) at 500 L h^{-1} and cone gas flow (argon) at 50 L h^{-1}. The specific detection parameters applied for each compound are shown in supplementary material (Table SM1).

2.4. MAE extraction and clean up

The microwave oven used for the extraction was a 6 EVAP rotor, and 6 MF100 vessels were purchased from Anton-Paar (Graz, Austria).

The procedure was carried out using 100 mg of fish sample with the addition of 2.5 mL of acetonitrile as extractant. After 10 min of applying 200 W of power, the vessels were cooled for 10 min before opening. Then, the extracts were filtered through a 0.20 μm filter to avoid solids and they were passed through a phospholipid removal cartridge in order to remove interferences. Finally, the extract was dried with nitrogen and reconstituted with 1 mL of acetonitrile to obtain a more concentrated solution before being injected into the UHPLC-MS/MS system.

3. Results and discussion

3.1. MAE optimization

To obtain the maximum extraction efficiencies, all the parameters involved in the MAE procedure were carefully optimized. A 2^3 experimental design was employed to study the effect of each variable under the influence of the others. Two distant levels were initially tested for three variables: 5 and 10 min of extraction time, 300 W and 800 W of power and 4 mL and 8 mL of methanol as extractant solvent.

This first approach showed that better recoveries were obtained using 10 min of extraction time, while the hardest conditions of power (800 W) provided poor results. For that, a subsequent experimental design had to be drawn to test mild conditions. Regarding the extractant volume, the normalised extraction efficiencies were similar for both tested values, so the lowest value is preferred to obtain a higher preconcentration.

Taking into account this previous picture, the following 2^3 experimental design was set to enhance the response: time, 12 min and 14 min; power, 100 W and 200 W; solvent volume, 2.5 mL and 5 mL. Regarding time, tested times did not provide better recoveries than 10 min, so the lower value was selected to not enlarge the procedure unnecessarily. In contrast, the weakest conditions for the power did not seem to be enough to facilitate the extraction of the analytes from the matrix, so 300 W was fixed. Among the tested volumes, 2.5 mL of solvent provided the better relation for the signal-concentration in the normalised recoveries.

Finally, the efficiencies of different solvents as extractants were compared. Methanol, acetonitrile and a mix of both (50:50, v/v) were tested. As seen in Fig. 2, the best recoveries were obtained using acetonitrile as extraction solvent from fish tissue samples.

In summary, the best conditions for the target BUVSs in fish samples were 10 min of time at 300 W using 2.5 mL of acetonitrile as extractant.

3.2. Analytical parameters

Extraction efficiencies, linearity, precision and detection/quantification limits (Table 3) were calculated to validate the optimized method for the determination of target BUVSs in fish muscle and viscera.

To overcome the matrix effect, calibration curves in fish muscle and viscera in the range 1–500 ng g^{-1} (except for UV-326 and UV-327 starting at 5 ng g^{-1}) were built using the matrix match calibration approach. Linear correlation coefficients higher than 0.992 were obtained for all the target compounds in both matrices.

Absolute recoveries were tested at two concentration levels (20 ng g^{-1} and 200 ng g^{-1}) in three replicate fish samples. The results were in the range of 13.1–75.4% and 22.9–72.8% for muscle and viscera, respectively. As can been observed in Table 3, similar results were obtained for the lowest concentrations and for the viscera samples. The poorest absolute recoveries were provided by the most polar compound, UV-P.

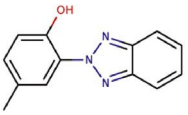
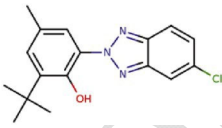
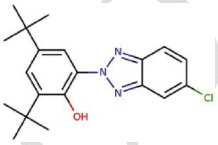
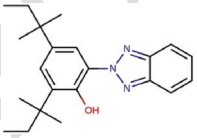
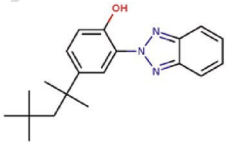
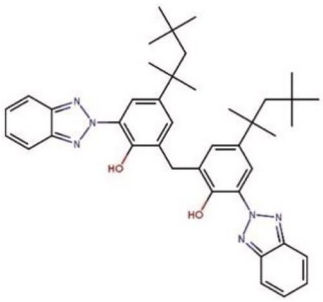
To evaluate both intra-day and inter-day precision, six replicates spiked at two different concentrations (20 ng·g-1 and 200 ng·g-1) were performed. The obtained values, expressed as the relative standard deviation (RSD), ranged from 0.21% to 6.06% for intra-day and from 1.12% to 21.3% for inter-day. All these results showed that the accuracy and precision of this method was adequate. In general, better reproducibility was obtained for muscle samples than for viscera, probably because the higher fat content of this matrix produces a greater interaction between the target compounds and sample matrix, which makes the extraction with organic solvent difficult.

