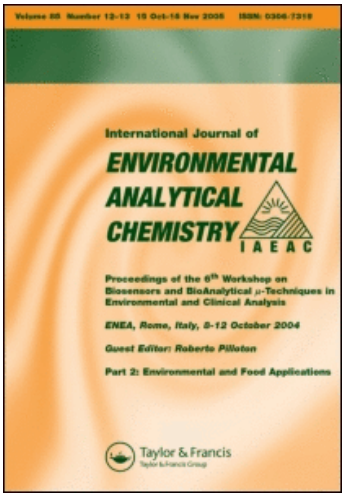

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Performance of different extraction media for the ultrasonic-assisted extraction of nonylphenol and nonylphenol mono- and diethoxylates from sediments

Emiliano Romero Ale[a]; Paola Babay[a]; Jorge Magallanes[a]; Elena Becquart[a]; Griselda Polla[b]; Eduardo Gautier[a]
[a] Comisión Nacional de Energía Atómica, Argentina [b] Gerencia de Química, Argentina

To cite this Article Romero Ale, Emiliano , Babay, Paola , Magallanes, Jorge , Becquart, Elena , Polla, Griselda and Gautier, Eduardo(2009) 'Performance of different extraction media for the ultrasonic-assisted extraction of nonylphenol and nonylphenol mono- and diethoxylates from sediments', International Journal of Environmental Analytical Chemistry, 89: 14, 1005 — 1021
To link to this Article: DOI: 10.1080/03067310903006960
URL: http://dx.doi.org/10.1080/03067310903006960

Intern. J. Environ. Anal. Chem.
Vol. 89, No. 14, 10 December 2009, 1005–1021

Taylor & Francis
Taylor & Francis Group

Performance of different extraction media for the ultrasonic-assisted extraction of nonylphenol and nonylphenol mono- and diethoxylates from sediments

Emiliano Romero Ale[a], Paola Babay[a], Jorge Magallanes[a], Elena Becquart[a], Griselda Polla[b] and Eduardo Gautier[a]*

Comisión Nacional de Energía Atómica; [a]Gerencia de Química; [b]Departamento de Física – Av. Gral. Paz 1499, B1650KNA-San Martín, Prov. de Buenos Aires, Argentina

(*Received 12 September 2008; final version received 27 April 2009*)

A systematic study was undertaken to optimise and compare the performance of different extraction media employing ultrasonic-assisted extraction (USE), for the recovery of nonylphenol (NP) and nonylphenol mono- and diethoxylates (NP1EO and NP2EO, respectively) from different spiked sediments (sand, clay and soil). Dichloromethane (DCM) and ethyl acetate (EtAc) were used alone or with methanol (MeOH). Normal phase high performance liquid chromatography with fluorescence detection (HPLC-FL) was used for separation and quantification. In a first stage, a screening Plackett-Burman experimental design was used as a multivariate strategy to evaluate the effects of three variables (solvent polarity, analytes concentrations and sonication time), at two levels each, on spiked clay. Solvent polarity was found to be the most influential factor, especially on the recovery of NP2EO. In a second stage, based on the screening results, USE time was set at 5 min to evaluate the performances of a 1 : 1 MeOH : DCM and a 1 : 1 MeOH : EtAc mixture on spiked sand, clay and soil. The 1 : 1 MeOH : EtAc mixture led to highly satisfactory recoveries for every analyte, statistically comparable to those yielded by a 1 : 1 MeOH : DCM mixture (NP > 85%, NP1EO and NP2EO > 90%). Due to similar interaction observed between each single sediment and the 1 : 1 MeOH : EtAc extractant, a composite substrate made of sand + soil + clay was spiked in a third stage. Extractant composition was then evaluated in order to find out the minimum volume of MeOH that could be used without loss of efficiency. It was found that 100% EtAc matched our aims ($\%R > 80$ and the lowest use of DCM and MeOH). USE protocol was tested on real sediments. The compounds were quantified by HPLC-FL and the identities were confirmed by gas chromatography-mass spectrometry.

Keywords: nonylphenol; nonylphenol ethoxylates; ultrasonic-assisted extraction; Plackett-Burman design; montmorillonite

1. Introduction

Alkylphenol ethoxylates (APnEO) are surfactants belonging to the group of non-ionic surfactants. They are widely used in domestic (cold cleaners for cars, household cleaners), cosmetics (emulsifiers, solubilisers) and industrial products (latex paints emulsifiers, pesticides formulations, industrial cleaners, pulp and paper manufacturing).

*Corresponding author. Email: gautier@cnea.gov.ar

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310903006960
http://www.informaworld.com

Most of APnEO are discharged in municipal and industrial wastewaters, which enter the sewage treatment plants. During sewage treatment APnEO suffer biodegradation, leading to the formation of several sub-products. The main process of degradation involves shortening of the ethoxy chain, which yields the highly lipophilic di- and monoethoxylated derivatives (NP2EO and NP1EO, respectively) and the nonethoxylated nonylphenol (NP) [1–3].

Because of the amphiphilic nature of these molecules and the influence of alkyl chain and aromatic ring (lipophilic moiety), when the number of ethoxy groups (hydrophilic moiety) decreases, the aqueous solubility of the resultant compounds diminishes. These properties have been demonstrated by Ahel and Giger [4]. The degradation enhances their tendency to be associated with suspended particulate and organic matter (sediments in rivers, lagoons, coast waters and groundwater), soils and sludges. The association with suspended particles increases their mobility and dispersion favouring their bioavailability [5]. Different studies have demonstrated that NP2EO, NP1EO and NP are toxic for the biota, showing endocrine disrupting effects via estrogenic metabolism, as evaluated by Müller *et al*. [6].

Due to the remarks mentioned above, analytical methods for the determination of these compounds in a wide variety of matrices have been developed in recent years. Different analytical techniques, such as gas chromatography (GC) and high performance liquid chromatography (HPLC), have been widely employed and optimised for the determination of NP2EO, NP1EO and NP [1,7–9].

Analytical chemistry plays an important role in minimising or eliminating the use of hazardous substances in environmental studies. In this context, there exists an increasing concern in developing alternative extraction methods, such as microwave-assisted extraction (MAE) [8,10], ultrasonic-assisted extraction (USE) [11] and pressurised liquid extraction (PLE) [8]. All these extraction procedures have arisen in recent years as a fast and efficient alternative to the classical procedures of Soxhlet [12–14]. For instance, Blackburn *et al*. [15] employed USE with ethyl acetate to measure the lipophilic metabolites of APnEO in estuarine and river sediment samples. More recently, Nuñez *et al*. [16] reported the employment of a highly polar H_2O-MeOH system (30 : 70) for the recovery of the series NP to NP13EO, also applying ultrasonic-assisted extraction.

The objective of the present study was to evaluate the USE efficiency for the recovery of NP2EO, NP1EO and NP from spiked blank sediments. Three representative types of sediments (sand, clay and soil) were selected as generic model soils. Several extractants were assessed, looking mainly for less environmentally and toxicologically harmful alternatives in substitution of dichloromethane (DCM) and methanol (MeOH).

