



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 $\mu\text{g L}^{-1}$, 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 $\mu\text{g L}^{-1}$ (nonylphenol).

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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 $\mu\text{g L}^{-1}$, whereas the maximum allowable concentration (MAC) is 2 $\mu\text{g L}^{-1}$. In the case of 4-tert-octylphenol, the MAC is not applicable since the AA (0.01 $\mu\text{g L}^{-1}$) 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 ($\pm 1\%$ tolerance) and 4-n-nonylphenol (4-n-NP) 99.9% purity ($\pm 0.5\%$ tolerance) were from Riedel-de Haën (Seelze, Germany). Bisphenol A (BPA) 99% purity ($\pm 0.5\%$ tolerance), 4-tert-octylphenol (4-tOP) 97% purity ($\pm 0.5\%$ tolerance) and 4-octylphenol (4-OP) 99% purity ($\pm 0.5\%$ 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-d₄ (NP-d₄) 99.3% was obtained from CDN Isotopes (Pointe-Claire, Canada) and solution of bisphenol A-d₁₆ (BPA-d₁₆) 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 μ L 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 μ m 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 μ L), a binary solvent pump and a thermostated column oven. The chromatographic separation was carried out with a column Hypersil Gold C18 (150 mm \times 2.1 mm), 3 μ m 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-d₁₆ was used as BPA surrogate, whereas 4-NP-d₄ was used to determine APs.

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

for a suitable confirmation of the compounds for LC–MS/MS analysis. Furthermore, the relation between the transitions (MRM ratio) was also used as a criterion for the identification in samples analysis [21].

2.6. Blank contamination considerations

Blank contamination is a common problem in the determination of alkylphenols and bisphenol A at these low levels. In order to reduce blank problems, the use of detergents and plastic material should be avoided. All the glassware was carefully washed with acetone, Milli-Q water and methanol before use.

Blank analysis revealed the presence of NP at level of concentration $<5 \text{ ng L}^{-1}$. Consequently, procedural blanks were frequently checked and kept under control. In order to identify the source of NP, procedural blank (non-spiked seawater sample) and solvent blank (no sample) were compared. The responses obtained in both cases were similar; therefore, it can be concluded that blank contamination come mainly from the experimental process. Procedural blanks were analyzed in triplicate and the contribution of NP ($<5 \text{ ng L}^{-1}$) was subtracted from the samples in order to avoid overestimations in the results.

3. Results and discussion

3.1. Study of LC–MS/MS variables

Optimization of MS/MS settings was performed by direct infusion of individual standard solutions using MS software (Analyst 1.4, Applied Biosystems, Carlsbad, CA, USA). The relevant instrument settings for each compound are shown in Table 1. According to the Decision 2002/657/EC [21], two products ions were chosen for the suitable confirmation of each compound, except for 4-OP because of the presence of an interference in one of them [15]. The parent ion of BPA- d_{16} was 241 instead of 242 because it becomes BPA- d_{15} in water [22]. The ion spray voltage was the maximum level -4500 V . The rest of parameters of the source that have influence in the ionization were tested using a multivariable Plackett–Burman $2^7 \times 3/32$ randomized design with one dummy factor. The experimental matrix was obtained using the statistical program Statgraphics 5.0 for Windows (Table 2). The response in each experiment was measured as area of peak and the results were analyzed by means of the representation of the first and second order Pareto charts ($P=95.0\%$).

The Pareto charts (Fig. 1) obtained for each compound show that collision gas flow is the only significant factor for alkylphenols. No interactions between variables were observed. The selected value

Table 2

Variables and values of the Plackett–Burmann design used in the optimization of the MS/MS conditions.

Variables (units)	Minimum value	Maximum value
Modifier: ammonium acetate (mM)	0	10
Temperature ($^{\circ}\text{C}$)	200	650
Gas nebulizer flow (psi)	20	60
Curtain gas flow (psi)	10	30
Collision gas flow (psi)	0	10
Vertical distance between the capillary and source (mm)	5	10
Dummy	-1	1

of collision gas was the maximum (12 psi) because of its positive influence. The values of the no significant parameters were selected according to the sign of the estimated effect as well other instrumental conditions: nitrogen was used as curtain gas (10 psi) and nebulizer gas (35 psi). The auxiliary gas (35 psi) was air. The temperature of the source was fixed at 650°C and the vertical distance between the capillary and the source was 5 mm.

