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Assessment of the presence of UV filters and UV stabilizers in stranded dolphin blubber

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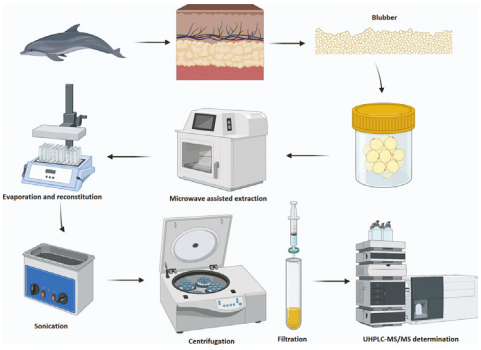
HIGHLIGHTS

- UV filters and stabilizers can be biomagnified through the marine trophic chain
- They were evaluated for the first time in blubber of the dolphin *Tursiops truncatus*
- A method based on microwave-assisted extraction combined with UHPLC-MS/MS was applied
- OC, BP3 and IMC were detected in the range from 5.92 ± 0.04 to 107.99 ± 11.32 ng·g^{-1}
- Dolphins occupying a high level in the food chain can accumulate target compounds

GRAPHICAL ABSTRACT



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ABSTRACT

The presence of ultraviolet filters (UVFs) and stabilizers (UVSs) was evaluated for the first time in the common bottlenose dolphin (*Tursiops truncatus*). UVFs and UVSs are compounds of growing concern because their effects on the environment are not completely known. UVFs and UVSs are added to personal care products (PCPs), such as cosmetics and products related to sun care and once released to the aquatic ecosystem, marine organisms can bioaccumulate these substances.

This work aimed to determine the presence of 12 UVFs and UVSs in cetacean blubber samples to assess the pollution to which these animals of the highest trophic chain levels are exposed due to human activity. Analytical determinations were carried out using a method based on microwave-assisted extraction combined with ultrahigh-performance liquid chromatography and tandem mass spectrometry detection. The developed method was successfully applied to determine the target compounds in the blubber tissues of five necropsied common bottlenose dolphins.

Three of the 12 studied compounds, namely 2-ethylhexyl 2-cyano-3,3-diphenylprop-2-enoate (octocrilene, OC), 2-hydroxy-4-methoxybenzophenone (benzophenone 3, BP3) and 3-methylbutyl (E)-3-(4methoxyphenyl) prop-2-enoate (IMC), were detected in several samples. Of the identified compounds, OC was present in all the samples and at the highest concentration within the range from 52.61 ± 18.59 to 108.0 ± 11.32 ng·g^{-1}.

1. Introduction

Despite the high dilution power of seawater, it is a fact that the marine environment is currently exposed to increasing pollution caused by the

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discharge of massive quantities of chemicals. Some of this pollution is caused by emerging pollutants (EPs), such as pharmaceutical, personal care products (PCPs) or chemicals used in packaging products, which may be harmful for human health and the environment (Bo et al., 2015).

Pollutants like PCPs entering the marine environment can be done indirectly through wastewater discharges or directly through recreational activities like swimming or water sports (Sánchez-Quiles and Tovar-Sánchez, 2014; Astel et al., 2020). Given their diverse behavior and multiple sources of production, the detection and quantification of these pollutants are challenges for researchers. Although many studies have demonstrated the bioaccumulation of different compounds, available data are still very limited for some families of these pollutants.

UV Filters (UVFs) and UV stabilizers (UVSs) are compounds that are added to PCPs to protect skin and hair from sunlight, and also to other industrial goods like paint, wax, plastic or textiles to prevent the photodegradation of polymers and pigments (Gago-Ferrero et al., 2013). Indeed both UVFs and UVSs are considered EPs and their use is only regulated in some countries.

Several UVFs and UVSs have been studied in different matrices like water (Jeon et al., 2006; Montesdeoca-Esponda et al., 2012), beach sand (Gago-Ferrero et al., 2012a, 2012b; Sánchez-Quiles and Tovar-Sánchez, 2015; Rodriguez-Romero et al., 2021), sediment (Tsui et al., 2015; Zhang et al., 2011), seaweed (Pacheco-Juárez et al., 2019) and marine organisms (Gimeno-Monforte et al., 2020; Montesdeoca-Esponda et al., 2020).

With organisms, most studies have focused on fish (Balmer et al., 2005; Meinerling and Daniels, 2006; Zenker et al., 2008) and their adverse effects have been demonstrated, particularly for fertility and reproduction. Although the concentrations found in invertebrates are generally low, they can accumulate and be biomagnified through the trophic chain (Gago-Ferrero et al., 2012a, 2012b).

As cetaceans are top predators with a worldwide distribution situated at a high trophic level, these animals' tissues can present high concentrations of organic pollutants. The impact that pollutants can have on them depends on feeding strategy, diet, nutritional status, sex, age, etc. (Borrell et al., 2004; Das et al., 2021). Besides long life span, they possess high absorption capacity and slow elimination, which increase the accumulation of pollutants (García-Álvarez, 2017). Therefore, marine mammals are considered significant bioindicators of the ecosystem and public health (Bossart, 2011; Schaefer et al., 2015).

