Microchemical Journal 171 (2021) 106834

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Analysis of microplastics-sorbed endocrine-disrupting compounds in pellets and microplastic fragments from beaches

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ARTICLE INFO

Keywords:
Steroid hormones
Microplastics
Ultrasound-assisted extraction
Ultrahigh performance liquid chromatography
Mass spectrometry
Beaches

ABSTRACT

Microplastics present in marine ecosystems have become a major environmental issue. This problem is due to not only the harmful effects of microplastics on exposed organisms, but also to their ability to adsorb organic compounds, which can have a combined harmful effect. Steroid hormones are an important group of endocrine-disrupting compounds that display moderately lipophilic behavior and can, subsequently, be adsorbed on microplastics. For this reason, it is necessary to determine this type of contaminants adsorbed on microplastic waste.

In order to investigate this issue, this work presents an analytical method based on ultrasound-assisted extraction and ultrahigh performance liquid chromatography tandem mass spectrometry (UAE-UHPLC-MS/MS) to determine the presence of 13 steroid hormones adsorbed on pellets and microplastic fragments. The different variables affecting the extraction process, such as solvent volume and extraction time, were optimized. The method showed excellent recoveries (above 80% for most compounds), satisfactory repeatability values and limits of detection (LoD) between 0.07 and 27.5 $ng{\cdot}g^{-1}$. Once optimized, the developed method was applied to the microplastic fragment samples and pellets collected from eight different beaches on the Canary Islands (Spain). In most samples, at least one hormone was detected and up to seven different steroid hormones in some samples. The steroid hormone concentrations in the studied samples ranged from < LoQ to 157 $ng{\cdot}g^{-1}$.

1. Introduction

Plastic production has increased exponentially worldwide in recent decades. For this reason, the scientific community has determined that plastics and microplastics are an important threat for marine and aquatic environments [1–3]. Instead recycling plastics has increased in the last few years and the landfill restrictions of some European countries are regulated. The average recycling rate in the European Union (EU) is 42% [4]. This means that a large fraction of used plastics is not recycled and up to 10% of the annual plastics production enters seas [5].

Microplastics (MPs) are plastic particles with a size diameter <5 mm that can be produced as degradation fragments of major plastics or as raw material to manufacture plastics. These particles are pervasive in nature, as evidenced in a large body of scientific literature about the presence of MPs in marine ecosystems [6–8]. The density of MPs, and ocean currents and seabed topography, contribute to scatter these particles [9]. Indeed MPs have been found worldwide, even in isolate or remote areas, which indicates that oceans are considered the ultimate receptor of plastics [10,11]. Many different marine organisms, such as algae, zooplankton, or both benthic deposit and suspension feeders, are susceptible to be affected by MPs [12]. This pollutant, combined with other anthropogenic stressors, can affect marine habitats and biodiversity [13]. MPs also directly affect organisms by blocking gastrointestinal and breathing tracts [14] and could become a transport vector of other organic pollutants that may be adsorbed on them [15,16]. From the different organic pollutants adsorbed on MPs, the majority of studies have focused on persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls or organochlorine pesticides [17]. Nevertheless, many other organic compounds are considered emerging pollutants (EPs), whose sorption to MPs has not been studied as much as POPs.

Steroid hormones are a group of naturally- and synthetic-occurring endocrine disrupting compounds that has been detected in environmental waters around the world [18,19]. The main source of these compounds being released to the environment is wastewater because sewage treatment plants (STPs) are not designed to eliminate steroids during wastewater treatment [20]. Steroid hormones have been detected in marine waters at concentrations of $ng{\cdot}L^{-1}$, which are enough to

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https://doi.org/10.1016/j.microc.2021.106834
Received 27 July 2021; Received in revised form 7 September 2021; Accepted 9 September 2021
Available online 13 September 2021

harm exposed biota. For these reasons, these compounds have been added to different surveillance programs, such as the Watch List of the EU Water Framework Directive, which has been adding different steroid hormones to its successive updates [21]. Steroid hormones usually exhibit moderately high octanol–water ($\log K_{OW} > 2$) values, which indicates that they are likely to adsorb on organic matrices, such as the sediment or solid particles present in STP effluents like MPs. About this, Lu et al. (2020) [22] studied the adsorption and desorption of 17β-estradiol and 17α-ethynylestradiol on MPs in seawater and determined that the highest adsorption rates were reached at pH 8.0, and desorption capacity accounted for over 40% adsorption capacity. This is a serious ecological problem because the steroid hormones adsorbed on MPs can be transported through oceans, which would increase the combined harmful effect of both pollutant types.

Therefore, the aim of the present research was to develop an analytical method to study the presence of 13 steroid hormones absorbed on MPs waste taken from different beaches on the Canary Islands (Spain).

Traditional extraction methods for organic pollutants adsorbed on MPs, such as maceration or soaking, involve large organic solvent volumes and long extraction times (up to 24 h), which makes it difficult to follow these procedures as routine methods [15]. For this reason, ultrasonic-assisted extraction (UAE) could be a greener alternative to these extraction methods. This enviro-friendly technique is based on the formation of small bubbles that bring about local changes in pressure and temperature when they explode. Moreover, UAE takes shorter extraction times and uses smaller solvent volumes to extract analytes [23]. Among separation and detection techniques, liquid chromatography tandem mass spectrometry has been widely used to determine steroid hormones in environmental samples thanks to its selectivity and sensitivity [24].