Due to the influence of the mobile-phase composition on the ionization efficiency in LC–ESI–MS/MS three kinds of modifiers were studied: acetic acid (0.05%, v/v) [23], ammonium acetate (2.5 mM) [13,14,24] and ammonia (0.05%, v/v) [25]. Fig. 2 shows that ammonia highly increases the response of APs. Basic pH (>8)

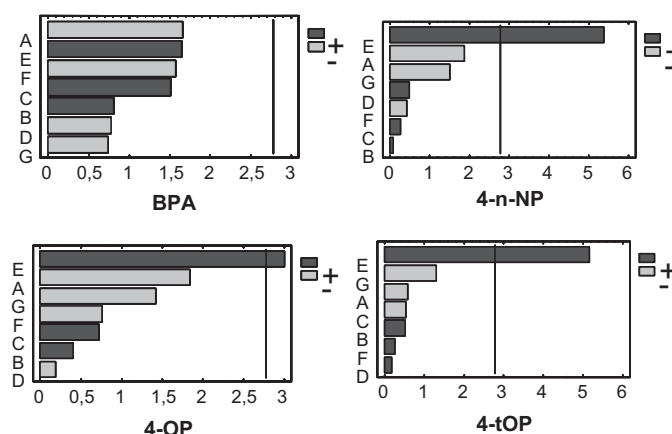


Fig. 1. Optimization of MS/MS conditions: First order Pareto Charts obtained for all compounds. Variables of the study were modifier of the mobile phase (A), temperature (B), gas nebulizer flow (C), curtain gas flow (D), collision gas flow (E), vertical distance (F) and dummy (G).

Table 1

Parent and fragment ions, retention times and MS/MS parameters for each compound.

Analyte	t_R (min)	Precursor ion (m/z)	Product ions (m/z)	Decustering potential (V)	Collision energy (V)	MRM ratio ^b
BPA- d_{16}	9.39	241 [M–D] [–]	142 ^a 221	–45	–44 –46	1.6 ± 0.1
BPA	9.40	227 [M–H] [–]	212 ^a 133	–40	–24 –32	15.7 ± 2.8
4-tOP	10.62	205 [M–H] [–]	133 ^a 116	–45	–30 –76	8.9 ± 0.3
NP	10.89	229 [M–H] [–]	133 ^a 116	–40	–38 –74	5.8 ± 0.1
4-OP	10.99	205 [M–H] [–]	106 ^a –	–50	–26 –	–
4-n-NP	11.23	219 [M–H] [–]	106 ^a 119	–45	–28 –44	22.4 ± 0.4
NP- d_4	11.23	223 [M–D] [–]	109 ^a 129	–45	–28 –46	14.4 ± 1.1

^a Quantitation ion.

^b Ratio and tolerances between quantification and confirmation MRM transitions according to Decision 2002/657/EC [21].

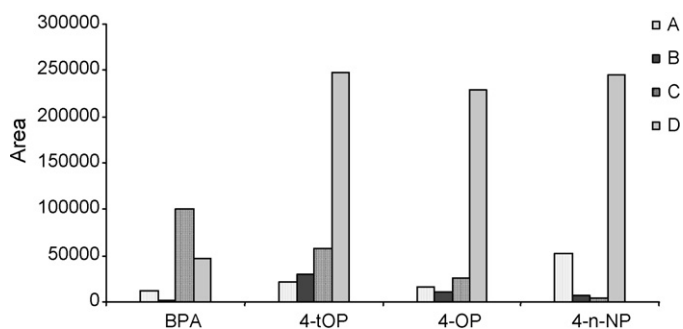


Fig. 2. Influence of the mobile phase modifiers on the signal: without modifier (A), acetic acid 0.05% (B), ammonium acetate 2.5 mM (C) and 0.05% ammonia (D) ($n = 2$).

contributes to the ionization of the target compounds, except in the case of BPA (the best modifier for BPA is ammonium acetate). In order to reach a compromise between these situations and achieve the low limits established by Directive 2008/105/EC for alkylphenols, ammonia (0.05%, v/v) was chosen as a modifier.