Limits of detection (LODs) and quantification (LOQs) were obtained from the signal to noise relation (S/N) for the analyte peaks, assuming minimum detectable S/N levels of 3 and 10, respectively. The LODs and LOQs ranged from 0.34 to 2.87 and 1.13–9.66 ng g^{-1} for muscle, and 0.60–2.43 and 2.00–8.09 ng g^{-1} for viscera, respectively.

3.3. Analysis of marine fish species

The optimized method was applied to study the presence of the target BUVSs in the muscle and viscera of three species of coastal fish samples taken close to three marine outfalls in the Gran Canaria island (Spain) during the 2-years of monitoring. A total of 144 samples were analysed, each one by triplicate, and the obtained positive results are

Table 2
Target compounds and main characteristics (Log K_{ow} obtained from ECOSAR Program, US EPA).

Abbreviation	CAS number	Common name IUPAC name	Log K_{ow}	Chemical structure
UV-P	2440-22-4	2-(benzotriazol-2-yl)-4-methylphenol	2.998	
UV-326	3896-11-5	2-tert-butyl-6-(5-chlorobenzotriazol-2-yl)-4-methylphenol	5.552	
UV-327	3864-99-1	2,4-ditert-butyl-6-(5-chlorobenzotriazol-2-yl) phenol	6.914	
UV-328	25973-55-1	2-(benzotriazol-2-yl)-4,6-bis(2-methylbutan-2-yl) phenol	7.252	
UV-329	3147-75-9	2-(benzotriazol-2-yl)-4-(2,4,4-trimethylpentan-2-yl) phenol	6.214	
UV-360	103597-45-1	2-(benzotriazol-2-yl)-6-[[3-(benzotriazol-2-yl)-2-hydroxy-5-(2,4,4-trimethylpentan-2-yl) phenyl] methyl]-4-(2,4,4-trimethylpentan-2-yl) phenol	12.46	

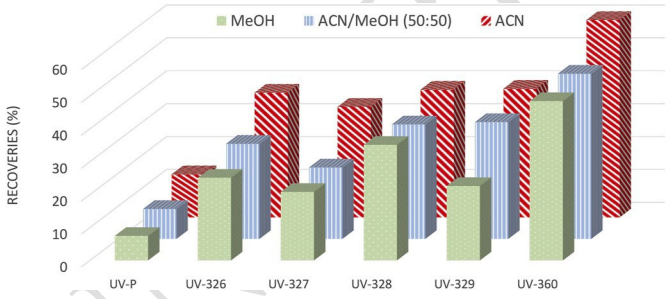


Fig. 2. Comparison of solvent efficiencies in the MAE for BUVSs (200 ng g^{-1}, dry weight in muscle).

shown in Fig. 3 and in the supplementary material (Table SM2).

Regarding the muscle samples, only three compounds were detected. UV-328 was the most frequently found compound, appearing in the three sampled species, while UV-326 was measured in two species and UV-360 was only found in *Boops boops* (Fig. 3a). The concentrations in this matrix ranged from 4.16 ng g^{-1} to 34.9 ng g^{-1}, and the maximum levels correspond to UV-326 and UV-328 in *Boops boops* (e.g., 29.8 ng g^{-1} of UV-328 in October 2017 in Las Palmas de Gran Canaria or 34.9 ng g^{-1} of UV-326 in April 2017 in Arinaga). All the data belong to the year 2017; no compounds were detected in 2016 and 2018.

A higher number of positive samples were obtained in the analysis of target BUVSs in viscera samples (Fig. 3b). Almost all the measured concentrations were below 10 ng g^{-1}, but some prominent results were recorded, such as 45.6 ng g^{-1} found for UV-328 in *Boops boops* from San Agustín in January 2017. No pattern of temporal variability was observed, although the best concentrations were obtained in January 2017. In this matrix, the most frequently measured compound was UV-329 with concentrations varying between 1.34 ng g^{-1} and 10.7 ng g^{-1} in the three studied locations. In the case of the viscera, the most polluted specie is not *Boops boops*, so future studies to observe accumulation in different tissues depending on the characteristics of the species would be helpful.