In this work, the optimization of a UAE procedure combined with ultrahigh performance liquid chromatography tandem mass spectrometry (UAE-UHPLC-MS/MS) to determine 13 steroid hormone residues absorbed on MPs was developed. To obtain the most suitable conditions for extracting the target compounds from MPs, a factorial experimental design was followed to jointly evaluate the effect of the variables involved in the process. After optimization, the method was successfully applied to samples of the microplastic fragments and plastic pellets taken from eight different tourist and remote beaches from the Canary Islands archipelago (Spain) to assess the presence of these steroid hormones in these microplastic residues. To the authors' knowledge, this is the first methodology to be developed to determine this type of endocrine-disrupting compounds adsorbed on MPs.

2. Materials and methods

2.1. Chemicals and reagents

The target steroid hormones (Table 1) were purchased from Sigma-Aldrich (Madrid, Spain) with purities over 99%. Stock solutions of single compounds were prepared in methanol at a final concentration of 1000 $mg{\cdot}L^{-1}$ and stored in a freezer at −20 °C in amber bottles. Then a logo working mixture of 10 $mg{\cdot}L^{-1}$ of the target steroid hormones was prepared by dissolving each standard in a proper volume of methanol. Working solutions were prepared daily from this mixture. HPLC-grade and LC-MS-grade methanol and LC-MS-grade water used as the mobile phase, and ammonia to adjust their pH, were obtained from Panreac Quimica (Barcelona, Spain). Ultrapure water was provided by a Milli-Q system (Millipore, Bedford, MA, USA). The polypropylene pellets used in the method optimization came from Sigma-Aldrich (Madrid, Spain).

2.2. Instrumentation

In order to extract the target analytes from MPs, an ultrasonic bath (VWR, Barcelona, Spain) was used and operated at a frequency of 45 kHz. After extraction, an ultrahigh-performance liquid chromatography system coupled to a triple quadrupole mass spectrometer (UHPLC-MS/MS) was employed to separate and detect analytes. This system consists of a binary pump to pulse the mobile phase, a 2777 autosampler capable of injecting up to 21 samples, a column oven and a triple quadrupole detector (Waters Acquity TQD) with an electrospray interface (ESI). They were all supplied by Waters (Barcelona, Spain). All the components were controlled by the Waters MassLynx Mass Spectrometry software.

Chromatographic separation was performed in the gradient mode using water + 0.1% of ammonia and methanol in an ACQUITY UHPLC BEH Waters C18 analytical column (50 mm × 2.1 mm, 1.7 μm) from Waters (Barcelona, Spain). The ionization and detection parameters were optimized in a previous article [25]. Briefly, estrogens were detected in the negative mode (ESI -) at a capillary voltage of −2.5 kV while progestogens, androgens and glucocorticoids were detected in the positive mode (ESI+) at a capillary voltage of 3 kV.

Table 1
Physicochemical properties of target steroid hormones studied.

		Log K_{OW}	Vapor pressure (mmHg)	Water solubility ($mg{\cdot}L^{-1}$)
Estrogens	17β-estradiol	4.01	$6.4{\cdot}10^{-9}$	3.0
	Estriol	2.45	$9.9{\cdot}10^{-12}$	27.3
	Estrone	3.13	$2.5{\cdot}10^{-10}$	3.0
Progestogens	Progesterone	3.87	$3.54{\cdot}10^{-4}$	8.8
	Norethisterone	2.97	$3.1{\cdot}10^{-7}$	7.0
	Norgestrel	3.48	$3.9{\cdot}10^{-10}$	2.1
	Megestrol acetate	3.75	$2.1{\cdot}10^{-10}$	6.5
Androgens	Testosterone	3.32	$1.7{\cdot}10^{-8}$	23.4
	Nandrolone	2.62	$3.5{\cdot}10^{-8}$	24.0
	Boldenone	3.09	$2.0{\cdot}10^{-9}$	20.0
Glucocorticoids	Cortisone	1.43	$3.0{\cdot}10^{-15}$	140.0
	Prednisone	1.46	$3.8{\cdot}10^{-13}$	77.5
	Prednisolone	1.62	$1.2{\cdot}10^{-13}$	223.0

Physicochemical values obtained from Pubchem database developed by National Center for Biotechnology Information (NCBI). https://pubchem.ncbi.nlm.nih.gov/

2.3. Sampling and sample pretreatment

The MPs samples were taken at the high-tide level on different beaches. Sample collection was normalized by using quadrants of 50x50 cm and a separation between them of at least 25 m. The top sand sample layer (5 cm deep) was obtained using a metal shovel and was sieved through a 1 mm mesh. At the laboratory, MPs were separated using forceps. One beach was studied on every surveyed island, except La Gomera and La Palma, where two different beaches were sampled.