The influence of the mobile phase flow in the ionization was also studied. Flows between 0.2 and 0.3 mL min⁻¹ were tested. The best result was obtained with a flow of 0.25 mL min⁻¹. No effect on the chromatographic separation was observed.

Finally, a chromatogram of a seawater sample spiked with 1.7 µg L⁻¹ of each compound and 0.5 µg L⁻¹ of surrogate standards

is shown in Fig. 3. A satisfactory separation is achieved in less than 12 min with these LC–MS/MS conditions.

3.2. Optimization of extraction parameters

Several organic solvents (200 µL) with different water miscibility (n-hexane, cyclohexane, dichloromethane, 1,2-dichloroethane, 1-octanol) [10,12,26] were tested as extractants of 30 mL of seawater spiked at a 1.7 µg L⁻¹ level of all compounds of study ($n = 2$). Because of their water immiscibility, the organic solvents tend to form a single micro drop when they are added to a water sample; therefore, an agitation (5 min, 1200 rpm) was needed to break down the drop of organic solvent and to improve the dispersion process. After that, centrifugation (3 min at 3500 rpm) was used to separate the two phases again. Finally, extracts 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.

As can be seen in Fig. 4, 1-octanol was the solvent that achieved a quantitative extraction of all compounds (included BPA, which was not extracted by any other solvent). The recoveries obtained with this solvent were higher than 100%, hence the influence of 1-octanol during the ionization was evaluated. The study showed that there was an increase in the signal when 1-octanol was present in the extract. For this reason, the same volume of 1-octanol employed as extractant solvent was added to the standards used for the quantitation. After that, recoveries between 95 and 102% were obtained.