The use of MeOH, a highly toxic solvent, has been widely reported for the extraction of our analytes; the same occurs with DCM [1,2,8,9,17,18]. However, the harmful toxicological and environmental profile of DCM is well known [19,20] and there are arguments that may imply its replacement by other solvents. Although EtAc has been less used than the widely employed DCM and MeOH, it may constitute a less toxic and more environmentally friendly alternative to the solvents mentioned above. Physicochemical properties shared by DCM and EtAc, such as water solubility, polarity and octanol-water partition coefficient (K_{ow}) [21,22], suggest that it could constitute a good alternative solvent.

The optimised USE procedure was applied on real samples, this being the first approach to the analysis of environmental solid samples in Argentina.

2. Experimental

2.1 *Reagents and instrumentation*

Nonylphenol mono- and diethoxylate technical mixture (Igepal® CO-210, Aldrich, Milwaukee, USA) and 4-nonylphenol (technical grade, nominal composition: 85% 4-NP, 10% 2-NP, Fluka, Buchs, Switzerland) were used as analyte standards for HPLC. 4-NP1EO and 4-NP2EO (both from Promochem, Wesel, Germany) were used as analyte standards for GC-MS determinations.

Methanol (MeOH), 2-propanol (2-PrOH), ethylacetate (EtAc) and dichloromethane (DCM) (LiChrosolv® Gradient Grade for liquid chromatography) and formaldehyde (37% v/v stabilised with 10% of methanol) were purchased from Merck (Darmstadt, Germany). Dimethylketone (DMK) p.a. grade was purchased from Dorwil S.A. (Buenos Aires, Argentina). Cyclohexane and hexane (Hex, 95% *n*-hexane/5% branched hexane) HPLC grade were Sintorgan (Buenos Aires, Argentina). Sodium sulphate (anhydrous) and sodium sulphite were p.a. reagents from Merck.

Glucose (α-D(+)-glucose anhydrous with approximately 4% of β-anomer p.a., Sigma, St. Louis, Missouri, USA) and Extra Pure Air and oxygen (TOC grade, Indura, Buenos Aires, Argentina) were used in Total Organic Carbon (TOC) determinations.

The substrates were obtained as follows: sand was bought from a local gardening store, clay was obtained from Porzio Logística S.A. (Buenos Aires, Argentina) and soil was collected from the surrounding laboratory park.

A Cleanson Ultrasonic Cleaner Model GU-500 (Buenos Aires, Argentina) operating at 25 kHz and 500 W was used for ultrasonic extraction.

Quantification of the analytes was performed with a high performance liquid chromatography (HPLC) system consisting of a Spectra SERIES P200 binary pump (Thermo Separation, San José, CA, USA) coupled to a fluorescence detector (Linear FLUOR LC305, USA). It was equipped with an APS-2 Hypersil® (Thermo Scientific, MA, USA) 5 µm particle aminosilica column of 250×4.6 mm with a 10×4.0 mm precolumn (UNIGUARD HPLC Column Protection System, Thermo Separation, San José, CA, USA), kept at 35°C in a CH-30 column heater by the use of a TC-50 temperature controller (Eppendorf, USA).

A Shimadzu GC-17A split-splitless gas chromatograph coupled to a MS-QP5050A mass spectrometer (Shimadzu Corp., Kyoto, Japan) was used to confirm identities of individual HPLC peaks.

Total organic carbon (TOC) determinations were carried out with a Shimadzu SSM-5000A Solid Sample Module for TOC-5000 (A) TOC Analyzer coupled to a non-dispersive infrared gas analyser (NDIR) (Kyoto, Japan).

HPLC data were obtained and analysed with the Konikrom version 5.2 software (Konik Instruments, Barcelona, Spain). GC-MS data were acquired and processed by GCMS solution version 1.10 software from Shimadzu Corporation (Kyoto, Japan). Statistical analyses were performed with STATISTICA version 7.1 (StatSoft's Inc., Oklahoma, USA).

2.2 *Substrates*

2.2.1 *Selection of the model substrates*

Sand, clay and soil were chosen as model substrates for our analyses because: (i) they constitute a representative and generic class of sediments and (ii) they are the main components of local river sediments and coasts [23].

These considerations are not only important for recovery studies. The analytes may have different mobilities and adsorption capabilities, depending on the morphology and composition of the material that they interact with. Therefore, the present substrate selection might also be useful to correlate the occurrence of the pollutants under study within the compartments of the environment. Natural sodium bentonite, a montmorillonitic clay, offers a complex mineral composition (mostly silicates) and morphology, organised in crystalline lattices capable of including solutes among them. This phenomenon was well described by Nagasaki *et al*. [24] and Barhoumi *et al*. [25]. Also Ding and Fann carried out recovery studies onto kaolin, another kind of clay [26]. On the other hand, sand possesses a simple pure silica crystalline surface with no organic matter content. Soil represents a more complex substrate, with an unorganised structure of minerals such as silicates and an important presence of organic matter, represented mostly by humic and fulvic acids (HA and FA). The presence of natural organic matter in sediments plays an important role in the sorption and desorption of hydrophobic organic contaminants, as described in a review by Weber *et al*. [27]. As a parameter to represent the organic matter content of the samples and to correlate it to recovery and adsorption values, TOC analyses were performed. Water content was also determined.

2.2.2 *Real samples*

The performance of the developed USE methodology was tested on sediments collected from the bank of a surface water course ('Jiménez brook drainage') placed 22 km SE from Buenos Aires city, in Quilmes town surroundings. It works as an auxiliary canal that avoids the overflowing of a main course ('Jiménez brook'), which runs through industrial and urban zones. Two non-sandy soils were taken by duplicate from different points of the bank (34°44′27″ S – 58°12′26″ W) in March 2007.

2.3 *Procedures*

2.3.1 *Model substrates collection, characterisation, conditioning and spiking*

All the operations described in samples collection, preservation and conditioning were mainly based on the reviews by Thiele *et al*. [1] and Theocharopoulos *et al*. [28].

To eliminate possible contaminants and organic residues, sand was rinsed several times with great volumes of water and then with smaller amounts of DMK. Once this was done, it was heated to 60°C overnight to favour complete evaporation of the solvent. Clay was used as provided.

Surface soil (up to 5 cm depth), was taken from an almost vegetation-free zone of the laboratory surrounding park, homogenised with a glass stick after eliminating any rock and vegetal, spread in a thin layer and placed in oven at 55–60°C up to constant weight (8–10 h). Finally, every substrate was passed through a copper sieve Tyler equivalent mesh 20 (opening 841 μm).

The TOC contents of the three model substrates was determined on dry basis by weighing 0.5 g into porcelain capsules previously cleaned with 10% hydrochloric acid and heated at 1150°C during 7 hours in order to eliminate carbon residues. Samples were heated at 900°C, with carrier gas flow rate at 150 mL min^{-1} and reading step of 290 s. Calibration was made with 0.025–0.05–0.1 g of glucose ($R^2 = 0.9998$) and results for every substrate were expressed as % TOC.

