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Analysis of endocrine disruptor compounds in marine sediments by in cell clean up-pressurized liquid extraction-liquid chromatography tandem mass spectrometry determination

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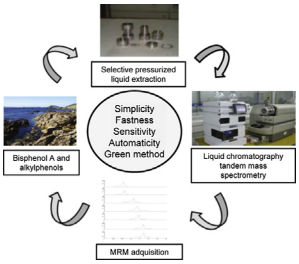
HIGHLIGHTS

- Simultaneous extraction and clean-up for BPA and APs analysis in marine sediments.
- Simple, fast, sensitive and automatic analytical methodology.
- Lower volumes of solvents, analysis time and waste generation (Green Chemistry).
- Fate and occurrence of BPA and APs in marine environment.
- Sediment toxicity. Evaluation of impact in aquatic biota.

GRAPHICAL ABSTRACT



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ABSTRACT

A less time-, solvent- and sorbent-consuming analytical methodology for the determination of bisphenol A and alkylphenols (4-*tert*-octylphenol, 4-octylphenol, 4-*n*-nonylphenol, nonylphenol) in marine sediment was developed and validated. The method was based on selective pressurized liquid extraction (SPLE) with a simultaneous in cell clean up combined with liquid chromatography-electrospray ionization tandem mass spectrometry in negative mode (LC-ESI-MS/MS). The SPLE extraction conditions were optimized by a Plackett–Burman design followed by a central composite design. Quantitation was performed by standard addition curves in order to correct matrix effects. The analytical features of the method were satisfactory: relative recoveries varied between 94 and 100% and repeatability and intermediate precision were <6% for all compounds. Uncertainty assessment of measurement was estimated on the basis of an in-house validation according to EURACHEM/CITAC guide. Quantitation limits of the method (MQL) ranged between 0.17 (4-*n*-nonylphenol) and 4.01 ng g^{-1} dry weight (nonylphenol). Sensitivity, selectivity, automaticity and fastness are the main advantages of this green methodology. As an application, marine sediment samples from Galicia coast (NW of Spain) were analysed. Nonylphenol and 4-*tert*-octylphenol were measured in all samples at concentrations between 20.1 and 1409 ng g^{-1} dry weight, respectively. Sediment toxicity was estimated and no risk to aquatic biota was found.

1. Introduction

Endocrine disruptor compounds (EDCs) have increasingly gained attention in the last years due to their potential adverse

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effects on the reproduction system of human and wildlife, as it was demonstrated in different in-vivo and in-vitro assays [1].

Among all EDCs, bisphenol A (BPA) and alkylphenols (APs) deserve special consideration due to their ubiquity in the environment. BPA is used in the production of polycarbonates, epoxy resins and flame retardants whereas APs are constituents of household and industrial products as herbicides, detergents and plasticizers [2].

The main sources of these EDCs into the environment are wastewater treatment plants discharges, where these compounds are scarcely removed [3]. Furthermore, their physical and chemical properties (log K_{ow} 3.9–4.48; K_{oc} 151–245 L kg^{-1} [2]) suggest these compounds tend to be associated with sediments and accumulated in organisms and humans.

In order to preserve aquatic environment and public safety, chemical pollution should be considered, controlled and minimized/eliminated, as it was set in the recent Directive 2013/39/EU [4] as regards priority substances in the field of water policy amending Directive 2000/60/EC and Directive 2008/105/EC. In this way, the accumulation of pollutants in the ecosystem, the losses of habitats and biodiversity and the damage of public health will be avoided. In this Directive, Environmental Quality Standards (EQS) in waters were established for 45 priority hazardous substances. Whereas BPA was not prioritized as a possible priority substance in the new legislation, the EQS of APs showed in Directive 2008/105/EC were maintained [5]. It is known that BPA and APs are one of the major contributors of estrogenic activity in waters and sediments [6]; consequently, and in our opinion, BPA should be incorporated in future researches.

Although the fate and occurrence of BPA and APs in freshwater are widely studied, seawater is still not well-documented [7]. Marine Strategy Framework Directive (Directive 2008/56/EC) [8] establishes a community action to ensure a good environmental status of marine ecosystem, including the evaluation of contamination by hazardous substances. Therefore, monitoring programs are required to assess chemical pollution.

In general, water monitoring is used to evaluate the presence of pollutants in marine environment, comparing measured concentrations with its water EQS [9]. However, depending on the physical and chemical properties of contaminants, other matrices such as sediments or biota could also be monitored. For example, in the case of compounds with log K_{ow} between 3 and 5 such as BPA and APs, sediment is preferred as an additional monitoring matrix [9]. However, no EQS for sediments were established at the moment by Directive 2013/39/EU due to the lack of data. For all the reasons commented before, analysis of BPA and APs in marine sediments should be encouraged.