Samples were taken from different beaches on six of the eight islands of the Canary Islands archipelago from July 2020 to January 2021 (Fig. 1). Beaches were located, from east to west, on the islands of La Graciosa (LGR), Gran Canaria (GC), Tenerife (TF), La Gomera (LG), La Palma (LP) and El Hierro (EH). Considering that the Canary Islands are a major tourist destination, some tourist beaches were studied: Las Canteras on Gran Canaria, Puerto Naos on La Palma or Valle Gran Rey on La Gomera. Other remote beaches or those used by locals were studied: Arenas Blancas on El Hierro or Playa Lambra on La Graciosa. Finally, considering that STP effluents are an important source of steroid hormones and MPs, some beaches like Playa del Inglés and Valle Gran Rey on La Gomera or Playa Grande on Tenerife were also studied because there are wastewater marine outfalls in their vicinities. Table 2 shows some information about the studied beaches.

Before the real samples analysis, virgin polypropylene pellets were spiked with a mixture of target analytes by diluting a proper amount of standard solution in methanol to optimize the extraction procedure. Then MP pellets were left at room temperature until the organic solvent had evaporated.

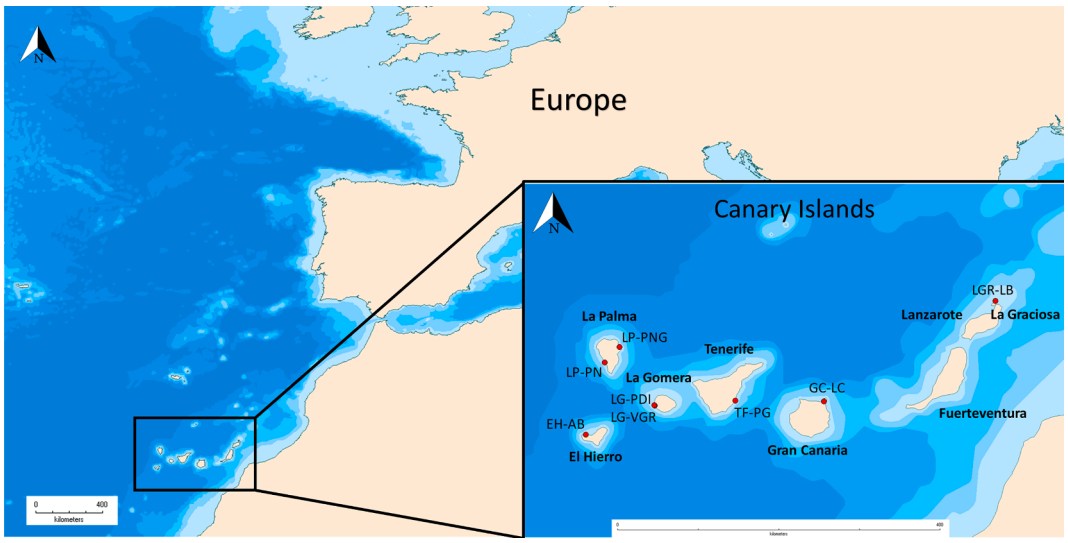

Fig. 1. Location of the surveyed beaches.

Table 2
Information of the studied beaches.

Island	Beach	Acronym	Coordinates	Touristic (T), Local (L) or Remote (R)	Some characteristics
La Graciosa	Playa Lambra	LGR-LB	29.2792, −13.4955	R	Sandy beach, no services
Gran Canaria	Las Canteras	GC-LC	28.1427, −15.4342	T	Sandy beach, full services, urban beach, the biggest of the capital of the island
Tenerife	Playa Grande	TF-PG	28.1524, −16.4318	L	Sandy beach, basic services, located in a small town. Windy area
La Gomera	Playa del Inglés	LG-PDI	28.1006, −17.3475	L	Volcanic sandy beach, no services but near a touristic area
	Valle Gran Rey	LG-VGR	28.0946, −17.3419	T	Volcanic sandy beach, some services, in a semi-urban area. Close to a port.
La Palma	Puerto Naos	LP-PN	28.5842, −17.9097	T	Largest beach of the island. Volcanic sandy beach. Many touristic services
	Playa de Nogales	LP-PNG	28.7600, −17.7407	R	Remote beach. Only be accessed on foot. Strong currents. Frequented by surfers
El Hierro	Arenas Blancas	EH-AB	27.7667, −18.1217	R	Remote white-sandy beach with easy access. No services

2.4. Extraction procedure

In order to follow the method optimization, extraction was done using 10 spiked virgin pellets with a mass of 300 mg ±10%. For the real samples, the same mass of pellets or fragments was used to perform the analysis. Sample weight was determined by considering that the amount of MPs is sometimes a limiting factor because their distribution is not consistent. MPs were placed in glass vials and the optimized amount of extraction solvent was added. Then vials were placed in an ultrasonic bath and extraction lasted the required time. After extraction, the organic solvent was transferred to a chromatographic vial and analyzed in the UHPLC-MS/MS system. For those extracts in which suspended solids were seen, the extractant was transferred to a glass test tube and centrifuged at 3000 rpm for 10 min before the chromatographic analysis.

