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# Pressurized liquid extraction followed by liquid chromatography–mass spectrometry for the determination of alkylphenolic compounds in river sediment

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**Abstract**

A new methodology based on pressurized liquid extraction (PLE) followed by LC–MS is presented for the simultaneous and unequivocal determination of alkylphenol ethoxylates (APEOs) and their degradation products, alkylphenols (APs) and alkylphenoxy carboxylates (APECs), in sediment samples. The protocol, applicable to a full range of APEO oligomers and degradation products, permits the sensitive and selective determination of APEOs ($n_{EO}$=1–15), APECs ($n_{EO}$=0–1) and APs at low ppb levels (LODs=1–5 μg/kg) in sediment samples. Optimization of the operational parameters of PLE clearly demonstrates that significant thermal losses of APs occur during extraction at elevated temperatures. The loss of octylphenol (OP) at 100 °C was 61.2% and of nonylphenol (NP) 40.0%, whereas other compounds were completely recovered. Thus, to avoid losses due to the volatility of alkylphenols, a low extraction temperature should be applied. The conditions that gave the best results for all target compounds were as follows: extraction solvent mixture, methanol–acetone (1:1, v/v); temperature, 50 °C; pressure, 1500 p.s.i.; two static cycles. Using PLE and a subsequent clean-up with solid-phase extraction (SPE), the simultaneous extraction of APEOs, APs and APECs from sediment samples was achieved yielding recoveries >70% and producing low MS background noise. The developed methodology was applied on a routine basis to the analysis of alkylphenolic compounds in sediment samples. APEOs and their persistent degradation products were detected in significant concentrations in sediments from Portuguese rivers, especially at sites situated in the proximity of industrial plants (mainly the textile industry). The total concentration of alkylphenolic compounds (APEOs+APs+APECs) ranged from 155 to 2400 μg/kg. Of all the alkylphenolic compounds, NP comprised 40 to 50% with concentrations up to 1172 μg/kg.
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*Keywords:* Pressurized liquid extraction; Endocrine disrupters; Alkylphenolic compounds

## 1. Introduction

The relevant chemicals identified as endocrine disrupters (EDs) can be divided into several groups: (a) organohalogens (e.g. dioxins, PCBs, etc.), (b) phenolics (bisphenol A and alkylphenols), (c) phthalates, (d) steroids, (e) pesticides and (f) phytoestrogens [1]. Among these compounds, phenolic EDs, i.e. APEOs and their degradation products, APs and APECs, deserve particular attention because of their

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massive use and ubiquitous occurrence in the environment. The global production of APEOs is well over 300 000 tons and they have been widely used in the last 40 years as detergents, emulsifiers, dispersants, antifoamers and pesticide adjuvants [2]. Approximately 60% of APEOs that enter mechanical and biological sewage and sewage sludge treatment are subsequently released into the environment, 85% being in the form of potentially estrogenic degradation products [3]. Given the relatively low polarity of some phenolic xenoestrogens (e.g. NP $K_{ow}$=4.48, $NP_1EO$ $K_{ow}$=4.17) [4] sorption to bed sediments appears to be a likely process. Under anaerobic or oxygen-deficient conditions, normal in the sub-surface layers of river sediments, these compounds are expected to undergo low photodecomposition and biodegradation. Thus, the implications of ED sorption on sediments can be significant in terms of the ecotoxicological impact and the determination of their concentrations is of great importance.

Over the years, a substantial amount of data has been produced for the water compartment, but significantly less attention has been devoted to the analysis of EDs in sediment samples. The analysis of alkylphenolic compounds in complex matrices, such as contaminated sediment, is a complicated task, not only because of the complexity of the samples, but also because of the low detection limits required. Therefore, there remains a need to improve the analytical methods used for the extraction and determination of alkylphenolic compounds, and especially to develop methods that permit simultaneous determination of alkylphenol ethoxylates and their potentially estrogenic degradation products.