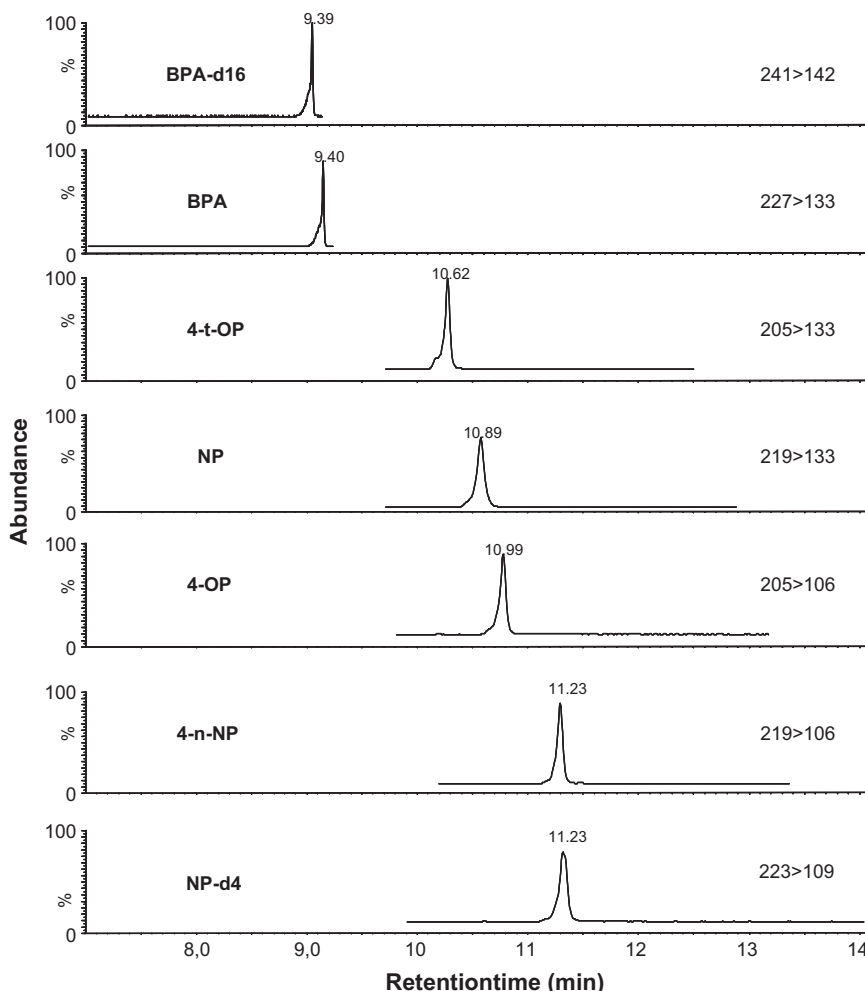


Fig. 3. LC–MS/MS chromatogram of a spiked seawater sample (1.7 µg L⁻¹ of each compound and 0.5 µg L⁻¹ of surrogate standards).

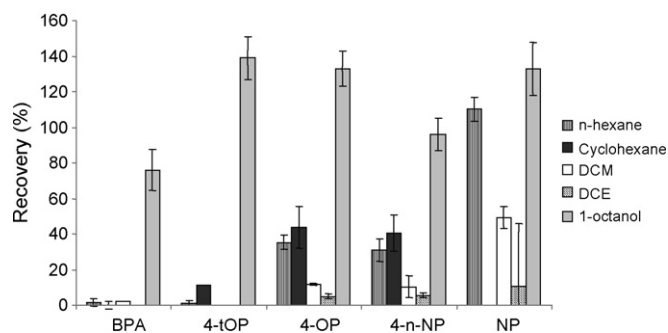


Fig. 4. Selection of extraction solvent in DLLME ($n=2$).

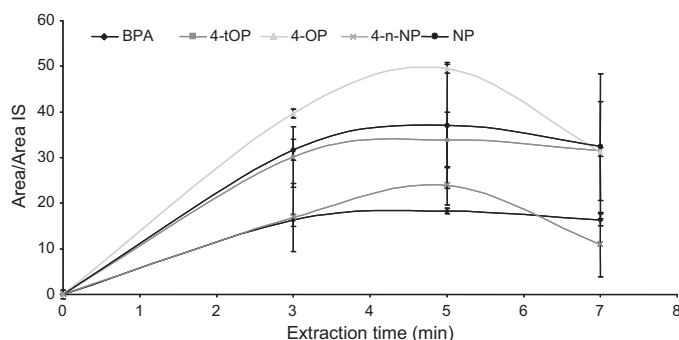


Fig. 5. Effect of DLLME extraction time ($n=2$).

The extraction time (agitation time) was also studied. As observed in Fig. 5, the response was highly increased until 5 min and kept constant after this time. Consequently, 5 min was selected as extraction time.

Finally, the volume of 1-octanol was tested. Different volumes between 50 and 300 μL were assayed and 100 μL were enough to extract analytes (Fig. 6).

3.3. Matrix effect

Ion suppression and enhancement originating from matrix is common in electrospray (ESI); therefore this phenomenon was studied. The slopes of the calibration plots built for standard analytes with 100 μL of 1-octanol ($1\text{--}100\ \mu\text{g L}^{-1}$) and for the standard additions performed on the seawater samples ($0.03\text{--}3.33\ \mu\text{g L}^{-1}$) were compared using a test t -Student ($P=95.0\%$) [27]. The statistical