These results demonstrate the widespread dissemination of BUVSs in the marine ecosystem. Although the volume of obtained data is not enough to apply a statistical analysis, we can observe that the highest frequencies of detection for both muscle and viscera samples corresponds to fish taken in the San Agustín marine outfall. This fact is in agreement with the results obtained when seawater and sediments from the same marine outfalls were studied previously (Montesdeoca-Esponda et al., 2019). This trend might be related to tourist activities, since this is one of the most popular locations on the Gran Canaria Island for tourism. The amount of floating population was higher between October and April; the high tourist season in the Canary archipelago, and its effect could be noticeable both for the increase in sewage in wastewater treatment plants and for the direct entry of BUVSs in very crowded

Table 3
Analytical parameters for the developed MAE-UHPLC-MS/MS method.

	LOD (ng g^{-1})		LOQ (ng g^{-1})		Absolute recoveries (%)			
	Muscle	Viscera	Muscle	Viscera	Muscle		Viscera	
					20 ng g^{-1}	200 ng g^{-1}	20 ng g^{-1}	200 ng g^{-1}
UV-P	1.46	1.31	4.86	4.36	14.5	13.1	24.0	22.9
UV-326	2.87	2.43	9.56	8.09	46.9	37.9	48.1	48.3
UV-327	2.75	2.37	9.16	7.89	40.9	33.6	53.3	57.4
UV-328	0.88	1.18	2.93	3.93	39.0	38.8	56.7	50.2
UV-329	0.34	0.60	1.13	2.00	45.5	39.1	56.0	57.0
UV-360	1.27	1.46	4.23	4.86	75.4	60.3	72.8	68.4
	Precision (RSD, %)							
	Intra-day				Inter-day			
	Muscle		Viscera		Muscle		Viscera	
	20 ng g^{-1}	200 ng g^{-1}	20 ng g^{-1}	200 ng g^{-1}	20 ng g^{-1}	200 ng g^{-1}	20 ng g^{-1}	200 ng g^{-1}
UV-P	2.76	0.28	3.94	5.26	2.30	2.21	11.3	7.37
UV-326	6.06	3.08	4.20	3.30	21.3	5.96	9.66	20.9
UV-327	0.57	0.76	2.88	2.51	1.12	4.59	8.15	12.6
UV-328	0.44	0.56	2.40	4.41	1.62	1.59	14.6	17.8
UV-329	0.27	0.62	1.13	0.32	5.81	4.25	4.41	13.5
UV-360	2.44	0.21	1.77	0.86	5.96	5.80	14.7	21.1