For the extraction of APEOs and their degradation products from solid samples (sewage sludge, sediment, soil) different analytical protocols have been proposed. Soxhlet extraction and steam distillation, used almost exclusively in the 1980s and 1990s, have been replaced by efficient and versatile sonicated extraction systems [5,6] and supercritical fluid extraction (SFE) [7,8]. Recent works report the use of efficient semi- or fully automated continuous-flow-high-temperature sonication [9], subcritical hot-water extraction [10] and PLE [11,12]. Quantification is usually performed using gas chromatography (GC) and liquid chromatography (LC), coupled to different detection systems, mass spectrometry (MS) for both GC and LC and fluorescence (FL) detection for LC. However, only a few methods permit the simultaneous extraction and determination of the parent compounds and degradation products. For example, steam distillation is limited to the less-polar components such as APs and APEOs with a few ethoxy units, while subcritical hot-water extraction gives high extraction yields for polar carboxylic degradation products.

The objective of this work was to optimize and validate an analytical protocol for the simultaneous determination of a full range of alkylphenolic compounds in sediments, based on PLE and LC–MS analysis, stressing in particular the optimization of the most important extraction parameters affecting the extraction efficiency. The procedure was designed to isolate and quantify parent compounds (APEOs, $n_{EO}$=1–15) and their main degradation products (APs and APECs, $n_{EO}$=0–1) providing sensitivity and selectivity of detection and avoiding tedious derivatization and clean-up steps. Finally, the applicability of the method was demonstrated by analysing fresh-water sediments from Portugal, performed as part of a systematic monitoring program carried out with the objective to determine *hot spot* areas with contamination by endocrine disrupters.

## 2. Experimental

### 2.1. Materials and standards

All solvents (water, acetonitrile, methanol, hexane, 2-propanol, acetone and dichloromethane) were HPLC grade and were purchased from Merck (Darmstadt, Germany). Analytical-grade sodium acetate and sodium sulphate were from Panreac (Barcelona, Spain).

The standards used in this study were laboratory synthesized nonylphenol monoethoxylate ($NP_1EO$), nonylphenol diethoxylate ($NP_2EO$), octylphenol monoethoxylate ($OP_1EO$) and octylphenol diethoxylate ($OP_2EO$) [13]. Additionally, technical mixturesM of polyethoxylated surfactants alkylphenol polyethoxylates Findet were from Kao (Barcelona, Spain). The mixture of OPEOs contained oligomers with an average of nine ethoxy units (Findet S8Q/21), while mixtures of NPEOs contained chain

isomers and oligomers with an average of four (Findet 9Q/16) and 10 ethoxy units (Findet 9Q/22), respectively.

Alkylphenoxy carboxylates ($NP_1EC$ and $OP_1EC$) and alkylphenoxy ethoxy carboxylates ($NP_2EC$ and $OP_2EC$) were synthesized according to the method described by Marcomini et al. [14] High-purity (98%) 4-heptylphenol (which was used as the internal standard), 4-*tert*.-octylphenol (OP) and technical-grade 4-nonylphenol (NP) were obtained from Aldrich (Milwaukee, WI, USA).

Stock solutions (1 mg/ml) of individual standards and standard mixtures were prepared by dissolving accurate amounts of pure standards in methanol. Working standard solutions were obtained by further dilution of stock solutions with methanol.

### 2.2. River sediment collection

Samples of river sediment from different sampling points in Portugal were collected using a grab sampler, transferred to the laboratory at 4 °C, then frozen at −20 °C and finally lyophilized. The lyophilized samples were ground and homogenized using a mortar and pestle and then sieved through a 125 μm sieve. The freeze-dried samples were stored in precleaned glass bottles at −20 °C until extraction.

### 2.3. Pressurized liquid extraction

Extractions were carried out using a Dionex ASE 200 (Dionex, Idstein, Germany). For extraction, 5.00 g of freeze-dried sediment was mixed thoroughly with $Na_2SO_4$ and filled into a 11-ml stainless steel extraction cell. Different solvent mixtures and temperatures were tested (see Results and discussion) and the optimized conditions were as follows: a mixture of acetone–methanol (1:1, v/v) was used as extraction solvent, a temperature of 50 °C, a pressure of 1500 p.s.i., a heating time of 5 min, and two cycles of static extraction, with the time of each static cycle being 5 min. As a final step, the cell was purged with gaseous nitrogen. The total volume of the extract was ~20 ml. The extracts were concentrated to an approximate volume of 1 ml using a rotary vacuum evaporator at 30 °C and redissolved in 100 ml of HPLC water. Subsequent cleanup of extracts was performed by solid-phase extraction (SPE) using LiChrolute $C_{18}$ cartridges (Merck) as described elsewhere [13].