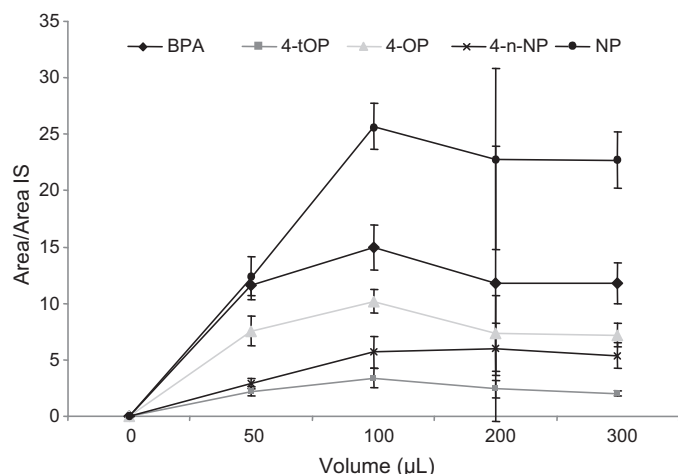


Fig. 6. Effect of extraction solvent volume in DLLME ($n=2$).

test showed that matrix had negative influence (suppression of the signal during the ionization) in the case of BPA, 4-n-NP and NP. Octylphenols did not present matrix effect. Surrogate internal standards (BPA- d_{16} and NP- d_4) were used to compensate this effect and satisfactory results were obtained for all compounds.

3.4. Method validation

There are not certificated reference materials available of these compounds in water, so the figures of merit of the method were evaluated analyzing spiked seawater samples.

Linearity ($r > 0.9989$) was evaluated between 0.09 and 50 $\mu\text{g L}^{-1}$ using seven calibration points ($n=2$). The response was linear between the MQL and 20 $\mu\text{g L}^{-1}$.

Precision and accuracy (Table 3) for each compound were determined at three different concentrations levels distributed over the linear range: low, medium and high concentration (0.09, 5 and 20 $\mu\text{g L}^{-1}$ in seawater samples). To evaluate the accuracy, the analytical recoveries of spiked samples were calculated. As it was explained before, the presence of NP in procedural blank was taken into account and the contribution of NP blank was subtracted from the spiked samples. Mean recoveries ($n=7$) ranged from 84 to 104% for all concentrations and all compounds. The repeatability was calculated as within-day RSD of concentrations, using seven replicates of spiking seawater samples analyzed with the proposed method during the same day and the same analyst and equipment. For intermediate precision, ten replicates of spiked seawater analyzed with the proposed method during different days along two weeks and the same analyst and equipment. In all cases, the repeatability and the intermediate precision of the method were satisfactory, with RSD < 10%.

Uncertainty of the analytical method was also estimated on the basis of in-house validation data according to EURACHEM/CITAC guide [28] for all compounds at three spiking levels. The main sources of uncertainty were identified and quantified and combined uncertainty (u_c) was calculated as follows:

$$u_c(y) = \sqrt{u_1^2 + u_2^2 + u_3^2}$$

where the uncertainties associated with the spiked sample (u_1), precision (u_2) and accuracy (u_3) were taken into account. The expanded uncertainty (u_{exp}) was estimated using the coverage factor (k) as 2 for a level of confidence of 95%.

$$u_{\text{exp}} = ku_c$$

Due to the fact that validation was carried out using spiked samples (certificate reference materials were not available), the preparation of the standards and the concentration spiked in sample (C_{sample}) had to be considered. The uncertainty associated with the spiked sample (u_1) was calculated considering the contribution of the following factors: standards, pipettes, flask and balance.

$$u_1 = C_{\text{sample}} \sqrt{\left(\frac{S_{\text{standard}}}{C_{\text{standard}}}\right)^2 + \left(\frac{S_{\text{pipette}}}{V_{\text{pipette}}}\right)^2 + \left(\frac{S_{\text{flask}}}{V_{\text{flask}}}\right)^2 + \left(\frac{S_{\text{balance}}}{m_{\text{standard}}}\right)^2}$$

The uncertainty associated with the precision (u_2) was expressed as a relative standard deviation obtained from the intermediate precision ($N=10$).

$$u_2 = \frac{S_{\text{intprec}}}{\sqrt{N}}$$

Finally, the uncertainty associated with accuracy was evaluated. At first, a test t -Student was used to determine whether the mean experimental recovery (X) was significantly different from

Table 3
Recovery, precision and uncertainty of target compounds in seawater with DLLME–LC–MS/MS.