In order to evaluate the pollution to which cetaceans are exposed, the presence of a variety of environmental pollutants has been studied, such as anthropogenic and natural organohalogen compounds (Vetter et al., 2001), different persistent organic pollutants (POPs) (Bachman et al., 2014), polybrominated diphenyl ethers (PBDEs) (Leonel et al., 2014) or isovaleric acid (Koopman et al., 2003). However, works studying the presence of EPs in cetaceans are very scarce. To the best of the authors knowledge, only three works have been conducted to determine UVFs and UVSs in dolphins (Table 1). UVFs have been determined in blood (Lu et al., 2019), and UVSs in the liver (Gago-Ferrero et al., 2013), blubber and muscle (Alonso et al., 2015). These results demonstrate that these compounds can accumulate in dolphins, which may have implications for their health and well-being. Overall, these studies highlight the importance of evaluating their presence in different tissues and organs of marine animals to better understand their effects on health and the environment.

As human health depends to a large extent on the ocean environment, and cetaceans can be considered bioindicators of the ecosystem, this work aimed to assess for the first time the presence of 12 UVFs and UVSs in blubber tissue samples taken from stranded dolphins. The selected compounds presented log K_{ow} within the 6–12 range (Table 2), which indicates that they are highly non polar compounds that tend to bioaccumulate in adipose tissues.

Skin comprises animal epidermis, dermis and hypodermis (Martín Díaz, 2019). This last and deepest skin level in cetaceans is also known as blubber, which is a dense vascularized layer of fat held by a structure of elastic and collagen fibers. Blubber is considered the primary and most important site of fat and energy storage in cetaceans. It performs several other important functions, such as serving as an efficient and adjustable thermal insulator, providing buoyancy or helping the animal's hydrodynamic locomotion (Struntz et al., 2004; Iverson, 2009). Yet given its high fat content, this layer can also concentrate very high levels of toxic lipophilic compounds that enter the body through feeding. Thus dolphins have been proposed as sentinels to evaluate aquatic ecosystem health and to identify damaging environmental trends (Bossart, 2011). The common bottlenose dolphin species (*Tursiops truncatus*) has already been studied on the Canary Islands to check the presence of POPs (García-Álvarez et al., 2014a, 2014b) and metals (García-Álvarez et al., 2015).

The complex matrix requires implementing an efficient extraction procedure to separate target compounds from the matrix. In this case, the selected technique was microwave-assistant extraction (MAE), which is a good choice for isolating pollutants from solid samples because it is efficient, reduces sample processing times and allows many samples to be prepared in a single step. In addition, MAE uses smaller volumes of organic solvents than other methodologies, and facilitates the control of parameters like time, temperature and power (Esteve-Turrillas et al., 2004). After the extraction procedure, the separation and detection of target compounds were carried out with ultrahigh-performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS). The developed method was applied to analyze blubber samples from five common bottlenose dolphins stranded on the coasts of the Canary Islands (Spain).

2. Materials and methods

2.1. Reagents and consumables

Twelve UVFs (6) and UVSs (6) compounds were analyzed. Table 2 shows their characteristics.

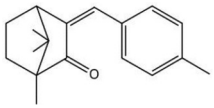
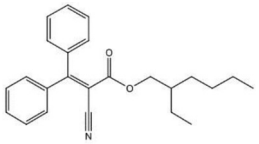
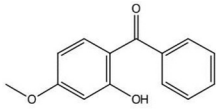
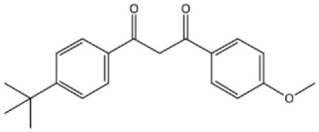
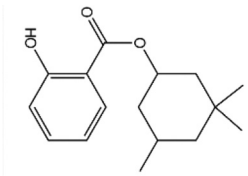
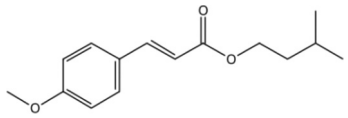
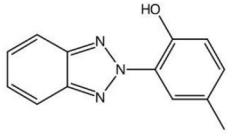
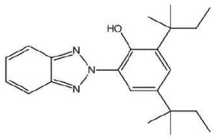
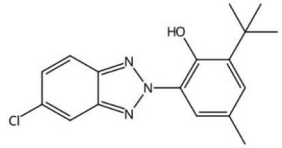
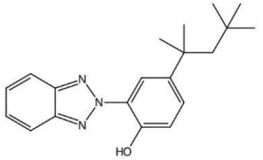
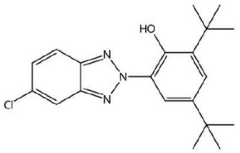
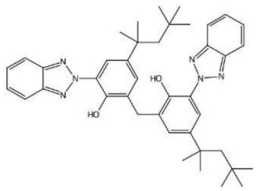
The studied UVFs were: 4-Methylbenzylidene camphor **(4-MBC)**; 2-Hydroxy-4methoxybenzophenone **(BP3)**; 2-ethylhexyl 2-cyano-3,3-diphenylprop-2-enoate **(OC)**; 1-(4-*tert*-butylphenyl)-3-(methoxyphenyl) propane-1,3-dione **(BMDBM)**; 3-methylbutyl (E)-3-(4-methoxyphenyl) prop-2-enoate **(IMC):** 3-methylbutyl (E)-3-(4methoxyphenyl)prop-2-enoate **(HMS)**. The selected UVSs compounds were: 2(benzotriazol-2-yl)-4-methylphenol **(UV-P)**; 2-tert-butyl-6-(5-chlorobenzotriazol-2-yl)4-methylphenol **(UV-326)**; 2,4-ditert-butyl-6-(5-chlorobenzotriazol-2-yl) phenol **(UV327)**; 2-(benzotriazol-2-yl)-4,6-bis(2-methylbutant-2-yl) phenol **(UV-328)**; 2(benzotriazol-2-yl)-4,6-bis(2-methylpentan-2-yl) phenol **(UV-329)**; 2-(benzotriazol-2yl)-6-[[3-(benzotriazol-2-yl)-2-hydroxy-5-(2,4,4-trimethylpentan-2-yl) phenyl] methyl]4-(2,4,4-trimethylpentan-2-yl)

Table 1
Methods used to determine target UVFs and UVSs in different dolphin tissues.