Blanks of the substrates were analysed in order to find out if there were significant amounts of NP or NPnEO present before spiking. Results revealed no significant chemical background interfering with detection of the analytes.

Portions of around 2.5 g of the substrate were placed into 100 mL beakers and spiked with 1 mL of a solution containing NP2EO, NP1EO and NP (in hexane) in order to obtain the desired concentrations. For the screening design (see Section 3.1 below) two analyte levels, around 0.5 and 500 μg per g of substrate, were applied (NP2EO 0.503 and 503, NP1EO 0.662 and 662, and NP 0.476 and 476 μg g^{-1}, respectively). In view of the results of the screening runs, only one spiking level (around 500 μg g^{-1}) was assessed in the subsequent experiments (see Section 3.2 below). Spiked samples were allowed to dry naturally under hood for 30 min. Then, 1 mL of 2-PrOH as co-solvent and 1 mL of MeOH were simultaneously added to favour the homogeneous distribution and interaction of the analytes within the whole substrate. After that, samples were dried in an oven at 55–60°C to allow complete solvent evaporation (3 h approximately). The drying temperature was selected in accordance to that used by Marcomini and Giger, thus minimising analyte losses [12]. Samples were stored at 4°C until extraction (not before 36 h).

For extraction, the spiked substrates were quantitatively transferred to clean beakers. The beakers where the samples were spiked were kept for the solubilisation of the analytes which might have not been adsorbed by the substrate. These fractions, named 'residues' from now on, were taken into account in order to calculate the recovery on the basis of the effectively retained portion onto the substrates and, at the same time, to reflect adsorption capabilities of every sediment. On the other hand, control solutions without substrate were prepared to estimate possible losses of analytes during thermal drying, following the same procedures. Blanks of substrates revealed no chemical background interfering with the separation or detection. Thus, no clean-up step was incorporated. All analyses were done at least in duplicate.

2.3.2 *Real samples collection and conditioning*

Environmental samples were collected with a steel shovel and placed in 500 cm^3 glass flasks. As it was proved for these compounds in river sediments and other natural matrices, degradation by micro-organisms may undergo if samples are not stabilised by chemical and/or temperature means [29]. Thus, 10 mL of formaldehyde were added to the sample and mixed with a glass stick. Flasks were then covered with aluminum foil and capped, kept initially at 4°C and stored at −18°C until the conditioning step. For conditioning, around 60 g of sediments were submitted to drying at 55–60°C until constant weight (around 30 h), homogenised and passed through 20 mesh sieve. Storage at −18°C took place until USE was carried out. For recovery assays, portions of 2.5 g of dry sample were spiked as described in Section 2.3.1 above, in order to obtain concentrations of the analytes at the levels initially found by HPLC-FL analysis.

2.3.3 *Ultrasonic extraction*

Samples and blanks were sonicated applying three extraction cycles of 2, 5 or 20 min each depending on the analysis stage. Bath temperature was kept at 29 ± 1°C by continuously sensing this variable with a laboratory thermometer and manually mixing hot and cold water when necessary. The ratio of extractant volume to extracted mass was 3 (7.5 mL of solvent per cycle). After each cycle, the extracts were transferred to a 25 mL volumetric flask. When suspended material was present in the liquid phase, an additional step of centrifugation (5 min at 3500 rpm) was included. Once the whole operation was completed, 2.5 mL of extractant were added to the extraction beaker and then centrifuged in order to rinse out the substrate. This aliquot was incorporated to the same flask and extracts were made up to volume. Aliquots of the extracts were dried under mild N_2 flow at 50°C and then redissolved in the HPLC elution mixture (0.15–1.5 mL of 4.1% 2-PrOH in hexane) for subsequent determination of the three analytes. Residues were treated with one single aliquot of 10 mL of extractant, sonicated along with the samples for three sonication cycles of 5 min each.

2.3.4 *Normal phase HPLC analyses*

Elution of individual compounds was carried out isocratically by normal phase HPLC-FL analysis at a flow rate of 0.65 mL min^{-1}. The mobile phase was a solution of 4.1% 2-propanol in hexane. Column temperature was set at 35°C. Fluorescence detection was performed at excitation wavelength $\lambda_{ex} = 230$ nm and at emission wavelength $\lambda_{em} = 300$ nm, following our previous procedures [7]. Detection limits, estimated as the concentration for a blank signal of $B + 3S_B$ (where B and S_B are the blank signal mean value and standard deviation for 10 injections of the solvent), were 0.8 μg L^{-1} for NP1EO and NP2EO, and 1.3 μg L^{-1} for NP (injection volume = 50 μL). Calibration curves were made for concentrations of 0.20, 0.40, 0.67, 1.65 and 3.63 μg mL^{-1} of NP, 0.19, 0.38, 0.64 and 1.60 μg mL^{-1} of NP1EO, and 0.12, 0.24, 0.39, 0.98 and 2.17 μg mL^{-1} of NP2EO dissolved in mobile phase. In all cases, linear coefficients (R^2) were $\geq$0.995.

2.3.5 *GC-MS analyses*

Although application of GC separation is limited by the volatility of the analytes, alkylphenol ethoxylates of short ethoxy chain can be measured directly without the necessity of derivatisation [1].

In the present work, GC-MS was proposed to confirm the identity of the compounds of interest previously separated and quantified by HPLC-FL. For the real samples in this study, every HPLC peak attributed to the analytes was collected separately as eluted, as many times as necessary in order to obtain detectable amounts for GC-MS analysis. HPLC solvent was gently evaporated under N_2 flow at 30–40°C and redissolved in a certain volume of cyclohexane (10–100 μL) for direct GC-MS determination. Compounds were separated in gas phase by means of a Zebron ZB-1 capillary column (60 m × 0.32 mm i.d. × 0.50 μm film thickness, Phenomenex, USA). Individual determination of NP, NP1EO and NP2EO isomers was carried out following our previous procedures [7]. Briefly, instrumental conditions employed for the separate measurement of the three compounds of interest were as follows: injector and detector temperature, 300°C; oven temperature programme, (a) 80°C (1 min)/6°C min^{-1}/280°C (5 min) for NP, and (b) 160°C

(4 min)/3°C min^{-1}/280°C (5 min) for NP1EO and NP2EO; column pressure, 90.5 kPa; split ratio, (a) 1 : 10 for NP, and (b) 1 : 5 for NP1EO and NP2EO. Injection volumes ranged from 1.0 to 2.0 μL. Detection was performed by monitoring the mass fragments in the selected ion-monitoring mode (SIM), allowing in all cases to distinguish at least 14 isomer peaks. Positive identification was made by means of coincident retention times of analytes and standards. In addition, every peak in the samples was checked for a matching abundance pattern of four (one target plus three qualifier) ions. External calibration was employed.

3. Results and discussion

3.1 *Preliminary screening design*

In order to study the effect of different variables on analytes recovery, a Plackett-Burman design was applied on spiked clay samples. This should allow us to obtain reliable results with the minimum number of experiments, saving time and reagents. Similar treatments have been applied by Chee *et al*. where, among other advantages, no necessity of repetitions of experiments are mentioned [13].