Analyte	Concentration in sample ±Uncertainty ^a (μg L ⁻¹)	Accuracy (%)		Precision (%)	
		Recovery (n = 7)		Repeatability (n = 7)	Intermediate precision (N = 10)
BPA	0.09 ± 0.014	100		9	9
	5 ± 0.97	100		6	8
	20 ± 3.7	104		7	6
4-t-OP	0.09 ± 0.015	84		5	6
	5 ± 1.1	91		9	10
	20 ± 4.5	94		9	9
4-OP	0.09 ± 0.016	96		10	9
	5 ± 1.0	94		5	10
	20 ± 3.2	94		5	10
4-n-NP	0.09 ± 0.009	103		2	3
	5 ± 0.87	91		9	7
	20 ± 2.2	101		3	4
NP	0.09 ± 0.014	101		8	9
	5 ± 0.72	103		9	9
	20 ± 2.7	94		8	4

^a Expanded uncertainty (U) about the mean with coverage factor 2 (95% of confidence).

the theoretical spiked value (μ) of concentration, according to the equation:

$$u_3 = \frac{S_{\text{rec}}}{\sqrt{N}}$$

$$t_{\text{exp}} = \frac{|X - \mu|}{u_3}$$

The t_{exp} values were compared with the 2-tailed theoretical value ($n - 1$ degrees of freedom at 95% of confidence, where n is the number of replicates used to estimate recoveries). If these values are higher or equal than the theoretical value ($t_{4,95\%}$), experimental mean is significantly different from the theoretical spiked value; therefore, the uncertainty associated to the accuracy (u_3) must be taken into account. The t_{exp} values were higher than $t_{4,95\%}$ (1.94) only for alkylphenols at level of concentration of 0.09 μg L⁻¹. Table 4 shows all values of these individual uncertainties, the uncertainty combined and the relative expanded uncertainty (%) of target compounds in seawater at different concentrations.

The method detection limit (MDL) and method quantitation limit (MQL) were estimated as $X_b + 3SD_b$ (X_b average and SD_b

standard deviation of the seven blank seawater samples) and $X_b + 10SD_b$, respectively. In addition, MQL were experimentally verified by spiking seawater sample at a level of concentration close to the estimated of each compound ($n = 5$). Recoveries ranged from 90 to 110% and RSD < 20% therefore the values calculated for the MQL were successfully verified (Table 5).

The MQL obtained allow the determination of 4-t-OP and 4-n-NP at the levels established by the legislation (Directive 2008/105/EC) with satisfactory precision and accuracy. Moreover, in the case of 4-n-NP the MQL is lower than the 30% of the Environmental Quality Standards, which is requested by the Directive 2009/90/EC.

The analytical method proposed for seawater samples was also evaluated for the analysis of alkylphenols and bisphenol A in other types of water (surface and tap water). The use of surrogate standards compensates the possible matrix effect and therefore, adequate results were obtained for both kinds of waters. Precision and accuracy were calculated at one level of concentration (1.5 μg L⁻¹) in order to check the possible applicability of the method. Recoveries obtained ($n = 5$) ranged from 95 to 104% in surface water and from 98 to 105% in tap water. Repeatability was

Table 4
Combined (u_c) and relative expanded uncertainties ($u_{\text{exp}}\%$) estimated for each analyte considering the uncertainty associated to the spiked samples (u_1), to the precision (u_2) and to the accuracy (u_3).