Matrix	Target compounds	Extraction procedure	Determination technique	Concentration found (ng g^{-1})	Reference
Liver	OC	PLE (pressurized liquid extraction)	UPLC-ESI-MS/MS	89–782 (lw)	Gago-Ferrero et al., 2013
Blubber	4-MBC	Sonication	HPLC-ESI-MS/MS	8.65–855	Alonso et al., 2015
Muscle	OC			50–11,130 (lw)	
Blood	UV-328	LLE (Liquid-Liquid extraction)	UPLC-ESI-MS/MS	-	Lu et al., 2019
	UV-329			0.64–0.86 (ww)	

lp: lipid weight; ww: wet weight.

Table 2
Characteristics of the target compounds.

Structure	Compound	Structure	Compound
UVFs			
4-Methylbenzylidene camphor 1,7,7-trimethyl-3 - [(4-methylphenyl) metilen] biciclo [2.2.1] heptan-2-ona 	***4MBC*** MF: $C_{18}H_{22}O$ MW: 254.37 g/mol CAS: 36861–47-9 *Log* K_{ow} = 4.95	***2-ethylhexyl 2-cyano-3,3-diphenylprop-2-enoate*** 	***OC*** MF: $C_{24}H_{27}NO_2$ MW: 361,48 g/mol CAS: 6197-30-4 *Log* K_{ow} = 6.88
2-Hydroxy-4-methoxybenzophenone 	***BP-3*** MF: C14H12O3 MW: 228,24 g/mol CAS: 131–57-7 *Log* K_{ow} = 3.79	***1-(4-tert-butylphenyl)-3-(methoxyphenyl)propane-1,3-dione*** 	***BMDBM*** MF: C20H22O3 MW: 310,39 g/mol CAS: 70356–09-1 *Log* K_{ow} = 4.51
(3,3,5-trimethylcyclohexyl) 2-hydroxybenzoate 	***HMS*** MF: C16H22O3 MW: 262,34 g/mol CAS: 118–56-9 *Log* K_{ow} = 6.16	***3-methylbutyl (E)-3-(4-methoxyphenyl) prop-2enoate*** 	***IMC*** MF: C15H20O3 MW: 248,32 g/mol CAS: 71617–10-2 *Log* K_{ow} = 4.33
UVSs			
2-(benzotriazol-2-yl)-4-methylphenol 	***UV-P*** MF: C13H11N3O MW: 22525 g/mol CAS: 2440-22-4 *Log* K_{ow} = 2.99	***2-(benzotriazol-2-yl)-4,6-bis(2-methylbutant-2-yl) phenol*** 	***UV-328*** MF: C22H29N3O MW: 351,49 g/mol CAS: 25973–55-1 *Log* K_{ow} = 7.25
2-tert-butyl-6-(5-chlorobenzotriazol-2-yl)-4methylphenol 	***UV-326*** MF:$C_{17}H_{18}ClN3O$ MW: 315,80 g/mol CAS: 3896-11-5 *Log* K_{ow} = 5.55	***2-(benzotriazol-2-yl)-4,6-bis(2-methylpentan-2-yl) phenol*** 	***UV-329*** MF: C20H25N3O MW: 323,43 g/mol CAS: 3147-75-9 *Log* K_{ow}^{b} = 6.21
2,4-ditert-butyl-6-(5-chlorobenzotriazol-2-yl) phenol 	***UV-327*** MF: $C_{20}H_{24}ClN_3O$ MW: 357,88 g/mol CAS: 3864-99-1 *Log* K_{ow} = 6.91	***2-(benzotriazol-2-yl)-6-[[3-(benzotriazol-2-yl)-2hydroxy-5-(2,4,4-trimethylpentan-2-yl) phenyl] methyl]-4-(2,4,4-trimethylpentan-2-yl) phenyl*** 	***UV-360*** MF: $C_{41}H_{50}N_6O_2$ MW: 658,87 g/mol CAS: 103597–45-1 *Log* K_{ow} = 12.5

MF: Molecular formula; MW: Molecular Weight; CAS: Chemical Abstracts Service number; Log K_{ow}^{b}: Octanol-water partition coefficient.
All the information was obtained from SciFinder.

phenyl (**UV-360**). All the target compounds were obtained from Sigma-Aldrich (Madrid, Spain).

A stock solution of 250 mg L^{-1} was prepared in acetone and stored in a glass container at 4 °C in the dark, while daily standards were prepared in methanol. The solvents used as extractants and mobile phases (water, methanol 99.9 %, acetone 99.9 % and hexane 99.0 %), were obtained from Panreac Quimica (Barcelona, Spain). Phree Phospholipid Removal Solid Phase Extraction (SPE) cartridges (1 mL) were bought from Phenomenex España (Madrid, Spain) and the 0.2 μm syringe polyethylene terephthalate (PET) filters were supplied by Macherey-Nagel (Dueren, Germany). The diatomeus earth used to dry samples was obtained from Mcherey-Nagel (Duren, Germany).