### 2.4. Liquid chromatography–mass spectrometry

The HPLC system consisted of an HP 1100 autosampler with a 100-μl loop and an HP 1090 A LC binary pump, both from Hewlett-Packard (Palo Alto, CA, USA). HPLC separation was achieved on a 5-μm, 250×4 mm I.D. $C_{18}$ reversed-phase column (LiChrospher 100 RP-18) preceded by a guard column (4×4 mm, 5 μm) of the same packing material from Merck. The injection volume was set at 25 μl and the flow-rate was 1 ml/min.

The separation was performed under gradient elution conditions using methanol (A) and water (B). Non-ionic surfactants (OPEO and NPEO), detected in positive-ion (PI) mode, were separated using the following solvent programming: initial conditions were held constant at 30% A for 3 min, then increased linearly to 80% A in 7 min, then increased linearly to 90% A in 5 min and kept isocratic for 15 min. For compounds detected in negative-ion (NI) mode (APECs and APs) the initial conditions were held at 20% A for 5 min and then increased to 90% A in 15 min, which was held constant for an additional 10 min.

Detection was carried out using an HP 1040 M diode-array UV–Vis detector coupled in series with an LC-MSD HP 1100 mass-selective detector, equipped with an atmospheric-pressure ionization source and electrospray (ESI) interface. The operating parameters of the ESI-MS detector were as follows (PI/NI): drying gas flow, 12/11 l/min; drying gas temperature, 375/325 °C; nebulizer pressure, 55/60 p.s.i.; capillary voltage, 3500/5000 V; and fragmentation voltage, 60/100 V.

Under PI conditions, APEOs gave exclusively evenly spaced sodium adducts $[M+Na]^+$. Prior to analysis the extracts were fortified with 25 μ*M* sodium acetate (5 μl of a 5 m*M* aqueous solution) to avoid possible reduction in APEO ionization due to insufficient metal ion availability.

Under NI conditions, APs were detected as $[M-H]^-$, while for $AP_1ECs$ and $AP_2ECs$ the base ions (at a fragmentor voltage of 100 V) corresponded to $[M-CH_2COOH]^-$ and $[M-CH_2CH_2OCH_2COOH]^-$,

respectively, and $[M-H]^-$ ions, which are less abundant, were used for confirmation purposes. Ions monitored were: $m/z$ 205 (OP, $OP_1EC$ and $OP_2EC$, respectively), $m/z$ 219 (NP, $NP_1EC$ and $NP_2EC$, respectively) and for confirmation $m/z$ 263 ($OP_1EC$), $m/z$ 307 ($OP_2EC$), $m/z$ 277 ($NP_1EC$) and $m/z$ 321 ($NP_2EC$).

### 2.5. Quantitation

Quantitative analysis was performed in a selected ion monitoring (SIM) mode using external calibration. To check the influence of ion suppression on the MS detection of target compounds, 4-heptylphenol was used as an internal standard for NI mode. Under PI conditions, no internal standard was used since the results (see Results and discussion) showed very limited signal reduction due to ion suppression. $AP_1EO$ and $AP_2EO$ were quantified by using authentic standards of these compounds, while longer ethoxy-chain oligomers ($n_{EO}=3$–15) were quantified using technical oligomeric mixtures of APEOs. The oligomeric distribution of each sample was estimated in the full-scan mode and then quantitative analysis (under SIM conditions) was performed using a calibrant with an oligomeric distribution as similar as possible to the oligomeric distribution in the sample [15]. For example, samples showing the MS pattern of degraded APEOs (see Results and discussion) were quantified with Findet 9Q/16 (mixtures of NPEOs with an average of four ethoxy units), while samples showing the pattern of undegraded ethoxylates were quantified using Findet 9Q/22 (with an average of 10 ethoxy units) as a calibrant.

Initially, a series of injections of target compounds in the concentration range from 10 ng/ml to 25 μg/ml was used to determine the linear concentration range. Calibration curves were generated using linear regression analysis and over the established concentration range (0.05–10 μg/ml) gave good fits ($r^2>0.990$). Five-point calibration was performed daily, and the possible fluctuation in signal intensity was checked by injecting a standard solution at two concentration levels after each six to eight injections. Confirmation of compound identity in environmental samples was performed in a full-scan mode.