Concentration in sample (μg L ⁻¹)	Analyte	u_1	u_2	u_3	u_c	$u_{\text{exp}}\%$
0.09	BPA	0.009	0.002	–	0.019	20.6
	4-t-OP	0.009	0.001	0.002	0.018	20.5
	4-OP	0.009	0.003	0.002	0.019	20.2
	4-n-NP	0.009	0.001	0.002	0.018	20.6
	NP	0.009	0.003	0.003	0.019	21.2
5	BPA	0.50	0.13	–	1.03	20.3
	4-t-OP	0.50	0.18	–	1.06	21.3
	4-OP	0.50	0.14	–	1.03	20.6
	4-n-NP	0.50	0.10	–	1.02	20.0
	NP	0.50	0.13	–	1.03	20.4
20	BPA	2	0.4	–	4.05	20.3
	4-t-OP	2	0.5	–	4.11	20.0
	4-OP	2	0.2	–	3.99	21.9
	4-n-NP	2	0.2	–	4.00	20.6
	NP	2	0.3	–	4.02	20.1

Table 5

Method detection limits (MDL) and method quantitation limits (MQLs) calculated and experimentally verified, precision (RSD%) and recoveries (R%) obtained. Directive 2008/105/EC requirements for each compound.

Compound	Estimated limits		Verified limits			Directive values	
	MDL ($\mu\text{g L}^{-1}$)	MQL ($\mu\text{g L}^{-1}$)	MQL ($\mu\text{g L}^{-1}$)	RSD%	R%	AA ($\mu\text{g L}^{-1}$)	MAC ($\mu\text{g L}^{-1}$)
BPA	0.006	0.020	0.020	9	100	–	–
4-tOP	0.003	0.007	0.008	19	108	0.01	Not applicable
4-OP	0.003	0.006	0.006	10	95	0.01	Not applicable
4-n-NP	0.001	0.003	0.005	15	90	0.3	2
NP	0.010	0.029	0.030	9	104	0.3	2

<10% ($n=5$) in all cases. These values are comparable with previous works [16,18]. Consequently, the method could be used for the analysis of other kinds of water, although more assays are needed in order to complete the validation.

3.5. Comparison with other reported methods

A wide variety of analytical methodologies for the analysis of alkylphenols and bisphenol A in surface and sewage waters can be found in the literature. However, few researches about the analysis of these compounds in seawater samples were reported. As it was said before, sensitive methods are needed in attempt to achieve the low levels set in the Directive 2008/105/EC. As far as we know, the MQLs of the proposed DLLME–LC–MS/MS method are lower than the limits reported in previous works, in which a similar volume of sample was processed [10,17]. When higher volumes of sample were processed, e.g. 500 mL seawater [13,14], 4 L seawater [1] or >40 L seawater [15], MQLs are comparable. Nevertheless, these high volumes hinder the sampling, transport and storage of the samples.

Recovery yields are similar to other methods [29] and even improved in the case of NP; due to the presence of NP in blank analysis, higher recoveries (>120%) were obtained when this contribution is not considered [30]. Furthermore, the precision of the proposed method is comparable to common application in DLLME [10] and better than SPE, LLE or other microextraction techniques [8].

Table 6

Concentrations ($\mu\text{g L}^{-1}$) of alkylphenols and bisphenol A in seawater samples collected from different beaches of A Coruña (NW Spain).

Analyte	C1	C2	C3	C4
BPA	<MQL ^b	0.035	nd ^a	nd ^a
4-t-OP	<MQL ^b	0.11	nd ^a	<MQL ^b
4-OP	nd ^a	0.065	nd ^a	nd ^a
4-n-NP	nd ^a	0.059	nd ^a	nd ^a
NP	0.070	0.14	<MQL ^b	<MQL ^b

^a Not detected.

^b Below method quantitation limit.

3.6. Analysis of seawater samples

In order to demonstrate the feasibility and applicability of the proposed DLLME–LC–MS/MS method some seawater samples obtained from different sites of A Coruña (NW Spain) were analyzed. Sampling points were chosen in order to compare the presence and levels of these compounds in industrial zones (C1 and C2) and urban zones (C3 and C4). These compounds were found at concentrations shown in Table 6.