We covered three variables potentially affecting the extraction performance significantly: analytes concentration, sonication time and solvent polarity. Temperature, extracted mass and extractant volume were fixed variables.

The levels assigned to each variable were chosen on the basis of those reported by Petrovič and Barceló [30], Ahel and Giger [4] and Marcomini *et al*. [2]. These authors have also used DCM and MeOH as extractants, which have proved to be, together or separately, efficient for the extraction of our analytes. Samples were spiked with analytes concentrations covering ranges reported in the literature for real samples of different solid matrices [1–3,8,11,12,14,17,18,30–33]; levels assessed were around 0.5 and 500 μg of each analyte per g of substrate. Sonication times were of 2 and 20 minutes per cycle.

Analysing the values of the effects (E) and comparing them with the corresponding critical effects (E_{crit}), few statistically significant differences were observed. In the case of NP2EO, $E_{NP2EO\text{-}solvent}$ (40.24) > $E_{crit\ NP2EO\text{-}solvent}$ (13.99); by taking into account the sign and magnitude of this effect, we can say that when solvent polarity increases, a strong increment in the response for NP2EO takes place. This analyte appears to be more sensitive to solvent polarity than NP1EO and NP.

Regarding sonication time, a statistically significant difference was observed for NP1EO response, where $E_{NP1EO\text{-}time}$ (17.61) > $E_{crit\ NP1EO\text{-}time}$ (15.31). Analysing the sign and magnitude of this effect, we may say that the trend for the recovery is that it slightly increases by increasing sonication time.

No statistically significant differences ($E < E_{crit}$) in the recoveries were observed when the concentrations of analytes were varied. In view of these results, only one analyte concentration level (around 500 μg g^{-1}) was investigated in the subsequent optimisation experiments. On the other hand, the robustness observed in this regard should add confidence in future real samples analysis, where analytes concentrations are unknown and may vary widely.

A comparison of the chromatographic results of the extraction with both solvents is shown in Figure 1a.

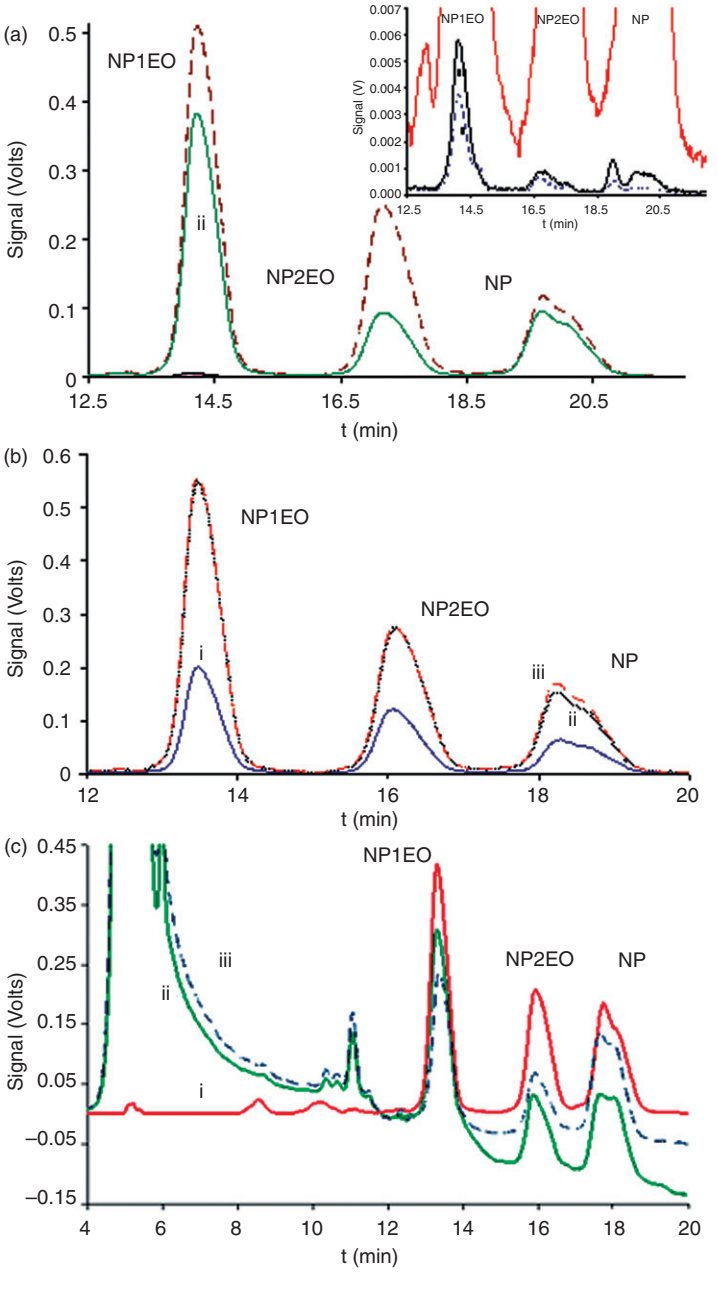

Figure 1. Comparative chromatograms of: (a) Regenerated USE extracts of clay spiked at a level of 0.5 μg g^{-1}: (i) MeOH as extractant; (ii) DCM as extractant. Inset: Expanded chromatograms of USE extract of clay blank samples: (i) MeOH as extractant, (ii) DCM as extractant. (b) (i) Direct injection of standard mixture, concentrations: NP1EO 0.28 μg mL^{-1}, NP2EO 0.29 μg mL^{-1}, NP 0.22 μg mL^{-1}; (ii) Regenerated USE extract of clay spiked at a level of 500 μg g^{-1}, with MeOH : DCM (1 : 1) as extractant; (iii) Regenerated USE extract of clay spiked at a level of 500 μg g^{-1}, with MeOH : EtAc (1 : 1) as extractant. (c) (i) Direct injection of standard: NP1EO 0.64 μg mL^{-1}, NP2EO 0.39 μg mL^{-1}, NP 0.66 μg mL^{-1}, (ii) USE-EtAc on Jiménez brook drainage I (2.5 g/25 mL; 10 mL/0.4 mL), (iii) Jiménez brook drainage II (10 g/100 mL; 15 mL/0.6 mL). Chromatographic conditions are those given in the Experimental section.

3.2 *Influence of different solvents on recoveries*

On the basis of Plackett-Burman results, the sonication time value was first modified. It was thought that it could be set at a value of twice the lower level used for the screening design, being not necessary to sonicate as much as 20 minutes per cycle. Thus, this variable was set at 5 min. Before proceeding to the assays onto different substrates with different extractants, the performances of MeOH and DCM were tested on spiked clay for this new sonication time. Duplicated analysis were done and results analysed by ANOVA.

Once again, low simultaneous recoveries for the three analytes were obtained with DCM ($\%R > 49$), and higher values ($\%R > 86$) were reached when MeOH was employed (lines 1 and 2 in Table 1), showing the predominant role of this variable on extraction efficiency. A sonication time of 5 min was proved to give satisfactory results; therefore, it was kept at this level for the subsequent optimisation experiments.

