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Detection of emerging contaminants (UV filters, UV stabilizers and musks) in marine mussels from Portuguese coast by QuEChERS extraction and GC–MS/MS

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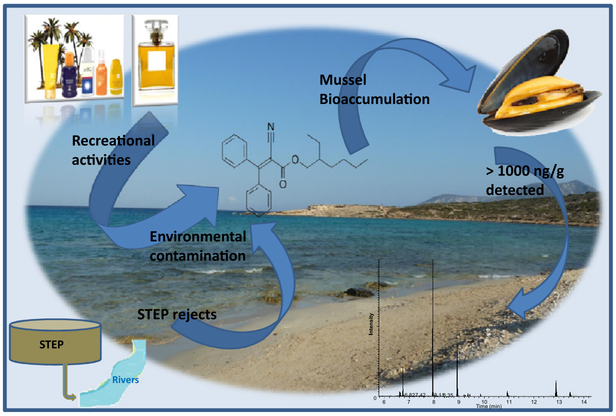
HIGHLIGHTS

- Wild mussels were a useful tool to monitoring of UV filters, stabilizers and musks.
- QuEChERS extraction was successfully applied to the determination of selected PCPs.
- Analysis by GC–MS/MS permitted us to obtain good levels of quantification.
- Results showed the differences due to recreational and tourist population pressure.
- The paper report for the first time the presence of UV filters in Portuguese coast.

GRAPHICAL ABSTRACT



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ABSTRACT

The UV filters and musk fragrances have come into focus because these compounds are contained and increasingly used not only in sunscreen products but also in many products of daily use, such as cosmetics, skin creams, plastics or varnish. In view of this, the main objective of the present work was to develop and validate a method for the determination of three UV filters, two UV stabilizers and four musks in mussel samples (*Mytilus galloprovincialis*). The procedure combined a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method with an analysis by gas chromatography–tandem mass spectrometry (GC–MS/MS). The methodology allowed the determination of target analytes at trace concentration levels (ng/g), with mean recoveries ranging from 91 to 112%. A monitoring study was conducted in four beaches in the Portuguese coast which are impacted by recreational activities and outflow of treated waste water effluents in rivers. The results are used to assess the occurrence of UV filters in comparison with UV stabilizers and musk fragrances which indicate other activities than bathing.

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1. Introduction

In recent years, personal care products (PCPs) have gained increasing interest due to their huge consumption and potentially harmful concentration in aqueous environment. Among these PCPs, UV filters and polycyclic musks showed potential endocrine disruption. In a recently study carried out in zebrafish it was observed that the exposure to these chemicals can induce a decrease in spermatocytes in the testes and a decrease in previtellogenic ovocytes in the ovaries (Zucchi et al., 2011). Up to date, no information on UV stabilizers' potential adverse effects is available. Polycyclic musks are used as fragrance ingredients in the PCPs and as washing and cleaning agents. UV filters and stabilizers are compound of sunscreens and cosmetics to prevent chemical degradation and skin damage under sunlight irradiation. They are also used as sunblocking agents for the protection of materials against UV such as plastics, adhesives and rubber.

In relation with the occurrence of PCPS in the aquatic environment, musk and UV filters have begun to be in the attention focus due to their high and widespread daily use. The main emission sources to environmental water are (i) direct way from recreational activities such swimming and bathing in beaches or (ii) indirect way via wastewater treatment plant (WWTP) release. Musk and UV filter compounds have been recently recognized as important organic contaminants of the aquatic environment (European Parliament, 2007). They were included in the analyte list as a point of interest in order to check for their presence in the aquatic environment as well. Moreover, due to their lipophilic properties (log Kow > 4.30), these substances have potential for bioaccumulation. The occurrence of UV filters is well documented in water samples (Moeder et al., 2010) and in fish (Mottaleb et al., 2009). Regarding marine mussels, 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) and octocrylene (OC) have previously been quantified up to 7000 ng/g and 200 ng/g dry weight, respectively, in samples collected from French coast (Bachelot et al., 2012). EHMC and OC have also been measured at above 200 ng/g and at 60 ng/g respectively in fish of a river basin in the south of Spain (Gago-Ferrero et al., 2013a, 2013b). Fent et al. (2010) reported EHMC in crustacean and mollusks in the range of 22 to 50 ng/g from Lake Greifen in Switzerland. Recently, Gago-Ferrero et al. (2013a, 2013b) mentioned the occurrence of OC in dolphins for the first time in marine mammals with concentrations from 89 to 782 ng/g lipid weight along the Brazilian coastal area. Bachelot et al. (2012) pointed to the bathing activities as the main input responsible for UV filter accumulation in mussels whereas Fent et al. (2010) obtained those substances that bioaccumulate downstream WTP releases. Up to date, there is no information on the levels of those contaminants in sites impacted by both bathing activities and sewage releases along the coast. In regard to musks, celestolide and galaxolide were found in green-lipped mussels (*Perna viridis*) at high levels 67 ng/g and 5460 ng/g, respectively (Shek et al., 2008), while the levels reported for cashmeran in sewage sludge were up to 330 ng/g (Herren and Berset, 2000). For UV stabilizers, UV-326 has frequently been detected in mussels from Asia-Pacific coastal waters (Nakata et al., 2012). However, to our knowledge, no previous study has reported the detection of this substance in organisms from Europe waters to date.