Due to the fact that phenolic compounds tend to bind tightly to the sample matrix, an exhaustive extraction technique is needed to separate them from sediment samples. A review of the state of the art in the analytical methodologies (from 2008 to the present) for the determination of these pollutants in sediments is shown in Table 1. Classic extraction techniques such as Soxhlet extraction [10–12] and sonication [13,14] were initially employed for this purpose. However, these techniques have been replaced by others which offer a great reduction in time and solvent consumption such as pressurized liquid extraction (PLE) [15,16].

Because of the complexity of this matrix, a subsequent clean-up step is required to enable the separation and detection of these compounds at low concentrations. Off line solid phase extraction using silica gel [10,12] and Florisil [11,13,14] are the most common clean-up. This procedure increases the analysis time and could produce blank contamination problems. Hence, alternative analytical methods which include simultaneous extraction and clean-up could be necessary.

One of these techniques is selective pressurized liquid extraction (SPLE) based on a PLE extraction with simultaneous in cell clean-up. This methodology was previously applied to the extraction of BPA and APs in sewage sludge following by the determination by gas chromatography mass spectrometry (GC–MS) [17]. However, the derivatization step to transform these compounds into volatile derivatives presents some disadvantages (such as more time for analysis or losses of analytes) and, consequently, greater error [2]. For this reason, liquid chromatography coupled with mass spectrometry should be employed in the analyses of these compounds [18].

The main objective of this paper is the development and validation of a novel, robust, accurate and sensitive analytical methodology for the determination of BPA and APs in marine sediments based on a selective pressurized liquid extraction (SPLE) with an in cell-clean up followed by the quantitation using LC-ESI-MS/MS. As far as we know, no previous works about the simultaneous extraction and clean-up of these pollutants in marine sediments using this methodology could be found in the literature. The main advantages of the proposed method were simplicity, automaticity, fastness and reduction in the analysis

Table 1
Analytical methodologies for the analysis of BPA and APs in sediments found in the literature (Web of Science^{TM}) from 2008 to the present.

Analysed compounds	Sediment type	Amount of sample (g)	Extraction technique	Extraction parameters	Determination	Recovery (%)	Method quantitation limit	Reference
NP	River	2 g	PLE	Acetone:MeOH (50:50 v/v) temperature 50 °C static time, 3 × 1 min	HPLC-UVVis	75–85	0.015 μg g^{-1} (LOD)	[15]
BPA, 4-*t*OP, NP	Marine	1 g	US-SBSE	1)MeOH, 30 min 2)6 h, 900 rpm	GC–MS	78–89	0.2–1.7 ng g^{-1}	[31]
BPA, 4-*t*OP and NP	Marine	NM	US-SPE	DCM:MeOH (7/3 v/v) C18 cartridges	LC–MS/MS	NM	NM	[25]
BPA, 4-*n*-NP, NP	River	5 g	Soxhlet-SPE	DCM, 30 mL, 20 min LC-Si cartridges	GC–MS	94–103	0.15–0.50 n g g^{-1}	[32]
BPA, NP	Marine	5 g	Digestion-LLE-SPE	Digestion in methanolic KOH hexane silica gel	GC–MS	94–98	NM	[33]
BPA, 4-*t*OP, NP	Marine	10 g	Soxhlet-SPE	DCM, 270 mL, 10 h silica gel	GC–MS	95–107	NM	[10]
BPA, NP	Marine	20 g	US-SPE	Acetone:MeOH (80:20 v/v), 2 h Florisil	LC–MS/MS	78–86	0.2–5.0 ng g^{-1}	[14]
4-*t*OP, NP	River	1 g	US-SPE	DCM:hexane (10 min) hexane:acetone (10 min) Florisil	GC–MS/MS	84–107	38–48 ng g^{-1}	[13]
4-*t*OP, NP	Marine	2.5 g	Soxhlet-SPE	DCM, 20 h Florisil	HPLC-FLD	NM	0.2–0.5 ng g^{-1}	[11]
4-*t*OP, NP	River	10 g	Soxhlet-SPE	DCM, 48 h silica gel	GC–MS	NM	NM	[16]
		10 g (+10 g sand)	PLE-SPE	Toluene:MeOH (1:6) temperature 100 °C static time, 5 min silica gel		NM	NM	[16]

DCM, dichloromethane; FLD, fluorescence detection; GC, gas chromatography; HPLC, high performance liquid chromatography; LC, liquid chromatography; LLE, liquid liquid extraction; MeOH, methanol; MS, mass spectrometry; NM, not mentioned; PLE, pressurized liquid extraction; SBSE, stir bar sorption extraction; SPE, solid phase extraction; US, sonication; UV–vis, ultraviolet–visible.