Although MeOH alone showed a good performance, we considered it mandatory to compare it against the mixture 1 : 1 MeOH : DCM to contrast literature procedures that normally employ a mixture of these two solvents [1,2,8,11,17,18]. This, at the same time, would allow us to systematically compare the performance of our proposed solvent, EtAc. Statistical analysis shown in ANOVA Table 2 (experiment a) for MeOH, DCM and the 1 : 1 mixture presents *p*-levels lower than 0.05, meaning that treatments are not comparable: methanol and MeOH : DCM mixture perform better than pure DCM.

3.2.1 *MeOH : DCM (1 : 1)* versus *MeOH : EtAc (1 : 1) on sand, clay and soil*

On the basis of the previous steps, and in order to compare the performance of a 1 : 1 MeOH : EtAc mixture with that of a 1 : 1 MeOH : DCM mixture, recovery experiments were carried out separately from spiked sand, clay and soil.

Recoveries were above 94% for NP2EO and NP1EO and higher than 85% for NP in all cases. All the values are summarised in Table 1 (lines 3–8). Statistical analysis shown in ANOVA Table 2 (experiments b–d) presents *p*-levels higher than 0.05, meaning that both

Table 1. Recoveries means of the different extractions by USE.

Extractant, technique and substrate	$\%R$ NP2EO	$\%R$ NP1EO	$\%R$ NP	Global $\%R$
DCM – clay	48.7 (8.65)	69.2 (4.13)	76.6 (2.89)	64.8 (2.95)
MeOH – clay	95.6 (1.49)	96.9 (1.54)	85.7 (3.48)	92.7 (2.17)
MeOH : DCM (1 : 1) – sand	98.6 (1.30)	96.3 (0.51)	88.0 (0.75)	94.3 (5.20)
MeOH : EtAc (1 : 1) – sand	98.1 (1.69)	95.9 (1.86)	87.4 (2.16)	93.8 (5.08)
MeOH : DCM (1 : 1) – clay	96.0 (3.05)	95.3 (1.93)	94.2 (5.20)	95.2 (2.89)
MeOH : EtAc (1 : 1) – clay	94.4 (1.53)	94.0 (1.12)	92.3 (1.28)	93.6 (1.49)
MeOH : DCM (1 : 1) – soil	95.8 (1.62)	95.2 (3.48)	85.4 (5.25)	92.1 (6.33)
MeOH : EtAc (1 : 1) – soil	95.2 (2.30)	93.8 (3.97)	86.8 (6.90)	91.9 (6.84)
I-MeOH : EtAc (100 : 0) – composite substrate	94.3 (3.51)	95.5 (1.88)	90.3 (1.43)	93.4 (2.96)
II-MeOH : EtAc (75 : 25) – composite substrate	93.9 (0.91)	95.4 (0.18)	89.7 (2.06)	93.0 (2.76)
III-MeOH : EtAc (50 : 50) – composite substrate	95.4 (0.77)	96.5 (1.97)	89.8 (2.81)	93.9 (3.45)
IV-MeOH : EtAc (25 : 75) – composite substrate	94.8 (1.25)	96.0 (1.17)	90.4 (0.96)	93.7 (2.73)
V-MeOH : EtAc (0 : 100) – composite substrate	94.4 (0.33)	95.1 (0.16)	91.1 (1.55)	93.5 (1.98)

Notes: In parentheses: % RSD ($n = 3$), except for extracts I to V ($n = 2$). Concentrations: NP2EO 503 $\mu g\,g^{-1}$, NP1EO 662 $\mu g\,g^{-1}$, NP 476 $\mu g\,g^{-1}$. Mass submitted to extraction: 2.5 g.
Sonication parameters: time (min per cycle) = 5 (3 cycles); temperature (°C) = 29 ± 1°C.

Table 2. One-way ANOVA analysis for individual analytes.

ANOVA	Effect	df Effect	MS effect	df Error	MS Error	F	*p*-Level
a. NP2EO	Extractant	2	2216.53	6	3.69	601.32	0.000000(*)
a. NP1EO	Extractant	2	724.16	6	10.24	70.723	0.000067(*)
a. NP	Extractant	2	189.57	5	11.30	16.771	0.006061(*)
b. NP2EO	Extractant	1	0.49	4	2.20	0.22	0.661021
b. NP1EO	Extractant	1	0.19	4	1.71	0.11	0.757420
b. NP	Extractant	1	0.56	4	1.11	0.51	0.516481
c. NP2EO	Extractant	1	3.62	4	5.33	0.68	0.456212
c. NP1EO	Extractant	1	2.47	4	2.25	1.10	0.353408
c. NP	Extractant	1	5.35	4	6.35	0.843	0.410486
d. NP2EO	Extractant	1	0.42	4	3.60	0.12	0.750562
d. NP1EO	Extractant	1	2.85	4	12.44	0.229	0.657378
d. NP	Extractant	1	3.26	4	27.98	0.116	0.750095
e. NP2EO	Sediment	2	10.96	6	3.22	3.40	0.102852
e. NP1EO	Sediment	2	4.50	6	6.06	0.74	0.515165
e. NP	Sediment	2	27.20	6	12.80	2.125	0.200579
f. NP2EO	Extractant	4	6.31	5	2.66	2.37	0.185036
f. NP1EO	Extractant	4	2.94	5	10.93	0.269	0.886484
f. NP	Extractant	4	0.61	5	2.84	0.22	0.919024

Notes: Variable = %*R*; df = degree of freedom; MS = mean square; $F = s_1^2/s_2^2$, where s_1^2 = MS Effect (between methods) and s_2^2 = MS Error (within method). Asterisk (*) refers to statistically significant effects at a level of significance of $p < 0.05$, which implies that treatments do not result equivalent.
a = DCM, MeOH and its (1 : 1) mixture on clay
b = MeOH : DCM (1 : 1) *vs*. MeOH : EtAc (1 : 1) on sand;
c = MeOH : DCM (1 : 1) *vs*. MeOH : EtAc (1 : 1) on clay;
d = MeOH : DCM (1 : 1) *vs*. MeOH : EtAc (1 : 1) on soil;
e = sand *vs*. clay *vs*. soil with MeOH : EtAc (1 : 1);
f = the five MeOH : EtAc mixtures (I-V) on compound substrate (sand + clay + soil).

treatments are comparable (variances are comparable, thus treatments are not significantly different). These results suggest that the widely employed MeOH : DCM mixture could be replaced by the 1 : 1 MeOH : EtAc one, thus meeting the first of our aims.

Chromatograms of the extracts from spiked clay, for both solvent mixtures, are depicted in Figure 1b.

3.2.2 *Minimisation of MeOH content in the extractant mixture with EtAc*

The minimisation of MeOH usage was another of the aims of the present work. For this purpose, five extracting solutions made with MeOH : EtAc at different volume rates (100 : 0, 75 : 25, 50 : 50, 25 : 75 and 0 : 100) were tested.