### 2.6. Method validation

The recoveries and overall method reproducibility were determined from triplicate analyses of spiked samples. A freeze-dried river sediment was spiked with 100 μg/kg of the composite standard solution of alkylphenolic compounds 72 h before analysis, and analysed by applying the method described above, together with a blank sample (non-spiked sample).

The detection limit (LOD) of the combined PLE–SPE–LC–MS procedure, achieved by the preconcentration of 5 g of river sediment, was calculated as the minimum amount of a compound present in the sample that produces a signal-to-noise ratio of 3, based on an injection of a 25-μl aliquot of the final 1 ml extract.

## 3. Results and discussion

### 3.1. Optimization of extraction parameters

To achieve fast and efficient extraction of the target compounds from a solid matrix using PLE, proper operational parameters and an appropriate extraction solvent or mixture of solvents, with polarities that closely match that of the target compounds, should be selected. It has already been reported that, due to the chemical characteristics of APEOs and their degradation products (reactivity, molecular size, polarity), an exhaustive extraction technique is required to recover all target compounds from sediments [16]. While some solvents are recommended for specific analyte classes, e.g. hexane for the extraction of lipophilic analytes (APs and short ethoxy chain APEOs), for the extraction of high oligomers and APECs more-polar solvents are needed. The solvents and solvent mixtures evaluated in this work were those reported to be effective with the Soxhlet or sonication extraction techniques: hexane–2-propanol (7:3, v/v), hexane–acetone (1:1, v/v), methanol–dichloromethane (7:3, v/v), methanol–acetone (1:1, v/v) and pure dichloromethane and methanol. The obtained recoveries are listed in Table 1. Using less-polar solvents (dichloromethane and hexane–2-propanol) the most hydrophilic compounds (APECs), with poor solubility in hexane or

Table 1
Recoveries (%, $n=3$) of target compounds using different solvents and solvent mixtures

| Compound | Hexane–2-propanol (7:3) | Hexane–acetone (1:1) | Methanol–acetone (1:1) | Methanol–dichloromethane (7:3) | Methanol | Dichloromethane |
|---|---|---|---|---|---|---|
| OP | 60 | 67 | 64 | 66 | 66 | 67 |
| NP | 75 | 69 | 74 | 74 | 68 | 76 |
| $OP_1EC$ | 39 | 87 | 80 | 49 | 77 | 11 |
| $NP_1EC$ | 42 | 89 | 79 | 58 | 75 | 15 |
| $OP_2EC$ | 43 | 81 | 85 | 48 | 80 | 21 |
| $NP_2EC$ | 50 | 88 | 89 | 55 | 86 | 35 |
| $OP_1EO$ | 83 | 90 | 90 | 87 | 86 | 89 |
| $OP_2EO$ | 81 | 94 | 89 | 91 | 91 | 90 |
| $OP_nEO$, $n_{EO}=3$–15 | 76 | 95 | 94 | 89 | 92 | 91 |
| $NP_1EO$ | 90 | 92 | 92 | 91 | 84 | 93 |
| $NP_2EO$ | 88 | 92 | 90 | 91 | 93 | 90 |
| $NP_nEO$, $n_{EO}=3$–15 | 79 | 95 | 94 | 90 | 94 | 80 |

PLE conditions: *m* (sample), 5 g; extraction cell, 11 ml; temperature, 75 °C; pressure, 1500 p.s.i.; two static cycles.

dichloromethane, exhibited low recoveries. The solvent systems that gave the highest extraction yields, without producing high MS background noise, were methanol–acetone (1:1, v/v) and hexane–acetone (1:1, v/v). Due to its compatibility with the subsequent cleanup step (reconstitution of a concentrated extract in water and purification by SPE), methanol–acetone (1:1, v/v) was chosen for further experiments.