3. Results and discussion

3.1. Optimization of the extraction procedure

Ultrasound-assisted extraction (UAE) uses the energy produced by ultrasounds to extract analytes from solid to liquid phases by cavitation. This methodology presents some key variables that must be optimized, such as extraction time and extractant volume. A factorial experimental design of these two variables at three levels (3^2) was performed. Factorial designs permit the required number of experiments to be reduced and, thus, limits the amount of reagent used and experimental times. The tested extraction times were 10, 30 and 50 min and the studied extractant volumes were 3, 5 and 7 mL of methanol. To avoid carryover effects, runs were randomized and surface responses were built using peak areas to know the optimum combination of these two variables. The spiked concentrations of the steroid hormones were calculated to obtain an extract at a final concentration of 300 ng·mL^{-1} to compare the peaks of the different runs. Fig. 2 shows the results of the four compounds under study that belonging to the four different steroid hormone families which were employed as representative compounds. The medium extraction time values (30 min) provided larger peak areas, while the maximum extraction rates were obtained with 5 mL for estrogens and glucocorticoids and 3 mL for progestogens and androgens. For this reason, 30 min was defined as the optimum extraction time and 4 mL were used as the compromised extractant volume.

After determining the optimum extraction conditions, methanol, acetonitrile, and a 1:1 mixture of both, were tested to optimize the extractant solvent. To compare extraction efficiencies, the average peak areas of the triplicate were normalized by dividing them by the value of the solvent with the best extraction efficiency. Fig. 3 shows the average normalized efficiencies for the four steroid hormone families. Methanol displayed the best extraction efficiency for progestogens and androgens,

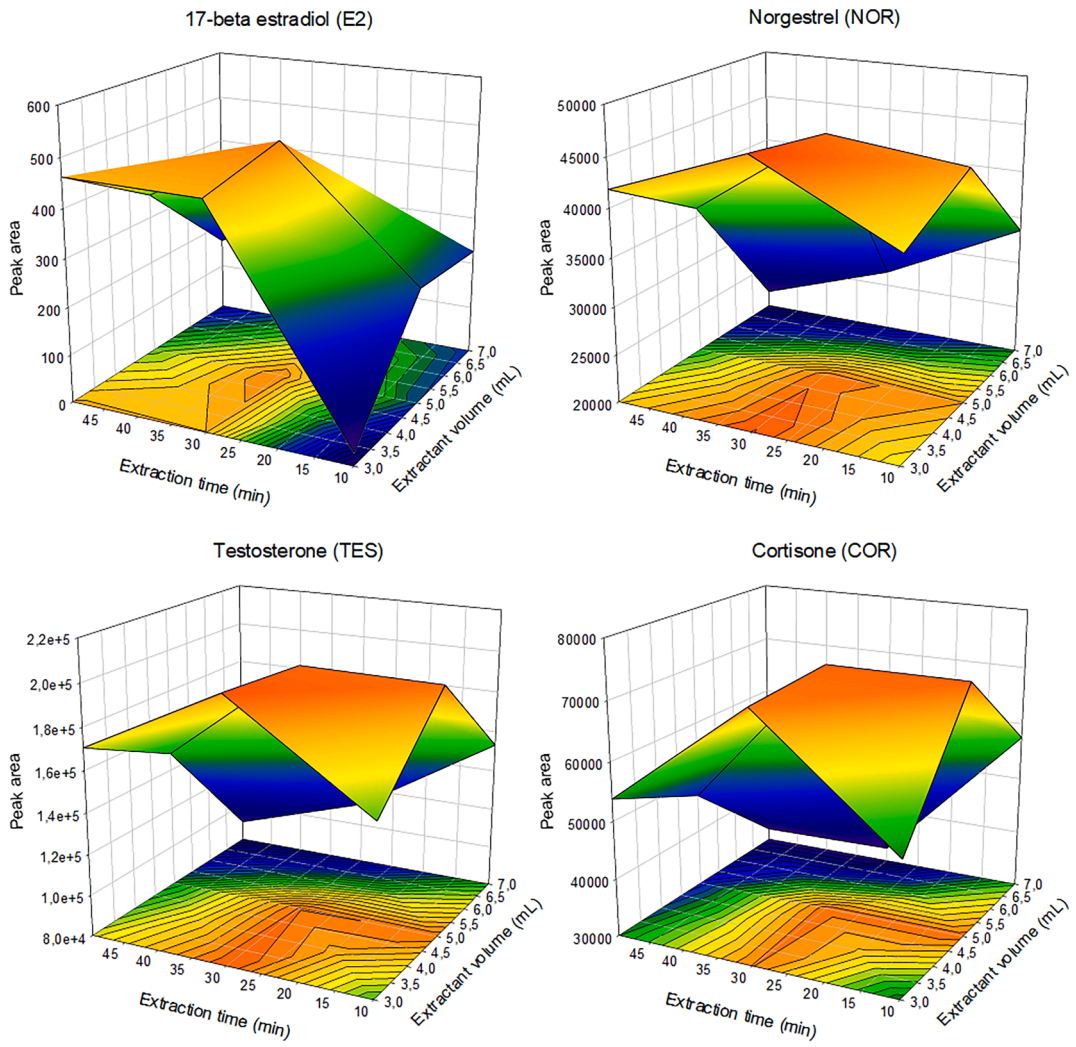

Fig. 2. Surface responses of UAE optimization for compounds from different steroid families.

