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Determination of alkylphenols and bisphenol A in seawater samples by dispersive liquid–liquid microextraction and liquid chromatography tandem mass spectrometry for compliance with environmental quality standards (Directive 2008/105/EC)

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ABSTRACT

A fast, simple, sensitive and green analytical chemistry method for the simultaneous determination of alkylphenols (4-tert-octylphenol, 4-octylphenol, 4-n-nonylphenol, nonylphenol) and bisphenol A in seawater was developed and validated. The procedure was based on a dispersive liquid–liquid microextraction (DLLME) of a small volume of seawater sample (30 mL) using only 100 μL of 1-octanol, combined with liquid chromatography–electrospray ionization tandem mass spectrometry in negative mode (LC–ESI-MS/MS). The matrix effect was studied and compensated using deuterated labelled standards as surrogate standards for the quantitation of target compounds. The analytical features of the proposed method were satisfactory: repeatability and intermediate precision were <10% and recoveries were around 84–104% 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 0.005 and 0.03 $\mu g\,L^{-1}$, therefore the levels established in the Directive 2008/105/EC were achieved. The applicability of the proposed method was demonstrated analyzing seawater samples from different sites of A Coruña (Northwest of Spain). The analyses showed the presence of all compounds at levels between 0.035 (bisphenol A) and 0.14 $\mu g\,L^{-1}$ (nonylphenol).

1. Introduction

Alkylphenols (APs) have been used in the production of herbicides, detergents and synthetic resin products. In addition, they are the biodegradation products of alkylphenols polyethoxylates, one of the main non-ionic surfactants in industrial applications [1]. Bisphenol A (BPA) is used as a monomer for the production of epoxy resins, phenol resins, polycarbonates, polyesters and lacquer coatings for food cans [2].

These compounds are considered synthetic endocrine-disrupting chemicals because they can alter immune functions, produce sexual dysfunction or cause cancer at low concentrations [3]. For all these reasons, alkylphenols (specifically 4-tert-octylphenol and 4-nonylphenol) have been included in the Water Framework Directive 2000/60/EC [4] and in the Directive 2008/105/EC [5] which sets the Environmental Quality Standards (EQS) for these compounds. The annual average (AA) for 4-nonylphenol in seawater samples (other surface waters) is 0.3 $\mu g\,L^{-1}$, whereas the maximum allowable concentration (MAC) is 2 $\mu g\,L^{-1}$. In the case of 4-tert-octylphenol, the MAC is not applicable since the AA (0.01 $\mu g\,L^{-1}$) is significantly lower than the values derived on the basis on acute toxicity. Therefore, it is considered protective against short-term pollution peaks in continuous discharges.

On the other hand, bisphenol A has not being legislated in water yet. However, it is included in the Annex II of the Directive 2008/105/EC as a future regulated substance in the “list of 33 priority substances” [5]. Furthermore, the Directive 2009/90/EC establishes that the quantitation limit of the method must be lower than 30% of the EQS (Directive 2008/105/EC) [6].

In order to support the implementation of the Directive 2008/105/EC, fast, simple and sensitive analytical methods are needed. Furthermore, the incorporation of these analytical methodologies in monitoring programs is mandatory in order to control the presence of pollutants in the environment.

Different chromatography techniques were used to determine alkylphenols and bisphenol A. Gas chromatography has been widely used [7–9]; however, due to the polarity and the low

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volatility of these compounds a derivatization step is frequently needed. In order to avoid losses of analytes and to simplify the experimental process, liquid chromatography is the most used technique.

Although liquid chromatography coupled with ultraviolet visible and fluorescence detectors are used for the determination of APs and BPA [10–12], these detectors do not have the inherent specificity of the mass spectrometer. Furthermore, a tedious sample preparation is required and interferences are common in the analysis of complex matrices [13].

Therefore, liquid chromatography coupled to mass spectrometry (LC–MS) is the most powerful tool to analyze APs and BPA in environmental matrices [1,13–16]. Besides its sensitivity and selectivity, the emergence of new ionization techniques which allow the soft ionization of a wide range of substances, such as electrospray (ESI) and atmospheric pressure chemical ionization (APCI), have encouraged the use of this technique in the last years.

An efficient sample preparation to remove possible interferences and preconcentrate the analytes is also mandatory to achieve the required levels. APs and BPA are commonly extracted by solid phase extraction (SPE) [1,13–15]. However, this technique is being replaced by other fast and simple techniques that minimize the waste of organic solvents according with the principles of the Green Chemistry.

Dispersive liquid–liquid microextraction (DLLME) [10,17], was introduced by Rezaee et al. [18] in 2006 and is based on the extraction of analytes in aqueous samples by an appropriated mixture of extraction solvent and dispersant agent producing a cloudy solution. Some of its advantages are short extraction time, ease of operation, low cost and high enrichment factors. The main problem is the correct selection of mixture of solvents because losses of analytes can take place. To avoid this situation, some authors suggest that dispersant agent can be eliminated using an adequate extraction solvent helped with an agitation step to achieve the formation of the cloudy [19].