2.2. Sample collection and preparation

All the bottlenose dolphin specimens (*Tursiops truncatus*) analyzed in this work had stranded on the coast of the Canary Islands. They were necropsied at the "Instituto de Sanidad Animal y Seguridad Alimentaria" (IUSA) of the University of Las Palmas de Gran Canaria (Spain). Blubber tissues were stored at −80 °C until the time of the analysis.

Triplicate samples of 100 mg of the blubber sample from each animal were weighed and 50 mg of diatomaceous earth were used to remove moisture from blubber samples. Then the optimized MAE-UHPLC-MS/MS procedure was applied.

2.3. Instrumentation

The microwave oven used for extraction was a TITAN MPS with 16 vessels (230 V, 50–60 Hz, 40 bar) which were purchased from Perkin Elmer (Madrid, Spain).

Determination of compounds was carried out by an ACQUITY-UHPLC (Waters Chromatography, Barcelona, Spain). It was 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 the components were monitored by the MassLynx Mass Spectrometry software.

2.4. Chromatographic and detection conditions

For the separation of compounds, an ACQUITY UHPLC Waters BEH C18 column (50 × 2.1 mm, 1.7 μm particle size) was used at a flow rate of 0.4 mL min^{-1} at 35 °C. The mobile phase consisted of water and methanol with 0.1 % formic acid. The gradient started with 25 % water: 75 % methanol and then reached 100 % methanol in 3 min, which was left until 5 min. At 6 min, the composition had gone back to the initial conditions and the system was allowed to equilibrate up to 7 min before the next injection. The ESI parameters for mass spectrometry detection were: capillary voltage at 4 kV, extractor voltage at 2 V, cone voltage at 30 V, radio frequency (RF) lens voltage at 1 V, desolvation and source temperatures of 150 °C and 450 °C respectively, desolvation gas flow at 500 L hr^{-1} and, finally, cone gas flow at 50 L hr^{-1}. Gas nitrogen was used as desolvation and argon as the collision gas. Retention times and conditions of mass spectrometry detection are shown in Supplementary material (Table S1).

2.5. Quality control

Analytical parameters were calculated for the developed method in a butter matrix, since is not possible to reproduce the store of analytes in the adipose tissues at laboratory. Linearity was evaluated using eight concentration levels within the 1–500 ng mL^{-1} range of each target compound. The limits of detection (LODs) and limits of quantification (LOQs) were obtained from the signal/noise (S/N) response of the individual compounds from the lowest point of the calibration curve by assuming minimum detectable S/N levels of 3 and 10, respectively. Precision and relative recovery were evaluated by adding known quantities of a mixture of standard to 100 mg of sample at three concentrations, 1, 2.5 and 5 μg g^{-1} dry weight (dw), to obtain 100, 250 and 500 ng mL^{-1} in the theoretical final extract, respectively.

Finally, to check for interferences due to the complexity of the matrix over the MS suppression, butter and blubber blanks were enriched at three levels (100, 250 and 500 ng mL^{-1}) after the MAE procedure and the resulting extracts were compared with standards prepared in methanol.

3. Results and discussion

3.1. Microwave-assisted extraction

The variables that affect the MAE procedure must be optimized to obtain the best extraction efficiencies for each compound. The involved parameters were temperature, time and extractant solvent. To study the influence of each one, experimental designs were carried out with the spiked samples to know their contribution to the analytical response and the relation among them.

Given the impossibility of spiking cetaceans' adipose tissues in the laboratory, some authors often decide to run the methodology in a similar matrix, such as butter or olive oil (García-Álvarez, 2017; Nakata et al., 2010). In this work, butter was used to optimize the extraction methodology by two experimental designs. First, a 2^3 design (two levels for three variables) was used: 5 and 10 min extraction times, 50 °C and 55 °C temperatures and hexane and acetone as extractant solvents (Table S2 in Supplementary material). 7 mL of extractant were added to 100 mg of the butter spiked with 5 μg·g^{-1} of the target compounds to obtain a theoretical final extract of 500 ng mL^{-1}. Next the MAE procedure was applied under the different conditions of the first experimental design. Three replicates were used per experiment. When extraction ended, the sample was transferred to a glass vial and the extractant was evaporated. The residue sample was then reconstituted with 1 mL of methanol and sonicated. As the sample became turbid after sonication, a centrifugation step at 3000 rpm for 10 min was run. Finally, the extract was filtered through a 0.20 μm syringe filter before being placed inside the UHPLC-MS/MS equipment.

The measured peak area for each experiment and per compound was processed in the Minitab software. The mean, standard deviation and relative standard deviation were calculated, and Pareto graphs were built. By way of example, Fig. 1 shows the graph obtained for BP3, OC and IMC compounds. The interpretation of the graph provides information about the influence of each variable, as well as their combined effect. The level represented by a red discontinuous line is the value at which the factor has a significant effect. Blue bars correspond to the factors that exceed that level of significance. Finally, gray bars denote those that do not exceed the significance level. As it can be seen, for these compounds the factor whose effect is more significant is time. The Pareto graphs for the rest of compounds are shown in Supplementary material (Figs. S1-S9), in which can be observed that time is significant for all the compounds, while temperature and extractant are significant for the most of compounds (specifically for 7 of the 12 target compounds).

After analyzing the Pareto graphs, a statistical study was conducted for the most influent variables (time and temperature) by calculating Pearson correlations, which allow establishing the trend of these effect, with 0 being no influence, −1 being the maximum negative effect and 1 being the maximum positive effect. Since the mildest influence was found for the variable extractant agent, hexane was chosen because it is more non polar and easier to evaporate. It can be concluded that time presented a positive correlation for all the compounds, except for UV-360 (Table S3 in Supplementary material). On the contrary, temperature correlated negatively for all the compounds except for UV-360.