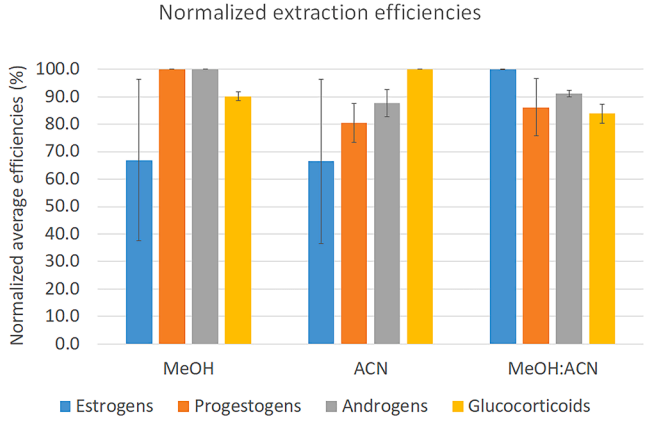

Fig. 3. Normalized extraction efficiencies with the different tested organic solvents.

and a normalized efficiency over 90% for glucocorticoids. Acetonitrile was the best extraction solvent for glucocorticoids, but for which normalized extraction efficiencies were below 90% for the other compounds. The methanol and acetonitrile mixture was the best option for estrogens, but the average normalized extraction efficiencies were lower for the other families than those using methanol. In previous studies, methanol has shown the best efficiencies to extract steroid hormones from different environmental and biological solid samples [25,26]. For these reasons, methanol was taken as the optimum extraction solvent.

3.2. Method validation

The chromatographic method's linearity was checked between 1 and 400 ng·mL^{-1} for androgens, progestogens and glucocorticoids, and from 10 to 400 ng·mL^{-1} for estrogens, using the external calibration curves that exhibited correlation coefficients (r^2) over 0.990.

After optimizing the extraction conditions, the analytical parameters of the UAE-UHPLC-MS/MS method were evaluated. The determination of the organic compounds from the environmental complex samples by mass spectrometry detection was extremely challenging, especially when by electrospray ionization (ESI) because of the analytical signal changes caused by the extracted interferences. This has been observed in different extraction and determination methodologies for the steroid hormones present in solid environmental samples [27,28]. The next step was to evaluate if the UAE extracts showed large differences than the standards prepared in pure solvent during mass spectrometry analytical detection. The extracts of virgin pellets were spiked with different concentrations of a mixture of steroid hormones and the signals of these extracts ($A_{spiked\_extract}$) were compared to those of the standards prepared in methanol ($A_{standard}$). Signal enhancement and suppressions were evaluated at 100, 500 and 1000 ng·g^{-1}. For most of the

compounds, checks were made to see that the changes in analytical signal were between −10 and 10% compared to the analytical signal of the pure standards. This would mean that the extracts obtained by the UAE method would acquire a greater similarity to the standards prepared in the pure solvent. Fig. 4 shows two chromatograms, where we can see that the extracts present similar peak areas and S/N ratios to the standards prepared in methanol.

After evaluating that the analytical signals were not affected by UAE extracts' composition, the capacity of the UAE method to extract hormones from microplastic samples, along with its repeatability were evaluated. Both parameters were studied using the virgin pellets contaminated by known concentrations of steroid hormones. To perform these studies, 300 mg (±10%) of virgin polypropylene pellets (10 pellets) were spiked with a proper volume of the steroid hormones mixture in methanol at three concentration levels: 100, 500 and 1000 ng·g^{-1}. After spiking, pellets were left for 1–2 h until methanol had evaporated before completing the UAE process. To calculate relative recoveries, the peak areas of the extracts of spiked pellets were compared to those areas obtained from the blank extracts spiked with the same concentration of the target analytes. Equation (1) shows how relative recoveries were calculated:

$$Recovery(\%) = \frac{Area_{spikedsample}}{Area_{spikedextract}} \quad (1)$$

As seen in Table 3, marked recoveries were obtained for most of the studied steroid hormones. In general, recoveries over 80% were obtained at the three concentration levels under study (100, 500 and 1000 ng·g^{-1}), except estriol, cortisone and prednisolone with recoveries over 68% at the low concentration point. Regarding repeatability, intraday and interday precisions were evaluated using six samples per analysis. The results showed that the relative standard deviations (RSD) were below 20% for most compounds and spiked levels.

In order to determine the optimized methodology's limits of detection and quantification (LoDs and LoQs), the signal-to-noise ratios equaling 3 and 10, respectively, were used. For the target analytes, the LoDs ranged from 0.07 to 27.5 ng·g^{-1}, while the LoQs were between 0.23 and 91.7 ng·g^{-1}.