The lower recoveries of OP and NP, as compared to other alkylphenolic compounds, might be the result of losses of these analytes due to their volatility. Most PLE applications operate in the 75 to 125 °C range, and the sample remains in the heated zone for 15–20 min, depending on the number of static cycles applied. Therefore, thermal decomposition and the volatility of the target compounds during transfer of the heated extract to the collection cell should be considered. The alkylphenolic compounds studied are not considered to be thermally labile, and decomposition is not expected to occur, even at temperatures higher than 100 °C. With PLE, oxidative losses are also minimized if solvents are degassed with nitrogen, however the relative volatility of the target compounds (in our case of OP and NP) could be a concern. To determine the losses due to volatization, the inert material ($Na_2SO_4$) used as drying and dispersing agent was spiked with the target compounds and extracted by applying extraction temperatures of 50 and 100 °C, respectively, with subsequent SPE cleanup. The recoveries obtained are shown in Fig. 1A. The results clearly demonstrate that significant thermal losses of APs occur during extraction at elevated temperatures. The loss of OP at 100 °C was 61.2% and of NP 40.0%, whereas other compounds were completely recovered. At 50 °C complete recovery of all target compounds was obtained. Thus, to avoid losses due to the volatility of alkylphenols, a low extraction temperature should be applied. However, this may lead to lower analyte recovery from the sediment matrix. Therefore, the extraction temperature should be carefully optimized taking into account the volatility of the target compounds. Fig. 1B shows the recoveries of selected target compounds from spiked river sediment obtained by applying different extraction temperatures. For relatively volatile compounds (OP, NP and low APEO oligomers) a decreasing extraction efficiency with increasing temperature was generally found, while for ionic nonvolatile compounds (APECs) the opposite effect was observed. For APECs the highest yields were obtained at 100 °C and a positive correlation between extractability and extraction temperature was found for the temperature range investigated. However, the increase in recovery of APECs was not too signifi-

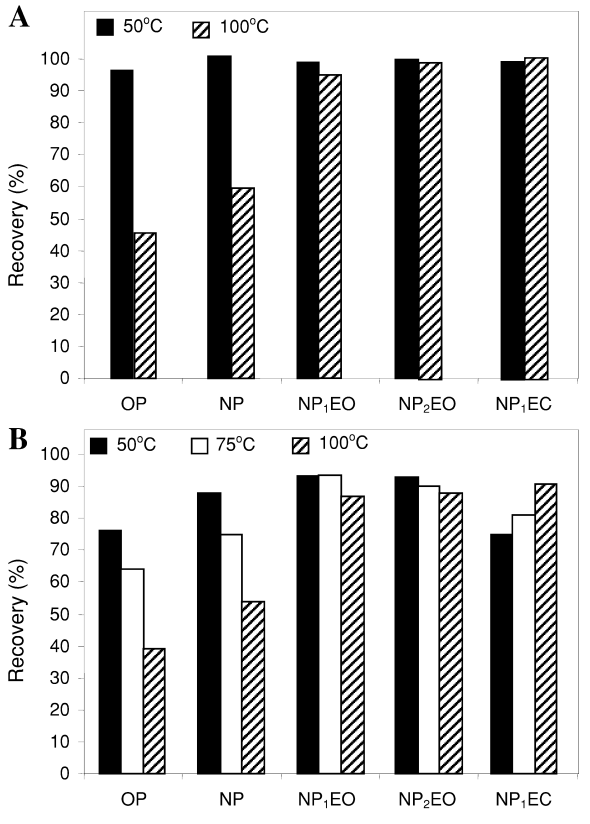


Fig. 1. Recoveries (%) obtained with PLE applying different extraction temperatures. (A) Spiked inert matrix, (B) spiked river sediment.

cant to justify extraction at an elevated temperature, which, at the same time, would cause substantial losses of APs.

Furthermore, it was found that MS detection is affected by high concentrations of co-extracted matrix substances. Suppression of the analyte signal, caused by high concentrations of matrix components, is one of the problems to be solved when analysing APEOs and their degradation products by MS. This signal irreproducibility, associated with matrix-induced suppression of the analyte, can be compensated, over a limited retention time window, by the use of an appropriate internal standard. However, the use of an internal standard cannot compensate for the sensitivity reduction. Optimization of the extraction parameters in order to achieve selective extraction of the target compounds and efficient extract cleanup (e.g. SPE) are the most direct means of obtaining maximum sensitivity and signal reproducibility. To obtain an insight into the influence of the extraction temperature on the purity of the obtained extracts and, consequently, on MS detection, the intensity of the signal of 4-heptylphenol (4-HP), which was used as an internal standard, in sediments extracts obtained by PLE applying different extraction temperatures was checked (Fig. 2). As can be seen, the ion suppression effect, directly related to the extractability of matrix components, displays a clear relationship with the extraction temperature. The signal