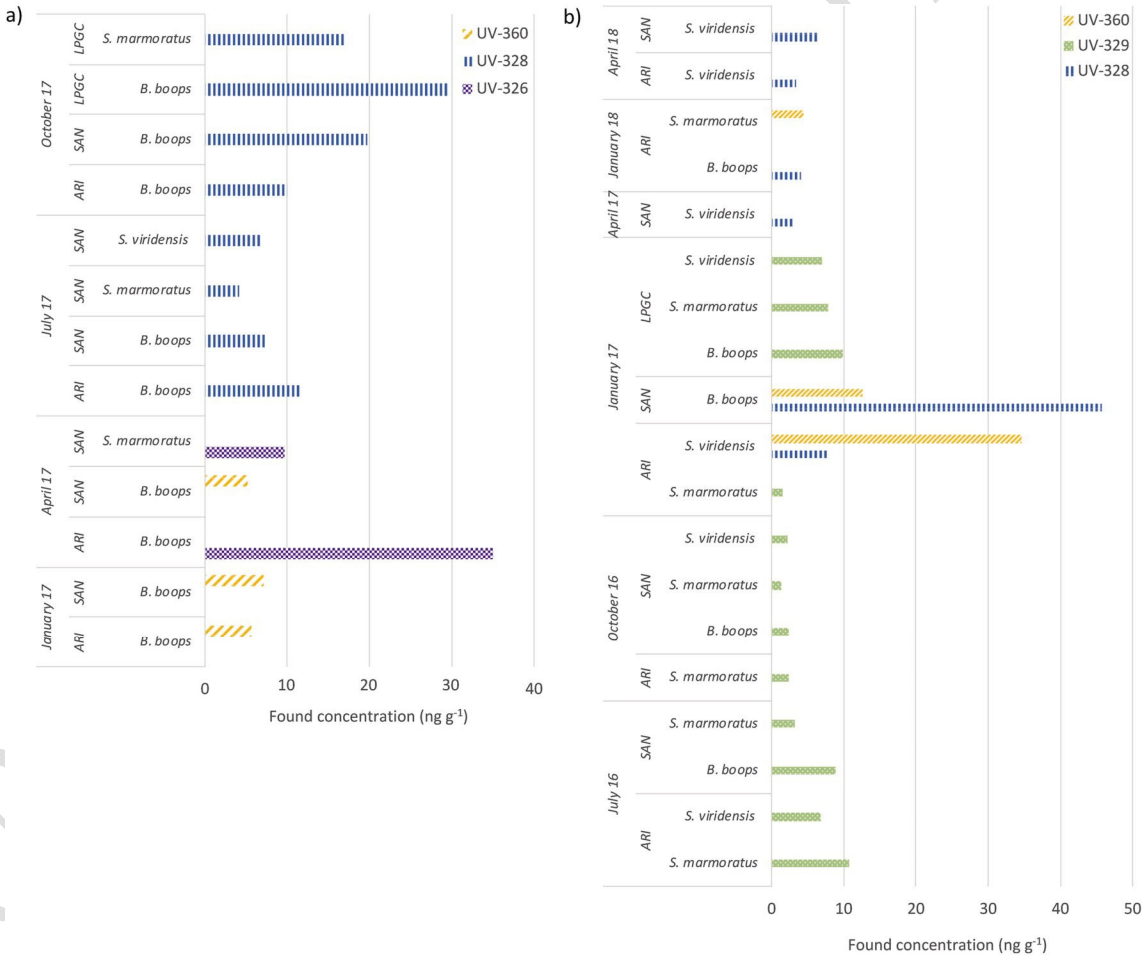


Fig. 3. Concentration of BUVSs in coastal fish samples: a) muscle; b) viscera (ng g^{-1}, dry weight).

beaches. A smaller number of positive samples was found in Las Palmas de Gran Canaria samples for both muscle and viscera samples.

The theoretical human health risk for the measured concentrations cannot be evaluated because, for the best of our knowledge, the reference dose for chronic oral exposure values is not available for target BUVSs.

4. Conclusions and future trends

A reliable determination of trace level compounds in biota matrices

requires a selective, sensitive and robust analytical method. The optimized MAE-UHPLC-MS/MS procedure provides satisfactory analytical parameters for such a complex matrix, allowing one to analyse both fish muscle and viscera. The application of the optimized method to coastal fish taken near marine outfalls showed the occurrence of BUVSs compounds in several species.

The most frequently found compounds were UV-328 in muscle and UV-329 in viscera samples, although clear spatial and temporal variability was not observed among the results. While the majority of positive results in muscle were obtained for *Boops boops*, in viscera, the accumulation in this specie was not outstanding.

Since BUVSs have already been measured in coastal seawater and sediment samples taken from the studied marine outfall areas (UV-326, UV-328 and UV-329 with concentrations in the range 67.01–2419 ng L^{-1} and 4.42–2162 ng kg^{-1} dry weight, respectively), their occurrence in fish feeding at these places is proven to arise from the discharge of sewage.

Although the levels found for target analytes are relatively low, taking into account that *Boops boops* and *Sphyraena viridensis* are consumed very much in the Canary Islands, their occurrence in such species must be carefully studied to determine the possible transference to humans. The reference dose for chronic oral exposure to these compounds is necessary in order to calculate the human health risk associated with the concentrations found.

To understand the behaviour of target analytes in the trophic chain and obtain a whole picture of their distribution in the Canary archipelago, it is necessary to enlarge the sampling and include other eatable marine organisms in future studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Sarah Montesdeoca-Esponda: Conceptualization, Methodology, Validation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **María Esther Torres-Padrón:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Visualization, Supervision, Funding acquisition. **Martin Novák:** Methodology, Validation, Formal analysis, Writing - review & editing. **Lucie Krchová:** Methodology, Validation, Formal analysis, Writing - review & editing. **Zoraida Sosa-Ferrera:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **José Juan Santana-Rodríguez:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2020.110805.