3.3. Determining steroid hormones in the microplastics from Canary Islands beaches

Microplastic fragments and pellets were taken from eight different beaches on the Canary Islands from July 2020 to January 2021. As shown in Table 4, 11 of the 13 analyzed target compounds were detected in at least one of the studied samples. No significant differences were observed between the concentrations for the same compound detected on the different beach types: tourist, local or remote. No hormones were detected in the fragment samples from Las Canteras on Gran Canaria (GC-LC), which is the most tourist beach of this study, and in the pellets from Playa Lambra on La Graciosa (LGR-LB), a remote beach located on the archipelago's smallest island. This could mean that beach type had no effect on the presence of the hormones adsorbed on the MPs that reached them. For the other beaches, at least one of the target analytes was detected at measurable concentrations, which meant that 60% of the studied samples presented at least one positive analysis. Furthermore, the steroid hormones from the four families under study were detected in different samples. Progestogens was the family of steroid hormones with the highest positive percentage as it was detected in 37% of the studied samples. Estrogens and glucocorticoids were detected in 33% and 20% of the studied samples, respectively. Finally, androgens were detected only on Arenas Blancas beach on El Hierro (EH-AB) in both studied samples (fragments and pellets).

Due to the differences in the plastic debris taken from the surveyed beaches, the detected concentrations of the target analytes were variable and mostly found in one of the triplicates performed. The most detected compound was norgestrel, with 37% of the samples had positive results and the highest concentrations of the target analytes. The norgestrel concentrations ranged from 19.2 to 157.2 ng·g^{-1} and positive analyses were obtained on all the beaches, except Las Canteras on Gran Canaria. For the other progestogens under study, megestrol acetate was not detected in any sample and norethisterone and progesterone were detected only on Arenas Blancas on El Hierro (EH-AB) at concentrations ranging from < LoQ to 26.1 ng·g^{-1}. The positive results obtained for estrogens came from the samples taken from three of the beaches, with concentrations ranging from < LoQ to 48.5 ng·g^{-1}. With androgens, two of the three studied compounds were detected only on one beach. In the samples from Arenas Blancas (EH-AB), testosterone and nandrolone were recorded in both plastic fragments and pellets at concentrations from 15.0 to 37.3 ng·g^{-1}. Finally, prednisolone was the only glucocorticoid to be found in the study. The highest glucocorticoid Looks concentration was obtained on El Hierro on Arenas Blancas (EH-AB), and the highest concentration of the detected analytes was found on this beach. This compound was also detected on the Playa Nogales and Puerto Naos beaches on La Palma (LP-PNG and LP-PN) at concentrations ranging from 38.8 to 53.2 ng·g^{-1}.

The samples from Arenas Blancas on El Hierro island (EH-AB) showed the highest positive analysis of the whole study. Specifically, up to seven steroid hormones were detected in both the microplastic fragments and pellets collected from this beach. This could be related to the fact that the Arenas Blancas beach is considered a hot spot of MPs

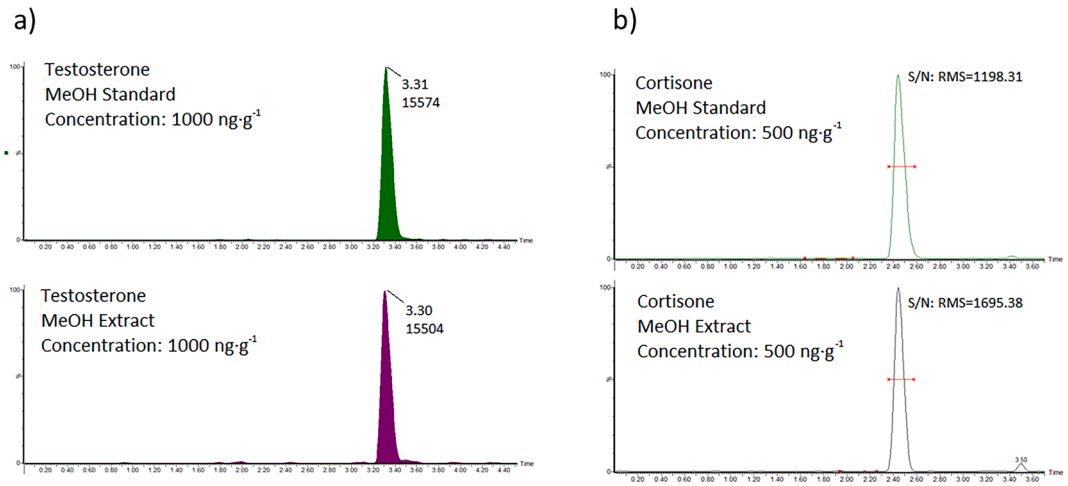

Fig. 4. Peak areas of testosterone at 1000 ng·g^{-1} (a) and the signal-to-noise ratio of cortisone at 500 ng·g^{-1} (b).

Table 3
Analytical parameters of the developed UAE-UHPLC—MS/MS method.