Based on the results of the correlations, a 3^2 experimental design was built with two variables and three levels: 10, 12 and 14 min and 50, 52 and 54 °C (Table S4 in Supplementary material). From the results of these experiments, it was concluded that the lowest tested temperatures and shorted times provided better results, as the contour graph corresponding

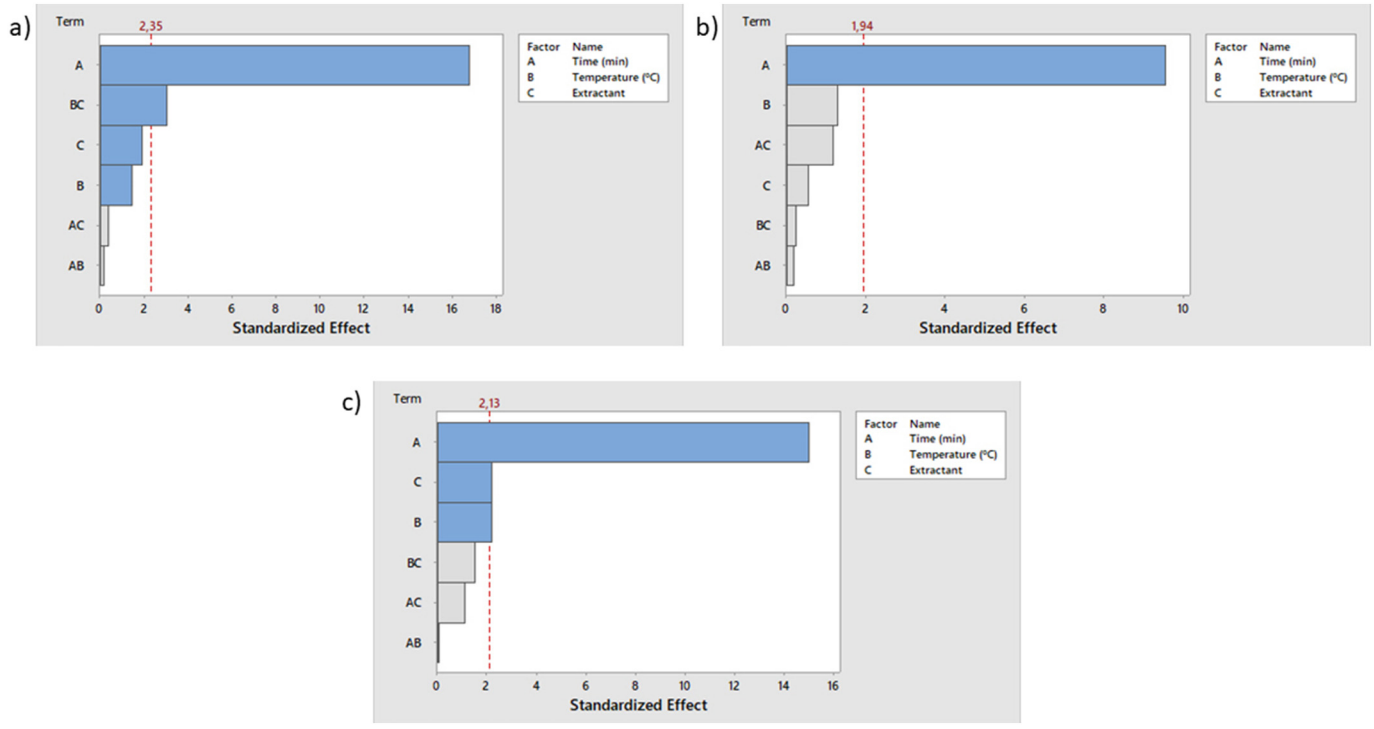


Fig. 1. Pareto chart of standardized effects of 2^3 experimental design for a) BP3; b) OC; c) IMC (500 ng·mL^{-1} in the final extract).

to the BP3, OC and IMC compounds reveals (Fig. 2). The same behavior was observed for most of the studied compounds (Figs. S10-S18 of the Supplementary material). In summary, the extraction conditions were as follows: 100 mg of sample, extraction with 7 mL of hexane for 10 min at 50 °C. After MAE, extracts were evaporated and reconstituted with 1 mL of methanol to obtain a preconcentration factor of 7 times. Then it was sonicated, centrifuged and filtered before being placed inside the UHPLC-MS/MS equipment.