On the other side, as a way to evaluate the effects on recoveries in a composite model substrate, simplifying analysis, assays were carried out onto a 1 : 1 : 1 mixture of sand, clay and soil. This could be done because ANOVA analysis showed no influence of substrate nature on USE-MeOH : EtAc (1 : 1) recoveries (see Table 2, experiment e).

Table 3. Adsorption values (expressed as %A) of the analytes onto different substrates and losses due to thermal treatment.

Parameter	NP2EO	NP1EO	NP
%A onto sand ($n=6$)	79.0 (5.14)	78.8 (5.01)	80.8 (4.76)
%A onto clay ($n=6$)	98.2 (1.89)	98.7 (1.49)	98.9 (1.39)
%A onto soil ($n=6$)	94.6 (3.31)	95.1 (3.21)	96.5 (2.52)
%A onto sand + clay + soil ($n=10$)	98.3 (1.82)	98.1 (1.94)	98.8 (1.03)
Thermal loss (%) ($n=11$)	1.15 (1.44)	1.92 (1.22)	10.3 (4.42)

Notes: In parentheses: % RSD.
Concentrations ($\mu g\,g^{-1}$): NP2EO 503, NP1EO 662, NP 476.

Table 4. Substrates properties.

Substrate	Property	Value
Sand	Water content (at 105°C)	0.1%
	Organic matter on dry basis (as TOC)	<0.1% ND
Clay	Water content (at 105°C)	8.3%
	Organic matter on dry basis (as TOC)	0.3%
Soil	Water content (at 105°C)	5.1%
	Organic matter on dry basis (as TOC)	3.1%

Note: ND: not detected.

All recoveries were over 94% for NP2EO and NP1EO, and over 90% for NP, with every extraction mixture (Table 1, lines 9–13). The ANOVA results confirmed this appreciation (Table 2, experiment f). This led to the final and most important conclusion of this work: the use of EtAc alone as extractant for these model substrates yields quantitative extracts. This behaviour was not observed for DCM alone, what sets up this procedure as a less ecotoxicologically hazardous one, by avoiding the use of the organochlorine solvent along with the use of highly toxic MeOH.

At this scenery, we considered that USE recovery studies on different model sediments had been optimised.

3.3 *Sediments characteristics and its influence on recovery and adsorption of the analytes*

For the spiking levels applied in this work, compositional and morphological differences of the chosen substrates did not affect extraction performances significantly. On the other hand, adsorption (%A) of every analyte onto every substrate, calculated by measuring the residues of the non-adsorbed analytes in the spiking beaker, showed that the three analytes were almost quantitatively adsorbed onto every single substrate and its composite mixture, except for the sand. Observed adsorption values were around 80% for every analyte in that substrate (Table 3); 20% of each analyte remained in the spiking beaker. Regarding organic matter content, TOC revealed only considerable values for soil as it can be seen in Table 4, where it can also be seen the water content of the different substrates.

4. Environmental samples analysis

4.1 *HPLC*

4.1.1 *Identification and quantification: matrix background considerations*

As an initial approach to real matrices, two soil samples conditioned and prepared as described in Section 2.3 above, were submitted to USE. Extracts were concentrated, redissolved in eluent and directly injected in the HPLC system (see Figure 1c) without any clean-up operation, similar to the Marcomini *et al*. procedure [12].

Quantitative results are summarised in Table 5. Expressing the concentrations measured for NP, NP1EO and NP2EO in terms of molar fraction (mmol of analyte/total amount of NPnEO, $n = 0$–2), they represent 0.6 : 0.3 : 0.1 (NP : NP1EO : NP2EO). Some other groups also reported that NP appears as the most abundant compound present in soil in comparison to other ethoxylated terms of the series [12,17]. This fact may be attributed to the ability of nonylphenol to bind strongly to humic acids from organic matter in soils and sediments [27,34], due to its high octanol/water partition coefficient [21].

On the side of the chromatographic performance and matrix background, analytes peaks appear well resolved, matching in shape and retention times with those of the standard. The fact that they appear mounted above a tailing did not seem to represent a problem at the level they were present. It was thought that this tailing might be due to the slow elution of different fractions of fulvic and/or humic acids (FA and HA), normally present in soils [35], which could be co-extracted from the soil samples during USE. As mentioned in the work by Masqué *et al*., HA and FA usually dye extracts in yellow to brown [36], the same as observed in our extracts. The minimisation of the effects of their potential interference is mainly reduced by means of selective detection tools, such as mass spectrometry and fluorescence detection [37]. During the pre-treatment step, these potential interferents can be removed by clean-up operations on alumina (EPA 3610B) [38], Florisil® (EPA 3620C) [39] and silica gel (EPA 3630C) [40] or by means of newer developed resins as refered by Cortázar and co-workers [8]. As mentioned above, no severe interferences were detected in HPLC-FL runs, and that is the reason why we opted not to perform any clean-up step.

The limits of detection (LOD) and quantification (LOQ) were calculated as the concentration giving a signal to noise ratio of 3 and 10, respectively. The evaluated signal was that obtained from a blank made from model soil (four samples) at the retention times of every analyte. To obtain them expressed on dry basis, the applied treatment was taken

Table 5. Method performance and samples results.

Parameter	Sample	NP2EO	NP1EO	NP
LOD ($\mu g\,kg^{-1}$, dry basis; $n = 4$)	Model Soil	0.06	0.13	0.14
LOQ ($\mu g\,kg^{-1}$, dry basis; $n = 4$)	Model Soil	0.18	0.45	0.46
Found values ($\mu g\,kg^{-1}$, dry basis; $n = 2$)	Jiménez brook drainage I	93 (0.76)	196 (0.36)	248 (4.9)
	Jiménez brook drainage II	72 (3.0)	136 (5.2)	254 (3.6)
Recovery values (%R, $n = 2$)	Jiménez brook drainage	90 (2.4)	90 (13)	95 (8.2)

Notes: In parentheses: %RSD. Values obtained for 2.5 g of sample (dry weight) made up to a final volume of 0.15 mL.

into account (2.5 g of dry soil in 25 mL of EtAc and those 25 mL concentrated to 0.15 mL). Method detection limits and quantification limits are included in Table 5.

4.1.2 *Recovery assays and USE performance*

To evaluate the performance of the proposed USE methodology, recovery assays were carried out. Samples were spiked in order to obtain concentrations of every analyte at the levels found in HPLC-FL analysis.

As can be seen in Table 5, mean recoveries for USE were above 90% for the three analytes, with acceptable % RSD ($<15\%$). These results satisfy our aims and are comparable, for instance, with those obtained by means of more expensive and complex apparatus such as MAE or PLE [8,17] or tedious and waste-generating techniques such as Soxhlet [12]. The employment of USE for river sediments by Ding and Fann [26] led to comparable results, as also reported by Blackburn *et al*. [15].

4.2 *GC-MS*

Identity of the HPLC collected fractions for every single analyte was evaluated by means of GC-MS analysis. At least two injections from every sample extract measured by HPLC-FL were performed (Figure 2).