	Compound	Linearity (r^2)	LOD ($ng \cdot g^{-1}$)	Recovery (%)			Intra-day repeatability (CV[a] %) n = 6			Inter-day repeatability (CV[a] %) n = 3x6		
				100 $ng \cdot g^{-1}$	500 $ng \cdot g^{-1}$	1000 $ng \cdot g^{-1}$	100 $ng \cdot g^{-1}$	500 $ng \cdot g^{-1}$	1000 $ng \cdot g^{-1}$	100 $ng \cdot g^{-1}$	500 $ng \cdot g^{-1}$	1000 $ng \cdot g^{-1}$
Estrogens	Estradiol	0.995	27.5	n.a.[b]	118.9 ± 30.1	91.3 ± 10.6	n.a.	19.9	6.27	n.a.	23.3	23.2
	Estrone	0.996	3.27	n.a.	85.4 ± 17.4	91.9 ± 5.7	n.a.	6.26	8.99	n.a.	12.0	14.5
	Estriol	0.997	10.5	n.a.	93.3 ± 19.5	105.9 ± 22.0	n.a.	13.9	15.1	n.a.	28.2	23.3
Progestogens	Norgestrel	0.997	0.76	95.8 ± 30.4	93.3 ± 7.3	100.3 ± 6.0	6.99	5.16	4.34	10.0	11.9	7.31
	Megestrol acetate	0.996	6.12	94.2 ± 31.4	82.7 ± 8.3	107.6 ± 6.2	8.18	6.18	7.02	10.4	10.2	11.5
	Norethisterone	0.990	0.43	102.0 ± 20.2	98.6 ± 9.0	103.3 ± 3.8	4.16	6.49	5.12	7.55	9.72	8.46
	Progesterone	0.998	0.10	95.4 ± 3.5	91.9 ± 6.9	99.5 ± 8.0	5.76	5.82	4.51	6.90	12.1	7.36
Androgens	Testosterone	0.998	0.07	90.5 ± 2.0	93.6 ± 6.2	99.8 ± 5.8	7.81	3.28	6.05	9.98	9.91	9.04
	Boldenone	0.994	1.08	n.a.	102.6 ± 22.2	104.9 ± 18.5	n.a.	12.0	4.05	n.a.	15.4	7.69
	Nandrolone	0.993	0.42	90.4 ± 12.3	93.5 ± 7.6	97.0 ± 4.1	5.35	2.77	4.75	9.30	10.1	9.32
Glucocorticoids	Prednisone	0.991	4.07	84.8 ± 26.7	98.5 ± 2.5	114.7 ± 16.0	3.53	18.4	10.3	25.0	20.4	15.3
	Prednisolone	0.991	0.68	68.1 ± 29.3	84.7 ± 4.2	91.2 ± 10.8	7.28	9.06	8.48	17.5	15.1	14.2
	Cortisone	0.998	0.20	77.9 ± 18.4	85.0 ± 8.5	92.2 ± 5.2	13.7	2.60	6.53	16.8	7.71	12.2

[a] Coefficient of Variation.
[b] Not analyzed.

Table 4
Range of concentrations ($ng \cdot g^{-1}$) of target steroid hormones in microplastics from different Canary Island beaches (n = 3).

	Compound	LGR-LB Fragments	LGR-LB Pellets	GC-LC Fragments	TF-PG Fragments	LG-VGR Fragments	LG-PDI Fragments	LP-PNG Fragments	LP-PN Fragments	EH-AB Fragments	EH-AB Pellets
Estrogens	Estradiol	n.d.[a]	n.d.	n.d.	<LOQ[b]	n.d.	n.d.	n.d.	n.d.	<LOQ	<LOQ
	Estrone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	21.6–24.0	n.d.	28.3–43.2	n.d.
	Estriol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	48.5	n.d.	n.d.	<LOQ-25.8
Progestogens	Norgestrel	154.8	n.d.	n.d.	117.1	113.3–121.4	111.3	108.1–157.2	n.d.	39.7	19.2–41.8
	Megestrol acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Norethisterone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
	Progesterone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.1–18.3	12.1–26.1
Androgens	Testosterone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	15.0–26.5	37.3
	Boldenone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Nandrolone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	26.4	30.9
Glucocorticoids	Prednisone	11.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Prednisolone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	38.8	53.2	66.3	n.d.
	Cortisone	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] not detected.
[b] below quantification limits.

because the amount of microplastic debris that arrive to this beach is comparable to those of the most contaminated of the Canaries archipelago [29]. This phenomenon could explain why the samples collected from this beach contained the most detected hormones and the largest number of positive analyses. For norgestrel, with the highest detected concentrations in most samples, positive results were obtained on the four beaches near a sewage outfall. As seen in Fig. 5, a sewage outfall is less than 1.1 km from the beaches Playa Grande (TF-PG), Valle Gran Rey (LG-VGR) and Playa del Inglés (LG-PDI). On Playa de Nogales on La Palma (LP-PNG), the sewage submarine outfall of an urban STP is located 5.8 km from the beach. Considering that STPs are one of the main sources of steroid hormones and norgestrel is a widely used contraceptive pharmaceutical, the proximity of these outfalls to beaches could explain the presence of norgestrel in MPs debris at these locations. This synthetic progestogen was detected in the samples collected on two beaches considered hot spots of MPs of the Canary Islands: Playa Lambra in La Graciosa (LGR-LB) and Arenas Blancas in El Hierro (EH-AB) [29,30].