MASE (microwave assisted solvent extraction) and PLE (pressurized liquid extraction) have been the most common techniques used to extract PCPs in aquatic organisms (Bachelot et al., 2012; Gago-Ferrero et al., 2013a, 2013b). Although some of these techniques are now automatized, they are long, time consuming and require a large volume of solvents. Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) but not automatized approach has been developed in 2003 for the extraction of pesticides in food (Anastassiades et al., 2003). In a recent publication, a modified QuEChERS procedure was validated and successfully applied to the determination of residues of two anticonvulsants in marine mussel (Martinez Bueno et al., 2013). The extraction and purification are key steps in decreasing matrix effect and optimizing extraction overall of the analytical procedure.

The analytical methods for the detection and quantification of emerging contaminants are generally based on gas chromatography (GC) or liquid chromatography (LC) coupled with mass spectrometry (MS). Choosing between GC and LC is based on the physicochemical properties and thermostability of the target analytes. For the analysis of the selected compounds (UV filter, UV stabilizers and musk fragrances), GC is more suitable because many of these compounds have high lipophily and are thermostable. Furthermore, selective ion monitoring (SIM) or tandem-mass-spectrometry (MS/MS) has been the most frequent working mode to efficiently remove interference from co-eluting peaks and to ensure sensitivity, facilitating the detection of trace levels of organic compounds (Mitjans and Ventura, 2005; Lee et al., 2005; Herren and Berset, 2000).

With this purpose in mind, this work reports the development and application of a sensitive method for the determination of three UV filters (EHMC, OC, OD-PABA), two UV stabilizers (UV-P and UV-326) and four musks (galaxolide, celestolide, cashmeran and musk ketone) in marine mussels (*Mytilus galloprovincialis*). Different salt extractors and clean up sorbent materials (Z-Sep-Plus, $CaCl_2$/PSA, Na_2SO_4/PSA/C18) were tested to obtain the best results, i.e. decreased matrix effects and optimize extraction performance of a QuEChERS procedure. Analyte identification and confirmation were performed using a triple quadrupole mass spectrometer (GC–MS/MS). The developed method was applied for the detection of analytes in mussel samples collected in four different sites in Algarve coast in the south of Portugal. The four selected sites receive bathing activities and were more or less impacted by indirect WWTP rejects. This paper reports for the first time the results on the presence of such PCPs in marine mussels from this region. The collected samples were a representative of different spatial, recreational and tourist population pressure levels during four months (in summer period) to account for temporal variations.

2. Materials and methods

2.1. Chemicals

The compounds selected for this study were nine organic pollutants belonging to three different categories: UV filter, UV stabilizer and musk. The UV filters were: 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC), octyldimethyl p-amino benzoic acid (OD-PABA) and octocrylene (OC). The UV stabilizers were: 2-(2-hydroxy-5-methylphenyl)benzotriazole (UV-P) and 2-*tert*-butyl-6-(5-chloro-2*H*-benzotriazol-2-yl)-4-methylphenol (UV-326). The musks were: galaxolide, celestolide, musk ketone and cashmeran. Their structure and physicochemical properties are presented in Table 1. They were purchased at analytical grade (purity > 90%) from: Sigma-Aldrich, Merck, Fluka and BLH SA. Chrysene-d12 was the reference compound used as a surrogate standard for extraction and injection monitoring due to the unavailability of internal standards of the target compounds. Physical properties and molecular weight (log Kow = 5.8 and m = 240 g/mol) of chrysene-d12 are close to the compounds studied. Furthermore, chromatographic peak of chrysene-d12 elutes between those of the studied compounds. The structure and physicochemical properties of chemicals are presented in Table 1. Stock standard solutions of individual compounds were prepared at a concentration of 1–2 mg/mL in ethanol. All the standard solutions were stored at −20 °C. Working solutions at different concentrations were prepared by appropriate dilution of the stock solution in heptane. To avoid contamination at low levels like environmental levels, gloves were worn during all the sampling preparation and glassware was cleaned twice with acetone and dichloromethane and dried at 300 °C in the oven.

Analytical-grade heptane, ethanol, methanol (MeOH), acetonitrile (AcN), sodium sulfate (Na_2SO_4) anhydrous and sodium chloride (NaCl) were supplied by Carlo Erba (Val de Reuil, France). Formic acid (purity, 98%) was obtained from Fisher Labosi (Elancourt, France). Sodium citrate dihydrate ($Na_3Cit{:}2H_2O$) and sodium citrate dibasic sesquihydrate