References

Benedé, J.L., Chisvert, A., Salvador, A., Sánchez-Quiles, D., Tovar-Sánchez, A., 2014. Determination of UV filters in both soluble and particulate fractions of seawaters by dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry. Anal. Chim. Acta 812, 50–58.

Cadena-Aizaga, M.I., Montesdeoca-Esponda, S., Torres-Padrón, M.E., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2020. Organic UV filters in marine environments: an update of analytical methodologies, occurrence and distribution. Trends Environ. Anal. Chem. 25 https://doi.org/10.1016/j.teac.2019.e00079.

Esteve-Turrillas, F.A., Aman, C.S., Pastor, A., De La Guardia, M., 2004. Microwave-assisted extraction of pyrethroid insecticides from soil. Anal. Chim. Acta 522, 73–78.

Gago-Ferrero, P., Diaz-Cruz, M.S., Barceló, D., 2012. An overview of UV-absorbing compounds (organic UV filters) in aquatic biota. Anal. Bioanal. Chem. 404, 2597–2610.

Kim, J.W., Isobe, T., Ramaswamy, B.R., Chang, K.H., Amano, A., Miller, T.M., Siringan, F.P., Tanabe, S., 2011a. Contamination and bioaccumulation of benzotriazole ultraviolet stabilizers in fish from Manila Bay, the Philippines using an ultra-fast liquid chromatography-tandem mass spectrometry. Chemosphere 85, 751–758.

Kim, J.W., Ramaswamy, B.R., Chang, K.H., Isobe, T., Tanabe, S., 2011b. Multiresidue analytical method for the determination of antimicrobials, preservatives, benzotriazole UV stabilizers, flame retardants and plasticizers in fish using ultra high performance liquid chromatography coupled with tandem mass spectrometry. J. Chromatogr. A 1218, 3511–3520.

Langford, K.H., Reid, M.J., Fjeld, E., Øxnevad, S., Thomas, K.V., 2015. Environmental occurrence and risk of organic UV filters and stabilizers in multiple matrices in Norway. Environ. Int. 80, 1–7.

Loos, R., Marinov, D., Sanseverino, I., Napierska, D., Lettieri, T., 2018. Review of the 1st Watch List under the Water Framework Directive and Recommendations for the 2nd Watch List. EUR 29173 EN, Publications Office of the European Union, Luxembourg, ISBN 978-92-79-81839-4. https://doi.org/10.2760/614367, 2018.

Lu, Z., De Silva, A.O., McGoldrick, D.J., Zhou, W., Peart, T.E., Cook, C., Tetreault, G.R., Martin, P.A., De Solla, S.R., 2018. Substituted diphenylamine antioxidants and benzotriazole UV stabilizers in aquatic organisms in the great lakes of north America: terrestrial exposure and biodilution. Environ. Sci. Technol. 52, 1280–1289.

Lu, Z., De Silva, A.O., Zhou, W., Tetreault, G.R., de Solla, S.R., Fair, P.A., Houde, M., Bossart, G., Muira, D.C.G., 2019. Substituted diphenylamine antioxidants and benzotriazole UV stabilizers in blood plasma of fish, turtles, birds and dolphins from North America. Sci. Total Environ. 647, 182–190.

Lu, Z., Peart, T.E., Cook, C.J., De Silva, A.O., 2016. Simultaneous determination of substituted diphenylamine antioxidants and benzotriazole ultra violet stabilizers in blood plasma and fish homogenates by ultra high performance liquid chromatography-electrospray tandem mass spectrometry. J. Chromatogr. A 1461, 51–58.

Mao, H., Li, H., Li, Y., Li, L., Yin, L., Yang, Z., 2020. Four typical personal care products in a municipal wastewater treatment plant in China: occurrence, removal efficiency, mass loading and emission. Ecotoxicol. Environ. Saf. 188 https://doi.org/10.1016/j.ecoenv.2019.109818.

Molins-Delgado, D., Silvia Díaz-Cruz, M., Barceló, D., 2015. Removal of polar UV stabilizers in biological wastewater treatments and ecotoxicological implications. Chemosphere 119, 51–57.