No signs of interference came out during separation, which allowed an easy identification and quantification of the endocrine disrupting compounds (EDCs) under study by comparing retention times and abundances of selected ions in standard solutions and collected fractions. One target and three qualifier ions were selected to check out the abundance pattern for each peak [7]. The differences found between the values measured by HPLC-FL and GC-MS were lower than 15% (5.4, 12 and -0.6% for NP2EO, NP1EO and NP, respectively).

It is worthy mentioning that, for soils and sludge, it is recommended to apply an elemental sulphur and/or sulphide removal, due to their proved and potential interference during GC separations. They saturate columns, generating tailings, consuming derivatisation agents and saturating some type of detectors (as, for instance, Electronic Capture Detectors) [41,42]. In our study, we collected each peak separately, thus we might talk of 'HPLC cleaned-up fractions', that would reduce drastically the potential interference from sulphur or sulphide presence during GC-MS measurements. In this particular case, in the light of the performance showed by GC-MS analysis, a clean-up for the removal of these species was concluded as not necessary. However, it should not necessarily be excluded in future analyses.

Limits of detection were calculated on the basis of the sum of isomers (TIC) for the retention times interval of every analyte, as the concentration giving a signal to noise ratio of 3, and resulted $0.1\,\mu g\,mL^{-1}$ for NP2EO and NP1EO and $0.05\,\mu g\,mL^{-1}$ for NP.

4.3 *Applicability of the method*

In Argentina, there are no regulations related to the risk of nonylphenol and its ethoxylates in the environment. In order to get a reference about the applicability of the method, and to check the compliance of the found levels with existent regulations, we can

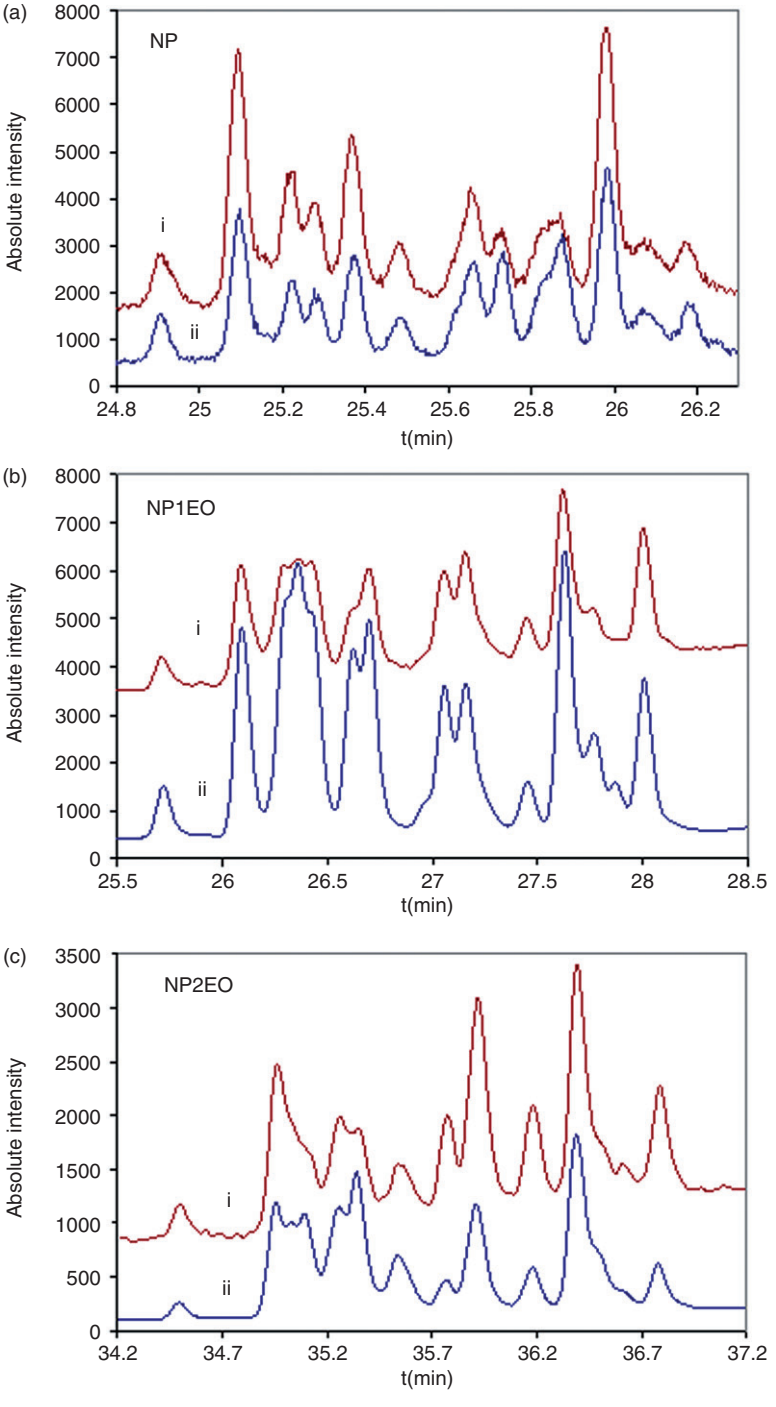

Figure 2. Comparative gas chromatograms (TIC) for (a) NP; (b) NP1EO and (c) NP2EO. (i) Standard solution of NP 2.4 μg mL^{-1}, NP1EO 5.0 μg mL^{-1} and NP2EO 5.0 μg mL^{-1}, respectively and (ii) HPLC collected fraction from Jiménez brook drainage extract I. Injection in cyclohexane. Instrumental parameters as in text.

contrast them, for instance, with the Dutch Environmental Risk Limits [43]. In that frame, Maximum Permissible Concentrations (MPCs) in sediments are (in $\mu g\,kg^{-1}$ dry weight) 105 for NP and 150 for NP1EO + NP2EO. As may be observed from the results in Table 5, the limits of quantification obtained fit with the determination of the compounds of interest at these environmentally relevant levels. At the same time, we may say that the amounts found in the samples widely exceed the MPCs; these first results give a primary evidence of a significant degree of contamination, which is in accordance with an unrestricted use of APnEO in the region.

5. Conclusions

The employment of sonication resulted in a viable alternative for the extraction of NP2EO, NP1EO and NP from three different model spiked sediments. With regard to the solvents, EtAc offered a remarkable performance, whenever it was used alone or in mixtures with MeOH. All this allows us to propose USE-EtAc as a good alternative for NP2EO, NP1EO and NP extraction from these widely occurring generic sediments.

Adsorption of the analytes has been observed to be stronger for clay and soil than for sand, most probably in accordance with sediments' structure and composition. Thermal treatment has produced a noticeable loss (around 10%) only for nonylphenol, the most volatile analyte, for the three sediments.

HPLC-FL showed sensitivity and selectivity at the same time, which was confirmed by means of GC-MS analysis. Although circumstantial conclusions showed no necessity of performing a clean-up step, these operations should be taken into account in case of interferences or irregularities being observed in forthcoming analyses.