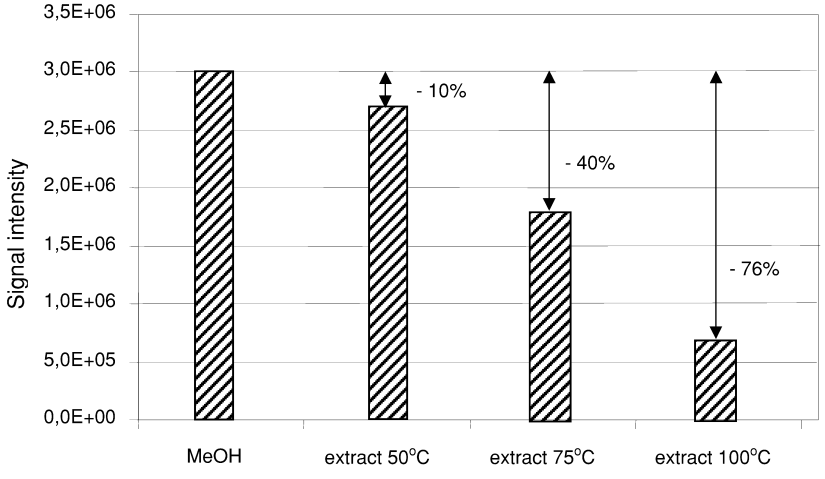


Fig. 2. Influence of extraction temperature on the signal intensity of 4-heptylphenol used as an internal standard.

intensity of 4-HP was reduced by 76% in the sediment extract obtained by PLE at 100 °C and by 40% at 75 °C. The signal reduction was limited to only 10% when the extraction temperature was 50 °C.

In order to compensate for the lower extraction efficiency at 50 °C the possibility of increasing the static time (from 5 to 10 min) and number of static cycles (two to four) was tested. However, it was found that the recovery rates of all target compounds were independent of the two parameters examined. Other parameters, such as pressure, flushing volume and purging time, have no effect on the extraction yields. Although at 50 °C no pressure is required to maintain a methanol–acetone mixture in the liquid state, a pressure of 1500 p.s.i. was applied, as recommended by the manufacturer, since the purpose of the pressure is not only to maintain solvents as liquids, but also to facilitate quick filling of the extraction cells.

Table 2
Recoveries, LOD and precision, expressed as the relative standard deviation (RSD) of triplicate analyses, of PLE under optimized operational parameters: extraction solvent, methanol–acetone (1:1, v/v); temperature, 50 °C; pressure, 1500 p.s.i.; two static cycles

| Compound | Recovery (%) | RSD (%) ($n=3$) | LOD ($\mu$g/kg)[a] |
|---|---|---|---|
| OP | 77 | 6.7 | 1 |
| NP | 89 | 11 | 1 |
| $OP_1EC$ | 73 | 6.2 | 1 |
| $NP_1EC$ | 74 | 5.4 | 1 |
| $OP_2EC$ | 79 | 7.1 | 2 |
| $NP_2EC$ | 79 | 4.7 | 2 |
| $OP_1EO$ | 94 | 9.8 | 5 |
| $OP_2EO$ | 94 | 3.6 | 2 |
| $OP_nEO$ $n_{EO}=3$–15 | 94 | 4.1 | 1 |
| $NP_1EO$ | 92 | 14 | 5 |
| $NP_2EO$ | 94 | 6.9 | 2 |
| $NP_nEO$ $n_{EO}=3$–15 | 97 | 5.9 | 1 |

[a] Concentration factor 10.

### 3.2. Method validation

The established protocol, PLE–SPE–reversed-phase LC–(ESI)-MS, was validated by the analysis of spiked sediment samples. The sediment used as matrix was from the Cardener river (Catalonia, NE Spain), and was collected at a sampling point upstream of known sources of pollution. Small concentrations of APs were found, and chromatogram traces obtained for spiked samples were subtracted from chromatograms of a blank sample analysed by a parallel assay.