BPA, 4-OP and 4-n-NP were only detected in industrial zones, whereas NP and 4-tOP were detected in all samples which show the ubiquity of these compounds. The higher concentration was found in sampling point C2 which is close to a wastewater treatment plant (WWTP). These values could be explained considering the degradation process of APEOs. In this sample, 4-n-NP and 4-OP were unexpectedly found, although they were not used in industrial

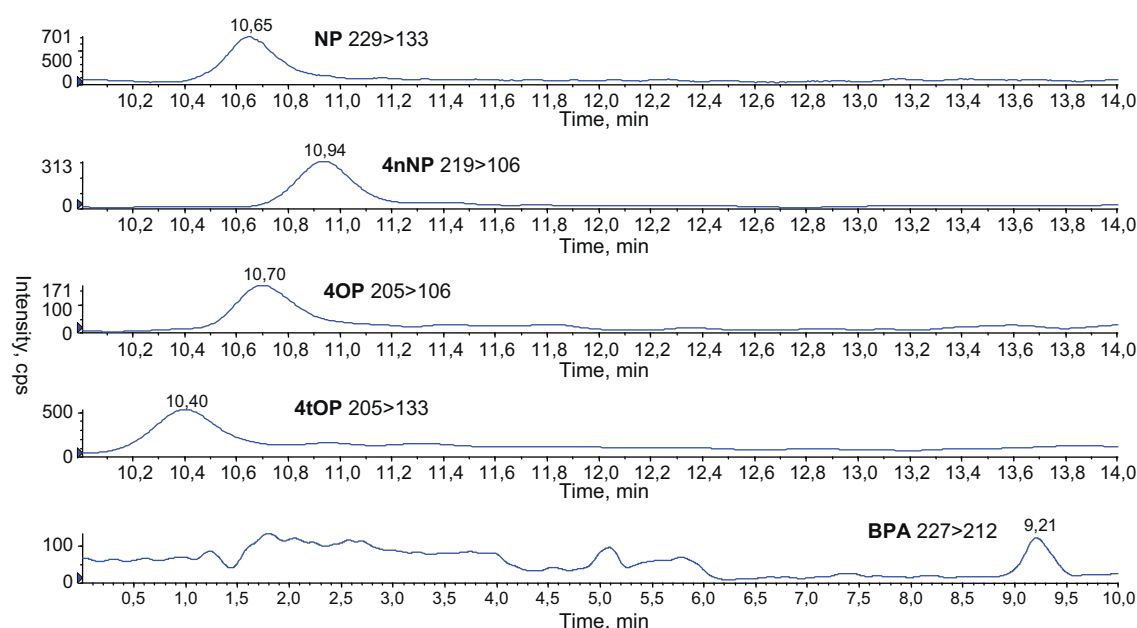


Fig. 7. Chromatogram of a seawater sample (C2) analyzed with the proposed method.

processes; therefore, the linear isomers should be also considered in degradation processes. The chromatogram of sample C2 was shown in Fig. 7. Only the concentration of 4-tOP and 4-OP were higher than the annual average (AA) set in Directive 2008/105/EC.

Not many data of alkylphenols and bisphenol A concentrations in seawater are available in the literature. Concentrations in this study are similar to the reported levels in other coastal zones in Spain [29] and Portugal [30]. However, these levels are lower than concentrations measured in Singapore, Thessaloniki Coast (Greece) and Jamaica Bay [8,20]. The largest population and the industrial activity in these zones can be the main reasons of those high results.

4. Conclusions

The analytical method proposed in this work allows the simultaneous extraction, identification and quantitation of alkylphenols and bisphenol A in seawater. This method is fast and simple and uses small volumes of organic solvent in agreement with the principles of the Green Chemistry. Furthermore, the application of a dispersive liquid–liquid microextraction without any dispersant agent simplifies the experimental process.

With the method proposed, satisfactory reproducibility, accuracy and low limits of quantification were obtained. Using only 30 mL of sample (which facilitates sampling, transport and storage) ultratrace levels of alkylphenols and bisphenol A can be determined allowing the compliance of the Directive 2008/105/EC.

The application of the proposed method to seawater samples proved its practicability. In addition, the presence of APs in some sites was evidenced and the concentration of 4-octylphenols exceeded the environmental quality standards showed in Directive 2008/105/EC. The method was also evaluated for the analysis of surface and tap water and satisfactory results were obtained.

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