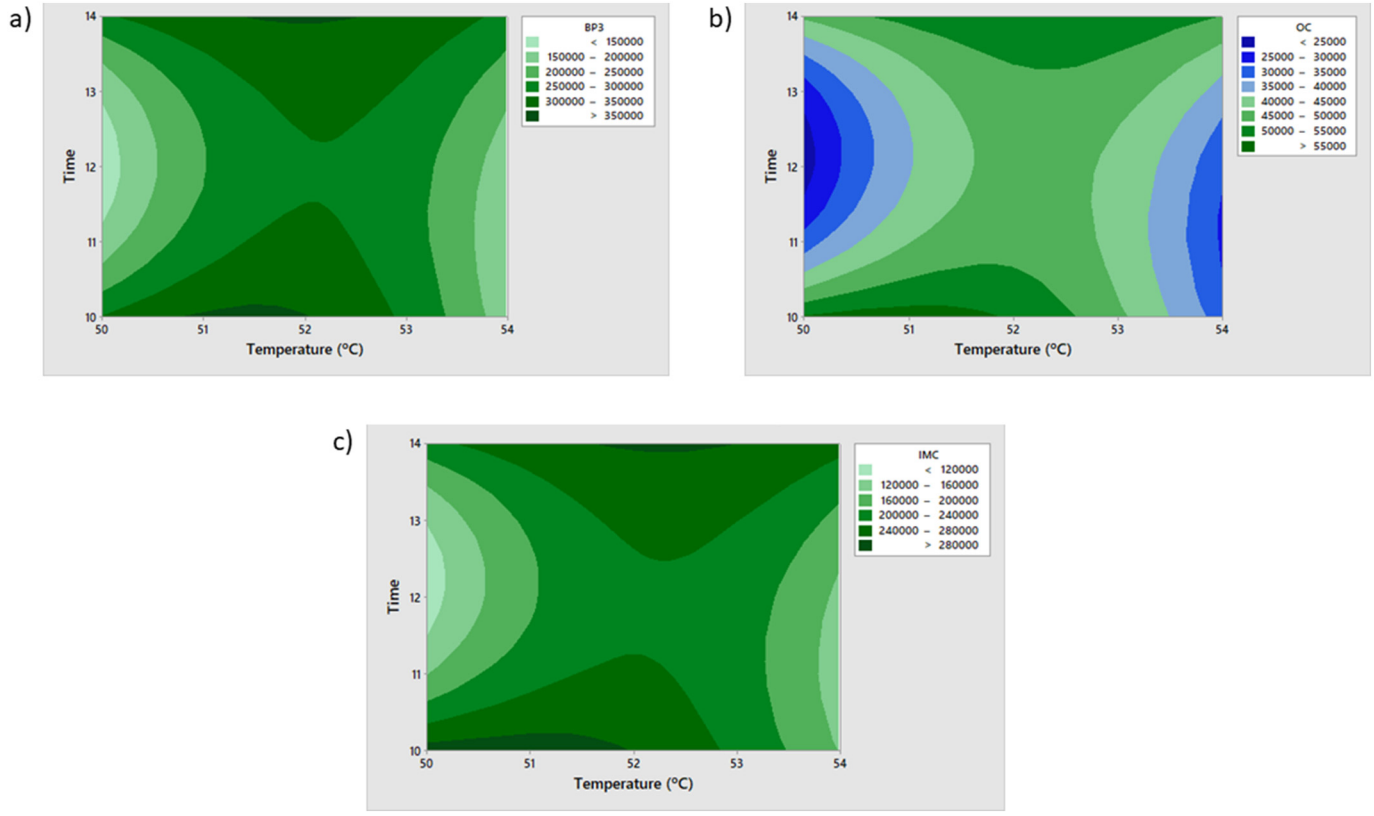


Fig. 2. Response surface of the effect of temperature and extraction time on the extraction for a) BP3; b) OC; c) IMC (500 ng·mL^{-1} in the final extract).

3.2. Method performance

Once the method was optimized for all the involved parameters, the analytical parameters (LODs/LOQs, precision, accuracy and matrix effect) were evaluated (Table 3).

External calibration curves were made using eight concentration levels within the range of 1–500 ng mL^{-1} of each target compound. Linear correlation coefficients higher than 0.99 were obtained for them all.

The LODs and LOQs were obtained from the S/N response of the individual compounds from the lowest point of the calibration curve by assuming minimum detectable S/N levels of 3 and 10, respectively. The LODs varied between 0.001 and 32.71 ng g^{-1}, while the LOQs fell within the range from 0.002 to 108.9 ng g^{-1}.

Precision and relative recovery were evaluated by adding known quantities of a mixture of standard to 100 mg of sample at three concentration levels, namely 1, 2.5 and 5 μg·g^{-1} (dw), to obtain 100, 250 and 500 ng mL^{-1} in the theoretical final extract, respectively.

Precision was tested from the relative standard deviation of three replicates, and it was lower than 20 % in most of cases. Some higher values were recorded for OC, BMDBM, IMC and UV-P, especially at lower concentrations.

Relative recoveries were calculated by comparing the samples spiked after and before the MAE procedure. They varied from a minimum of 55.20 % to a maximum of 97.90 %, except for HMS, UV-326, UV-327 and UV-328, which were not extracted or showed poor extraction, probably because they presented high log K_{ow}.

The matrix effect that such complex samples had in the detection system must also be considered. The standards prepared in methanol were compared to the extracts of butter and dolphin spiked after the MAE procedure at three levels (100, 250 and 500 ng mL^{-1}). High ionic suppression values (within the range from 73.43 % to 100 %) were obtained for the dolphin samples and revealed the complexity of matrix interferences. As the results were similar for butter, or even slightly higher, it can be concluded that the interferences of this substitutive matrix are comparable to dolphin samples and are appropriate for use in optimization.

Finally, butter and blubber blanks were enriched after the MAE procedure at three levels (100, 250 and 500 ng mL^{-1}), and the resulting extracts were compared with standards prepared in methanol to check the effect of the interferences due to the matrix over the MS suppression. The results obtained were similar in both cases (even slightly higher for butter), so we can conclude that the interferences from this surrogate matrix are comparable to dolphin blubber samples and are appropriate for use in optimization.

3.3. Monitoring the presence of UVFs and UVSs in blubber samples of stranded dolphins

Once validated, the developed method was applied to determine the target compound in the blubber samples of five common bottlenose dolphins (*Tursiops truncatus*) stranded on the coasts of the Canary Islands (Spain), whose information is presented in Table 4.