Limits of detection (LODs), calculated as $S/N=3$ for quantification in SIM mode, are given in Table 2. LODs (based on a concentration factor of 10) were from 1 to 5 $\mu$g/kg.

Recoveries of spiked compounds were between 73 and 96%. No change in the oligomeric distribution of APEOs extracted from spiked sediment as compared to a spiked solution was observed. The overall precision of the analysis was satisfactory with the RSD of triplicate measurements falling between 3.6 and 13.6%.

As compared to ultrasonic extraction, used in previous works [13], the extraction efficiency was similar for all compounds; however, using PLE, all extraction steps were performed automatically. Furthermore, the advantages of PLE are low solvent consumption (15–20 ml), short extraction time (maximum of 20 min per sample) and the intermediate centrifugation step, carried out after ultrasonic extraction, can be obviated, provided that the extracts are filtered at the time of PLE. The recoveries obtained in this study are comparable to, or better than, those previously reported. Valsecchi et al. [12] reported average recoveries of 85 and 87% for NP and NPEOs, respectively, while Shang et al. [11] reported recoveries of from 65 to 93% for NPEO oligomers.

### 3.3. Analysis of environmental samples

The developed analytical method was applied for the analysis of alkylphenolic xenoestrogens in sediments, collected during 2001, of different Portuguese rivers impacted with discharges of industrial effluents and agricultural run-off.

Throughout northern Europe (Scandinavian countries, UK, Germany) a voluntary ban on APEO use in household cleaning products began in 1995, and restrictions on industrial cleaning applications in 2000 [17]. However, mainly because of lower production costs, APEOs are still being used in southern European countries in substantial amounts in institu-

tional and industrial applications. The major users of APEOs are the textile, tannery and the pulp and paper industries.

Thus, information on the total concentrations of APEOs and their degradation products in environmental matrices is essential to assess the environmental impact of these compounds. Levels found in sediments from Portuguese rivers are shown in Table 3. The total concentration of alkylphenolic compounds (APEOs + APs + APECs) ranged from 155 to 2400 μg/kg. Of all the alkylphenolic compounds, NP comprised 40 to 50% with concentrations up to 1172 μg/kg. Concentrations of octylphenolic compounds were one to two orders of magnitude lower than those of nonylphenolic compounds due to the smaller usage of OPEOs. Acidic degradation products (APECs) were found in only one highly polluted sample (sampling site No. 3). Sediment is a modest sink for APECs due to their polarity and solubility in water, and NPECs found at this sampling point probably reflect the close proximity of discharges of treated industrial wastewater containing high concentrations of APEO degradation products. Generally, the highest concentrations of alkylphenolic compounds were found in industrial areas, therefore the elevated concentrations are likely to be attributed to uncontrolled discharges of industrial wastewaters. However, alkylphenolic compounds were also detected in areas without significant industrial activity, but with intensive agricultural practice, where one of the possible sources of alkylphenolic compounds is the use of sewage sludge as fertiliser.

Assessment of the degree of degradation of the parent surfactants permits identification of the main pollution sources (discharge of untreated, treated waste waters or sludges, respectively). The mass spectrum of NPEOs in untreated water is characterized by an oligomer distribution of a typical commercial mixture (maximum at NPEOs with nine EO units). During wastewater treatment the abundance of lower oligomers ($n_{EO}=1–4$) increases and biologically treated NPEOs contain only traces of oligomers with $n_{EO}=5–12$. Simultaneously, the concentration of NP and NPECs increases. In the samples analysed, two characteristic patterns of the oligomeric distribution of NPEOs were observed. One, found at sampling sites 1, 2, 3 and 6, (shown in Fig. 3A), shows MS spectra of degraded NPEOs, leading to the conclusion that the pollution source is the discharge of treated wastewaters or sludge. The other, found at sampling sites 4, 5 and 7 (Fig. 3B), is characteristic of non-degraded oligomeric mixtures, pointing to the uncontrolled discharge of wastewater without any treatment.