The main novelty of this paper is the development and validation of a simple, fast, sensitive and environmental friendly method for the simultaneous determination of APs and BPA in seawater based on dispersive liquid–liquid microextraction followed by the determination using LC–ESI-MS/MS. Although these compounds have been investigated in different environmental matrices, they are scarcely studied in seawater because of the difficulty of the matrix and the low levels of concentration [20]. Therefore, analytical methodologies are needed in order to investigate the distribution and partitioning of these compounds in marine ecosystem. This method enables the determination of these pollutants at ultratrace levels according to the restrictive legislation (Directive 2008/105/EC) analyzing only 30 mL of sample; consequently it can be an important tool for monitoring strategies to control the presence of alkylphenols and bisphenol A in seawater samples. As far as we know, other published works do not achieve these low limits or need to process higher volumes of samples which are difficult to manipulate and storage.

2. Experimental

2.1. Standards and reagents

Nonylphenol technical mixture (NP) 94% purity (±1% tolerance) and 4-n-nonylphenol (4-n-NP) 99.9% purity (±0.5% tolerance) were from Riedel-de Haën (Seelze, Germany). Bisphenol A (BPA) 99% purity (±0.5% tolerance), 4-tert-octylphenol (4-tOP) 97% purity (±0.5% tolerance) and 4-octylphenol (4-OP) 99% purity (±0.5% tolerance) were obtained from Sigma–Aldrich (Steinheim, Germany). All standard solutions (1000, 10 and 1 mg L^{-1}) containing all the compounds were prepared in methanol (SPS grade) from Romil Ltd. (Cambridge, United Kingdom) and stored at 4 °C.

As surrogate internal standards, 4-n-nonylphenol-2,3,5,6-d_4 (NP-d_4) 99.3% was obtained from CDN Isotopes (Pointe-Claire, Canada) and solution of bisphenol A-d_{16} (BPA-d_{16}) 99.5% in acetonitrile (100 mg L^{-1}) was from Dr. Ehrenstorfer GMBH (Augsburg, Germany).

For the extraction, 1-octanol Chromasolv® (grade HPLC 99%) was from Sigma–Aldrich Co. (Madrid, Spain). For the determination, methanol LC–MS PAI and ammonia (30%) for instrumental analysis were from Panreac (Barcelona, Spain). Water was purified with a Direct 5 Milli Q system (Millipore, Bedford, MA, USA). Seawater samples were used for the optimization and validation of the analysis method.

2.2. Sampling

Seawater samples were collected in amber glass containers and stored refrigerated at 4 °C before the analysis. Due to the low stability of APs and BPA, samples had to be analyzed within five days of their sampling.

2.3. Extraction

Aliquots of 30 mL of seawater samples were extracted as follows: 100 μL of 1-octanol was added as extractant solvent and the mixture was vigorously shaken using an agitation plate Vibrax-VXR by IKA (Staufen, Germany) during 5 min at 1200 rpm. Separation of two phases occurred upon centrifugation (Eppendorf 5804, Madrid, Spain) at 3500 rpm for 3 min. The fine droplets of 1-octanol were collected and the volume was adjusted to 1 mL with methanol due to the immiscibility of the 1-octanol with the LC mobile phase. To remove any solid particles that might interfere in the analysis and damage the equipment, the extract was passed through a 0.2 μm syringe filter of PTFE (Teknokroma, Barcelona, Spain) before LC injection.

2.4. Liquid chromatography–tandem mass spectrometry

LC analyses were performed using an Agilent HP-1200 Series LC system equipped with an autosampler (volume injected was 25 μL), a binary solvent pump and a thermostated column oven. The chromatographic separation was carried out with a column Hypersil Gold C18 (150 mm × 2.1 mm), 3 μm Thermo Fisher Scientific Inc. (Waltham, MA), using as mobile phase A (water) and B (methanol) with 0.05% of ammonia as modifier. A 14 min gradient was performed as follows: 20% B (1 min) to 100% B in 4 min; this percentage was maintained for 7 min and returned to initial conditions in 2 min. The system was re-equilibrated for 7 min between runs. The flow rate was 0.25 mL min^{-1} and the oven temperature was set at 40 °C.

The LC system is coupled to a mass spectrometer with a triple quadrupole detector (API 3200, Applied Biosystems, Carlsbad, CA, USA). It was equipped with an APCI/ESI source. All compounds were detected with ESI interface operating in negative mode.

2.5. Quantitation and quality control

Multiple-reaction monitoring (MRM) was chosen as acquisition mode because it allows high sensitivity and selectivity [1] and deuterated surrogate standards were used for the quantitation in order to correct matrix effect [14]. BPA-d_{16} was used as BPA surrogate, whereas 4-NP-d_4 was used to determine APs.

According to the Decision 2002/657/EC, four identification points (one precursor ion and two products ions) were required