Table 4
Information of the dolphins studied.

Sample code	Sex	Age range	Sexual maturity	Body condition	Stranding date	Island
CET1020	Female	Juvenile	Immature	Moderate	09/08/2019	Tenerife
CET1042	Male	Adult	Mature	Poor	23/11/2019	Tenerife
CET1103	Male	Juvenile	Immature	Moderate	13/06/2020	Gran Canaria
CET1133	Male	Adult	Mature	Poor	31/10/2020	Fuerteventura
CET1151	Male	Juvenile	Immature	Poor	21/02/2021	Tenerife

Three replicates of 100 mg of blubber from each sample were subjected to the optimized MAE-UHPLC-MS/MS procedure. The results are shown in Fig. 3a. As it can be seen, three of the studied UVFs compounds were found. OC was detected in all the samples and also presented the highest concentration of the analyzed compounds within the range from 52.61 ± 18,59 to 108.0 ± 11.32 ng g^{-1} (dw). BP3 and IMC were found in one sample at 5.920 ± 0.040 ng g^{-1} and 8.550 ± 1.190 ng g^{-1} (dw), respectively. No UVSs were detected.

The total target analytes concentrations as the sum of the single compound concentrations for each sample are shown in Fig. 3b. The total concentration in the bottlenose dolphins ranged from 54.01 to 113.9 ng g^{-1}, with the higher value corresponding to a male adult stranded on the Tenerife Island.

The presence of OC in all the analyzed samples is a major concern because it is a highly lipophilic compound (log K_{ow} 6.88) that is stable and resistant to degradation by sunlight (Cadena-Aizaga et al., 2020). Some studies show that it can trigger the production of potentially harmful free radicals (reactive oxygen species) when it releases absorbed energy (Gago-Ferrero et al., 2013). The results seem to demonstrate that dolphins can accumulate relatively high levels of organic pollutants in their bodies because they occupy a high trophic level in the marine food chain and their metabolic activity is relatively low (Tanabe, 2002). It is also important to mention the presence of two different compounds in the same specimen, namely BP-3 (5.920 ± 0.040 ng g^{-1}) and OC (108.0 ± 11.32 ng g^{-1}) in sample CET1042, and OC (33.59 ± 18.59 ng g^{-1}) and IMC (8.550 ± 1.190 ng g^{-1}) in sample CET1020. These results are worrying because they reflect that the same specimen can accumulate a variety of pollutants that could display a different behavior and produce several adverse effects. Moreover, the interactions between them could have synergistic effects and imply greater toxicity.

Given the few available data about the concentrations of UVFs and UVSs found in dolphin samples, no statistical or spatial analysis could be performed. When the concentrations are compared with other studies, found OC levels (52.61–108.0 ng g^{-1}) are much lower than those reported by Gago-Ferrero et al. (2013) and Alonso et al. (2015), who found up to 782 ng g^{-1} (liver) and 11,130 ng g^{-1} (blubber and muscle), respectively.

Table 3
Analytical parameters of the developed MAE-UHPLC-MS/MS procedure.

	ILOD (ng g^{-1})	ILOQ (ng g^{-1})	Precision (%)			Relative recovery (%)			Matrix effect (%)
			1 (μg g^{-1})	2.5 (μg g^{-1})	5 (μg g^{-1})	1 (μg g^{-1})	2.5 (μg g^{-1})	5 (μg g^{-1})	
4BMC	0.008	0.026	1.410	11.01	6.360	56.15	55.20	70.67	86.12–89.94
BP3	0.024	0.078	2.630	3.640	4.220	77.77	55.48	80.71	84.46–91.18
HMS	32.71	108.9	–	–	18.61	–	–	95.00	97.42–100.0
OC	0.062	0.205	24.66	0.540	13.33	68.25	60.08	62.20	83.39–87.44
BMBDM	20.12	66.98	22.01	4.030	27.89	70.95	46.91	68.21	80.58–86.12
IMC	0.040	0.132	24.20	0.500	13.47	60.03	61.52	76.42	86.20–90.42
UV-P	5.440	18.12	22.63	8.840	17.63	82.30	58.18	71.53	91.03–94.37
UV-326	0.225	0.751	–	–	19.52	–	–	90.17	83.85–100.0
UV-327	13.89	46.25	–	–	–	–	–	–	91.48–100.0
UV-328	1.713	5.704	–	–	8.460	–	–	83.42	86.71–95.66
UV-329	1.716	5.713	7.230	6.660	15.67	97.90	83.96	94.12	73.43–74.56
UV-360	0.001	0.002	24.15	9.450	16.05	28.47	14.47	24.83	94.97–97.29