4. Conclusions

Steroid hormones are a group of endocrine-disrupting compounds that cause concern because their physicochemical properties could be adsorbed on MPs and mesoplastics. This phenomenon enhances the toxic effects that marine biota could suffer when exposed to microplastic pollution. For the first time, this study optimized a methodology to

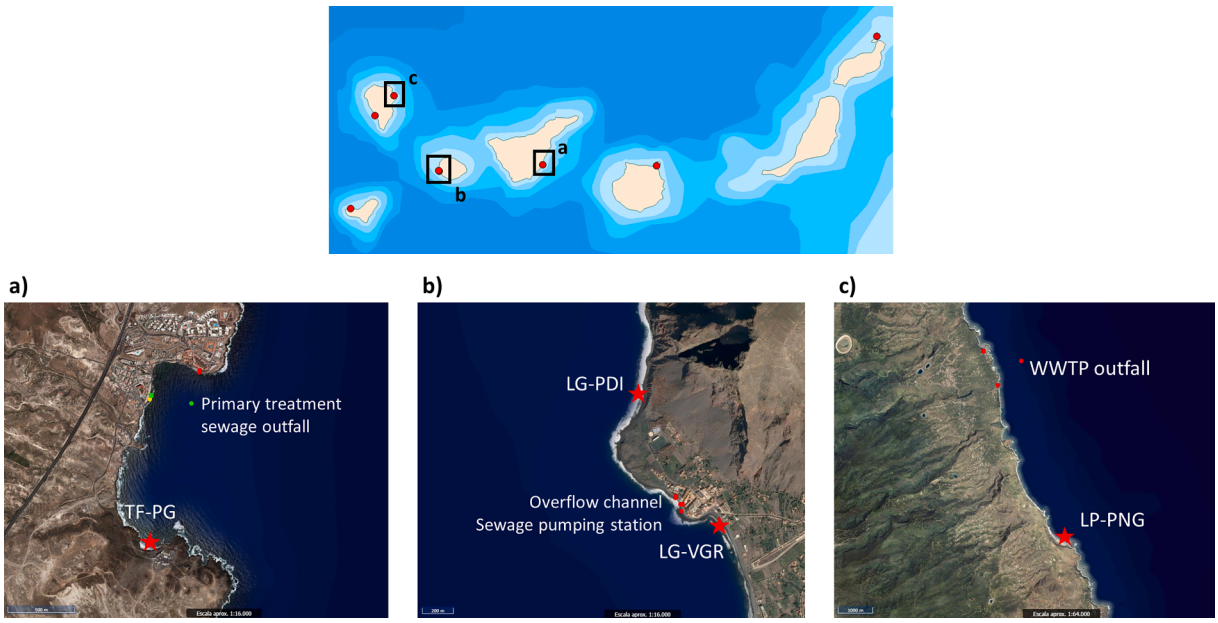

Fig. 5. Marine outfalls near the sample locations (indicated with a star) of Tenerife (a), La Gomera (b) and La Palma (c). Satellite images obtained from IDECanarias (https://visor.grafcan.es/visorweb/).

extract and determine steroid hormones residues adsorbed on MPs. The extraction methodology is based on UAE, which offers many benefits over traditional extraction methods, such as shorter extraction times and less organic solvent consumption. This extraction procedure was combined with UHPLC-MS/MS to obtain an analysis method capable of detecting steroid hormones at concentrations levels of few $ng{\cdot}g^{-1}$ (LoDs between 0.07 and 27.5 $ng{\cdot}g^{-1}$). The optimized methodology showed great recovery efficiencies (over 80% for most compounds) and good repeatability, with intraday and interday relative standard deviations ranging from 2.60 to 19.9 and from 6.90 to 28.2%, respectively.

The UAE-UHPLC-MS/MS optimized methodology was successfully implemented to work with MP residue samples from Canary Islands (Spain) beaches, and detected at least one of the 13 steroid hormones on eight beaches from five islands. The concentrations of the detected analytes ranged from < LoQ to 157.2 $ng{\cdot}g^{-1}$. The largest number of hormones detected on a beach coincided with those beaches considered to be hot spots for the presence of MPs. Likewise, the highest concentrations of steroid hormones corresponded to progestin norgestrel and were detected on those beaches closer to a submarine outfall. In addition, appreciable norgestrel concentrations were detected on the beaches considered to be hot spots for MPs contamination.

CRediT authorship contribution statement

R. Guedes-Alonso: Conceptualization, Methodology, Validation, Investigation, Data curation, Formal analysis, Writing – original draft. **Z. Sosa-Ferrera:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Resources, Writing – review & editing. **J.J. Santana-Rodríguez:** Conceptualization, Formal analysis, Funding acquisition, Supervision, Resources, Writing – review & editing.

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.

Acknowledgements

The funds to conduct this study were provided by Project MAC 2/1.1[a]/265 (IMPLAMAC) of Cooperation Program INTERREG MAC 2014-20. The authors also thank the EOMAR group from the Universidad de Las Palmas de Gran Canaria (ULPGC) for providing virgin pellets and some microplastic samples, as well as the AChem group from the Universidad de La Laguna (ULL) for also providing microplastic samples from other beaches.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2021.106834.

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