Montesdeoca-Esponda, S., Álvarez-Raya, C., Torres-Padrón, M.E., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2019. Monitoring and environmental risk assessment of benzotriazole UV stabilizers in the sewage and coastal environment of Gran Canaria (Canary Islands, Spain). J. Environ. Manag. 233, 567–575.

Montesdeoca-Esponda, S., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2013a. Microwave-assisted extraction combined with on-line solid phase extraction followed by ultra-high-performance liquid chromatography with tandem mass spectrometric determination of benzotriazole UV stabilizers in marine sediments and sewage sludges. J. Separ. Sci. 36, 781–788.

Montesdeoca-Esponda, S., Vega-Morales, T., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2013b. Extraction and determination methodologies for benzotriazole UV stabilizers in personal-care products in environmental and biological samples. Trends Anal. Chem. 51, 23–32.

Nakata, H., Murata, S., Filatreau, J., 2009. Occurrence and concentrations of benzotriazole UV stabilizers in marine organism and sediment from Ariake Sea, Japan. Environ. Sci. Technol. 43, 6920–6926.

Nakata, H., Shinohara, R., Murata, S., Watanable, M., 2010. Detection of benzotriazole UV stabilizers in the blubber of marine mammals by gas chromatography-high resolution mass spectrometry (GC-HRMS). J. Environ. Monit. 12, 2088–2092.

Nakata, H., Shinohara, R.I., Nakazawa, Y., Isobe, T., Sudaryanto, A., Subramanian, A., Tanabe, S., Zakaria, M.P., Zheng, G.J., Lam, P.K.S., Yim, E.Y., Min, B.Y., We, S.U., Viet, P.H., Tana, T.S., Prudente, M., Frank, D., Lauenstein, G., Kannan, K., 2012. Asia-Pacific mussel watch for emerging pollutants: distribution of synthetic musks and benzotriazole UV stabilizers in Asian and US coastal waters. Mar. Pollut. Bull. 64, 2211–2218.

Pacheco-Juárez, J., Montesdeoca-Esponda, S., Torres-Padrón, M.E., Sosa-Ferrera, Z., Santana-Rodríguez, J.J., 2019. Analysis and occurrence of benzotriazole ultraviolet stabilisers in different species of seaweed. Chemosphere 236. https://doi.org/10.1016/j.chemosphere.2019.124344.

Peng, X., Jin, J., Wang, C., Ou, W., Tang, C., 2015. Multi-target determination of organic ultraviolet absorbents in organism tissues by ultrasonic assisted extraction and ultra-high performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1384, 97–106.

Picot Groz, M., Martínez Bueno, M.J., Rosain, D., Fenet, H., Casellas, C., Pereira, C., María, V., Bebianno, M.J., Gomez, E., 2014. Detection of emerging contaminants (UV filters, UV, stabilizers and musks) in marine mussels from Portuguese coast by QuEChERS extraction and GC-MS/MS. Sci. Total Environ. 493, 162–169.

Rani, M., Shim, W.J., Han, G.M., Jang, M., Al-Odaini, N.A., Song, Y.K., Hong, S.H., 2015. Qualitative analysis of additives in plastic marine debris and its new products. Arch. Environ. Contam. Toxicol. 69, 352–366.

Rani, M., Shim, W.J., Han, G.M., Jang, M., Song, Y.K., Hong, S.H., 2017. Benzotriazole-type ultraviolet stabilizers and antioxidants in plastic marine debris and their new products. Sci. Total Environ. 579, 745–754.

Tovar-S□anchez, A., S□anchez-Quiles, D., Rodríguez-Romero, A., 2019. Massive coastal tourism influx to the Mediterranean Sea: the environmental risk of sunscreens. Sci. Total Environ. 656, 316–321.

Vimalkumar, K., Arun, E., Krishna-Kumar, S., Poopal, R.K., Nikhil, N.P., Subramanian, A., Babu-Rajendran, R., 2018. Occurrence of triclocarban and benzotriazole ultraviolet stabilizers in water, sediment, and fish from Indian rivers. Sci. Total Environ. 625, 1351–1360.

Wick, A., Jacobs, B., Kunkel, U., Heininger, P., Ternes, T.A., 2016. Benzotriazoles UV stabilizers in sediments, suspended particulate matter and fish of German rivers: new insights into occurrence, time trends and persistency. Environ. Pollut. 212, 401–412.