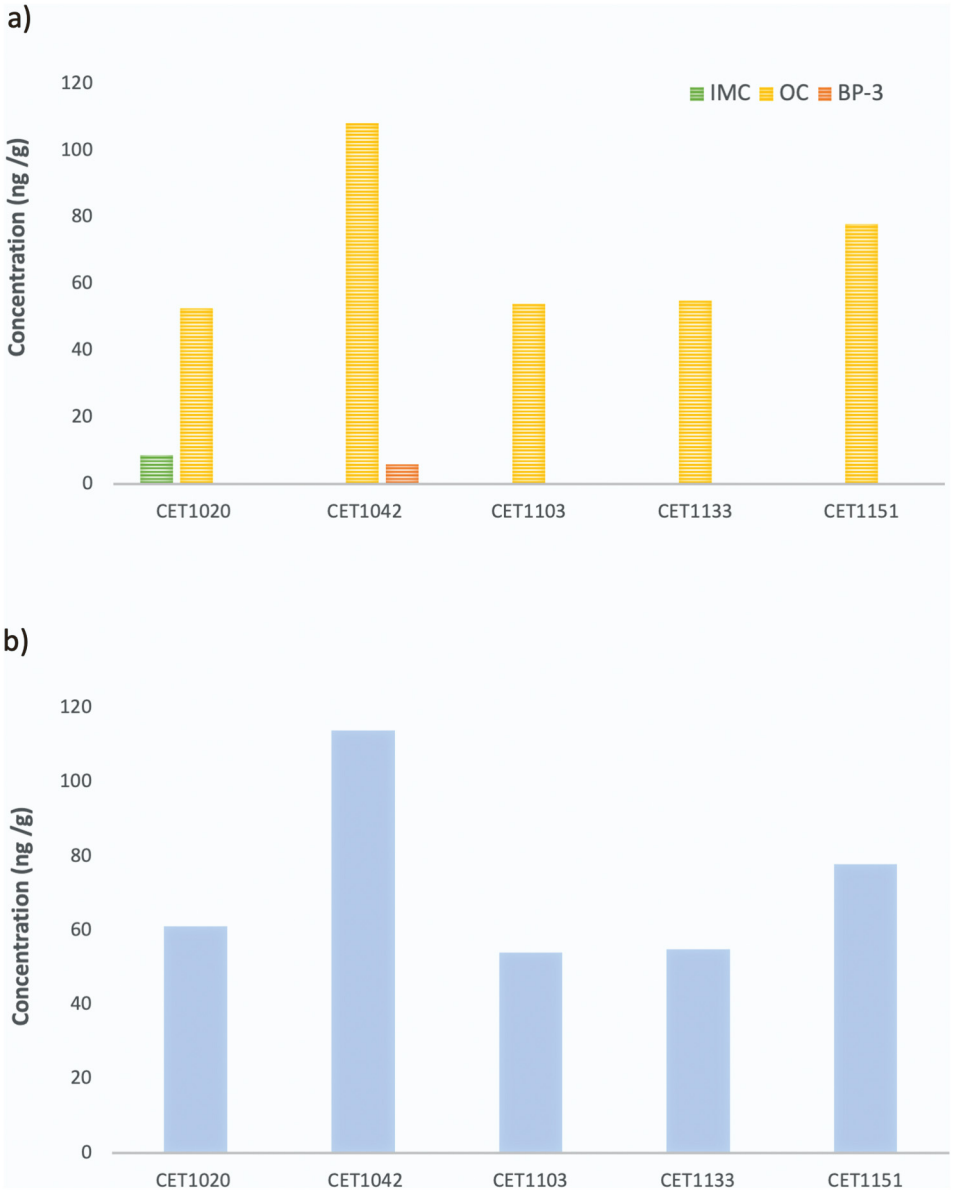


Fig. 3. UVFs and UVSs concentrations in the dolphin samples: a) individual concentrations of each compound; b) sum of the analytes found in each sample.

Alonso et al. (2015) also report 4-MBC in blubber and muscle within the 8.65–855 ng g^{-1} range, while Lu et al. (2019) found UVSs, with UV-329 at concentrations between 0.64 and 0.86 ng g^{-1} in blood (all the data are expressed as lipid weight, except those from Lu et al., which are expressed as wet weight).

4. Conclusions and future trends

Pollution by EPs such as UVFs and UVSs is increasing in oceans. As they are widely used as additives in PCPs, these compounds have become frequently detected ubiquitous environmental chemicals in different matrices. However, information on UVFs and UVSs concentrations in marine fauna, especially in marine mammal tissue, is scarce.

For this reason, their study in cetaceans is an important objective because knowing the possible impact of the human discharge of these compounds on wild animals is essential. As they are used as indicators of the state of the environment in which they live, the presence of these pollutants is alarming because of their possible effects on organisms and knowledge about the pollution level present in them is lacking.

Therefore, the MAE-UHPLC-MS/MS method that allowed seven of target compounds in the blubber of stranded dolphins to be determined simply and quickly was optimized and developed. This method is a valuable tool for monitoring a bigger number of samples and more kinds of cetacean species.

Regardless of quantitative determination accuracy, the developed method demonstrated the presence of three target compounds, and OC was the compound most commonly detected at the highest concentration. The fact that the other target compounds were not detected might because they are either absent in the studied organisms or related to the important matrix effects of the sample.

Further research is necessary to evaluate the pathways of the intake and metabolism of UVFs and UVSs, and their potentially toxic effects, in this long-lived long-range migratory species, which may be extremely susceptible to pollution.

CRediT authorship contribution statement

Emily González-Bareiro: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Sarah Montesdeoca-**

Esponda: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Supervision. **Jesús De la Fuente:** Conceptualization, Validation, Investigation, Resources, Writing – original draft, Supervision, Funding acquisition. **Zoraida Sosa-Ferrera:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision. **Manuel Arbelo:** Conceptualization, Investigation, Resources, Writing – review & editing, Funding acquisition. **Antonio Fernández:** Conceptualization, Resources, Writing – review & editing, Project administration, Funding acquisition. **José Juan Santana-Rodríguez:** Conceptualization, Validation, Resources, Writing – review & editing, Project administration.

Data availability

No data was used for the research described in the article.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jesus de la Fuente, Manuel Arbelo, Antonio Fernandez reports financial support was provided by European Union.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165041.

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