Table 3
Sediment sampling sites, their characteristics and concentrations of alkylphenolic compounds found

| Sampling point | Site description | TOC (%) | Concentration (μg/kg) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | NP<br>OP | $NP_1EO$<br>$OP_1EO$ | $NP_2EO$<br>$OP_2EO$ | $NP_nEO$<br>$OP_nEO$<br>$n_{EO}=3–15$ | $NP_1EC$<br>$OP_1EC$ | $NP_2EC$<br>$OP_2EC$ |
| 1. Ponte Ribeiro Pernes | Textile industry | 0.26 | 120<br>1.8 | 11<br><5 | 24<br><2 | 71<br>5.9 | <1 | <2 |
| 2. Ponte Moreira | Textile industry | 2.27 | 170<br>2.0 | 50<br><5 | 86<br><2 | 120<br>11 | <1 | <2 |
| 3. Formariz | Textile industry | 0.91 | 1170<br>8.6 | 78<br><5 | 86<br><2 | 240<br>8.9 | 395<br><1 | 350<br><2 |
| 4. Ponte Nova Barcelos | Agriculture | 0.21 | 58<br><1 | 20<br><5 | 27<br><2 | 190<br>5.7 | <1 | <2 |
| 5. Ria Aveiro | Agriculture/paper industry | 0.98 | 83<br><1 | 5.9<br><5 | 13<br><2 | 51<br><2 | <1 | <2 |
| 6. Monte da Vinha | Paper industry/ agriculture | 0.33 | 140<br>1.9 | 40<br><5 | 56<br><2 | 97<br>4.8 | <1 | <2 |
| 7. Ponte Sacavem | Agriculture/different industrial plants | 0.95 | 300<br>3.3 | 6.4<br><5 | 20<br><2 | 260 | <1 | <2 |

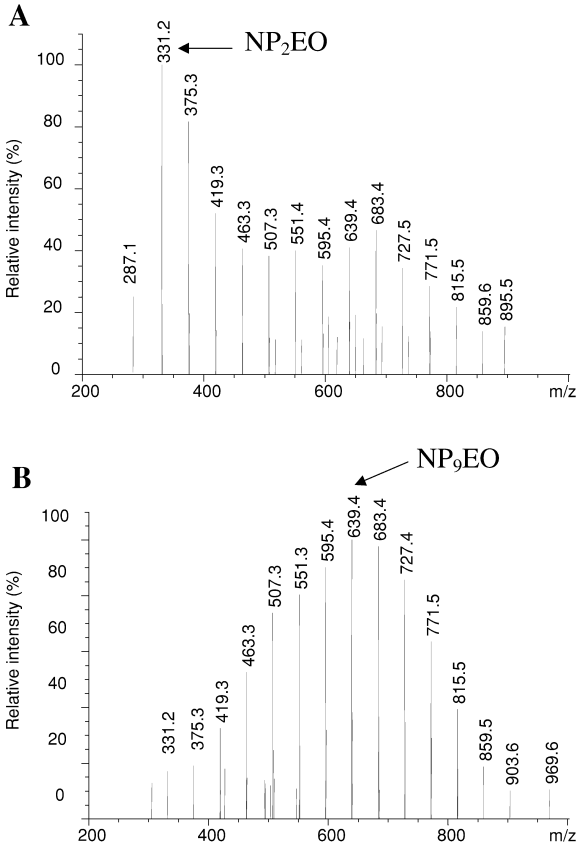


Fig. 3. ESI-MS spectra of NPEOs obtained under PI conditions of (A) sediments from sampling points 1, 2, 3 and 6, and (B) sediments from sampling points 4, 5 and 7.

## 4. Conclusions

Pressurized liquid extraction (PLE), with the binary solvent methanol–acetone (1:1, v/v) and applying a low extraction temperature (50 °C) to avoid losses due to the volatility of some target compounds, allows the efficient extraction of alkylphenolic compounds from sediment samples, reducing the time of extraction and the volume of solvent used and minimizing the ion suppression effect. The PLE–SPE–LC–MS methodology developed permits the simultaneous extraction and unequivocal determination of APEOs and their degradation products (APs and APECs) at the low μg/kg level. Environmental levels of alkylphenolic compounds in Portuguese freshwater sediments were from 155 to 2400 μg/kg (sum of APEOs, APECs and APs), indicating widespread pollution in both industrial and agricultural areas.

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