This single but relevant set of data constitutes the first one to be reported for solid environmental samples in Argentina. Further sampling and analysis are being carried out in order to increase method robustness and knowledge on the occurrence of these EDCs on solid matrices in Argentina.

At the view of these results, we could propose this USE-EtAc/HPLC-FL protocol as a suitable alternative for the extraction and quantification of NP, NP1EO and NP2EO from sediments.

Acknowledgements

This work was carried out as part of CNEA (CAC) Programme P5, Project PID-36-2. Financial support was provided by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) through project PICT 2003 13-14181. We would like to thank Daniel Cicerone for his help with TOC measurements and Rubén Fernández for his GC-MS analysis assistance. E. Romero Ale would like to thank Patricia Smichowski for her support and ANPCyT for his doctoral fellowship.

References

[1] B. Thiele, K. Günther, and M. Schwuger, Chem. Rev. **97**, 3247 (1997).
[2] A. Marcomini, F. Filipuzzi, and W. Giger, Chemosphere **17**, 853 (1988).
[3] G.G. Ying, Environ. Intern. **32**, 417 (2006).

[4] M. Ahel and W. Giger, Chemosphere **26**, 1461 (1993).
[5] W. Shiu, K.C. Ma, D. Varhaníková, and D. Mackay, Chemosphere **29**, 1155 (1994).
[6] S. Müller, P. Schmid, and C. Schlatter, Environ. Toxicol. Phar. **6**, 27 (1998).
[7] P.A. Babay, E.E. Romero Ale, R.F. Itria, E.T. Becquart, B. Thiele, and D.A. Batistoni, J. Environ. Monit. **10**, 443 (2008).
[8] E. Cortázar, L. Bartolomé, A. Delgado, N. Etxebarria, L.A. Fernández, A. Usobiaga, and O. Zuloaga, Anal. Chim. Acta **534**, 247 (2005).
[9] R. Liu, J.L. Zhou, and A. Wilding, J. Chromatogr. A **1038**, 19 (2004).
[10] M. Fountoulakis, P. Drillia, C. Pakou, A. Kampioti, K. Stamatelatou, and G. Lyberatos, J. Chromatogr. A **1089** (1–2), 45 (2005).
[11] X. Peng, Z. Wang, C. Yang, F. Chen, and B. Mai, J. Chromatogr. A **1116**, 51 (2006).
[12] A. Marcomini and W. Giger, Anal. Chem. **59**, 1709 (1987).
[13] K.K. Chee, M.K. Wong, and H.K. Lee, J. Liq. Chromatogr. R. T. **19**, 529 (1996).
[14] M. Song, Y. Xu, Q. Jiang, P.K.S. Lamb, D.K. O'Tooleb, J.P. Giesy, and G. Jiang, Environ. Intern. **32**, 676 (2006).
[15] M.A. Blackburn, S.J. Kirby, and M.J. Waldock, Mar. Pollut. Bull. **38**, 109 (1999).
[16] L. Núñez, E. Turiel, and J.L. Tadeo, J. Chromatogr. A **1146**, 157 (2007).
[17] V. Andreu, E. Ferrer, J.L. Rubio, G. Font, and Y. Picó, Sci. Total Environ. **378**, 124 (2007).
[18] R. Gibson, M.-J. Wang, E. Padgett, and A. Beck, Chemosphere **61**, 1336 (2005).
[19] J.P. Rioux and R.A.M. Myers, J. Emerg. Med. **6**, 227 (1988).
[20] Hazardous Substances Data Bank of the US Nat. Library of Medicine (2007). <http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~iLL2ff:1>
[21] M. Ahel and W. Giger, Chemosphere **26**, 1471 (1993).
[22] National Pollutant Inventory, Department of the Environment and Heritage of Australia (2006). <http://www.npi.gov.au/database/substance-info/profiles/34.html#npi-rank>
[23] A. Sarubi, M.G. Pittau, and A.N. Menéndez, 'Delta del Paraná: Balance de sedimentos' (2004). <http://www.fi.uba.ar/laboratorios/lmm/informes/it_delta_sedimentos_jun04.pdf>
[24] S. Nagasaki, Y. Nakagawa, and S. Tanaka, Colloids Surf. A **230**, 131 (2003).
[25] M. Barhoumi, I. Beurroies, R. Denoyel, H. Saïd, and K. Hanna, Colloids Surf. A **219**, 25 (2003).
[26] W. Ding and J.C.H. Fann, J. Chromatogr. A **1**, 79 (2000).
[27] W.J. Weber Jr, E.J. Leboeuf, T.M. Young, and W. Huang, Water Res. **35**, 853 (2001).
[28] S.P. Theocharopoulos, I.K. Mitsios, and I. Arvanitoyannis, Trends Anal. Chem. **23**, 237 (2004).
[29] B.V. Chang, C.H. Yu, and S.Y. Yuan, Chemosphere **55**, 493 (2004).
[30] M. Petrovič and D. Barceló, Trends Anal. Chem. **23**, 762 (2004).
[31] M. Jánská, M. Tomaniová, J. Hajlová, and V. Kocourek, Anal. Chim. Acta **520**, 93 (2004).
[32] C.G. Naylor, J.P. Mieure, W.J. Adams, J.A. Weeks, F.J. Castaldi, and L.D. Ogle, J. Am. Oil Chem. Soc. **69**, 695 (1992).
[33] R.C. Hale, C.L. Smith, P.O. de Fur, E. Harvey, E.O. Bush, M.J. La Guardia, and G.G. Vadas, Environ. Toxicol. Chem. **19**, 946 (2000).
[34] A. Hollrigl-Rosta, R. Vinken, M. Lenz, and A. Schaffer, Environ. Toxicol. Chem. **22**, 746 (2003).
[35] S.D. Kohl and J.A. Rice, Chemosphere **36**, 251 (1998).
[36] N. Masqué, R.M. Marcé, and F. Borrull, Chromatographia **48**, 231 (1998).
[37] J. Harmsen and P. Frintrop, in *Chemical Analysis of Contaminated Land*, edited by K.C. Thompson and C.P. Nathanail (Blackwell Publishing, Oxford, UK, 2003).
[38] The EPA SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, EPA Method 3610B 'Alumina Cleanup' (1996). <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3610b.pdf>
[39] The EPA SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, EPA Method 3620C 'Florisil Cleanup' (2007). <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3620c.pdf>

[40] The EPA SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, EPA Method 3630C 'Silica Gel Cleanup' (1996). <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3630c.pdf>
[41] I. Fernández-Escobar, M. Gibert, A. Messeguer, and J.M. Bayona, Anal. Chem. **70**, 3703 (1998).
[42] H. Tang, P. Heaton, and B. Brassard, Field Anal. Chem. Tech. **1**, 171 (1997).
[43] P.L.A. van Vlaardingen, R. Posthumus, and T.P. Traas, in RIVM report 601501019/2003: Environmental Risk Limits for Alkylphenols and Alkylphenol ethoxylates (Nat. Inst. of Public Health and the Environ., Bilthoven, The Netherlands).