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Evaluation of anti-pyrene and anti-fluorene immunosorbent clean-up for PAHs from sludge and sediment reference materials followed by liquid chromatography and diode array detection

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The determination of polycyclic aromatic hydrocarbons (PAHs) in complex matrices such as sludges or sediments was performed by off-line enrichment using four different immunosorbents (IS). These IS are anti-fluorene and anti-pyrene antibodies immobilized on silica. The method involves the extraction of PAHs from water samples onto either two anti-fluorene or two anti-pyrene IS followed by analysis using liquid chromatography with diode array detection (LC-DAD) and its validation using gas chromatography coupled to a mass spectrometer (GC-MS). The procedure also uses a solubilizer to limit unwanted adsorption on vessels or tubing. In order to be able to evaluate the four IS, we have analyzed environmental sediments and sludge reference materials containing PAHs. Sediments were extracted by sonication with dichloromethane–methanol (2:1) and the extracts were brought up to a volume of 100 mL of water in order to perform the extraction with the anti-fluorene and anti-pyrene IS. This method was optimized for 13 of 16 PAHs included in the Environmental Protection Agency US (EPA) priority list. The selectivity of the IS cleanup and the LC-DAD spectra allowed a good identification and quantification in the analysis of sediment and sludge complex samples containing the priority PAHs established by the EPA. The results obtained with the four IS were compared after determination of PAHs in complex sewage sludge and sediment reference matrices. Moreover, clean-up using IS was compared with conventional clean-up procedures and showed a better selectivity for PAHs by the IS procedures.

Introduction

Conventional clean-up procedures are not selective for the determination of organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs). These compounds are widespread environmental contaminants resulting from both natural and anthropogenic sources. Apart from small amounts of geochemical and biosynthetic origin (they are synthesized by some algae, bacteria, fungi and plants) PAHs are mainly anthropogenic. In general, all incomplete combustion at high temperature and pyrolitic processes involving fossil fuels (peat, coal, petrol) lead to PAH formation.[1]

PAHs are ubiquitous contaminants and are included in the European Union and/or US Environmental Protection Agency (EPA) priority list. EU regulations for six PAHs for drinking water indicate that they should not exceed 0.2 μg L^{-1} with a concentration limit of 0.02 μg L^{-1} for benzo[*a*]pyrene.[2] New EU regulations are being set up with maximum concentrations of three PAHs in the range 2–5 mg kg^{-1} in sewage sludge for soil amendment.[3]

Nowadays, immunological techniques are increasingly accepted tools in environmental analysis. For instance, the recently developed immunosensors,[4] ELISA and immunosorbents. In recent years, the use of extraction sorbents based on immunoaffinity has increased. Immunosorbents (IS) are based on antibody–antigen molecular recognition, that ensures high specificity and sensitivity. The IS are usually prepared by covalently immobilizing an antibody on the surface of a rigid or semi-rigid support.[5]

Immunoaffinity chromatography (or IS) combines the advantages of solid-phase extraction techniques with the selectivity obtained by binding antibodies into the adsorbent. Often sample preparation with conventional procedures involves numerous steps and it is difficult to find a selective sorbent. Using IS extraction, trace enrichment and clean-up are achieved in one step due to the selectivity of the antigen–antibody interaction. In order to improve and extend the results obtained in previous work,[6] we have evaluated four IS for the selective preconcentration of PAHs. We chose two different IS for two different classes of compounds covering a wide range of hydrophobicity (log*Kow* for compounds with 2–3 rings is 3.3–5.2 and log*Kow* for compounds with 4–6 rings is higher than 5). On the one hand, anti-fluorene IS was used to retain the less hydrophobic PAHs and on the other hand, anti-pyrene IS was used to retain the more hydrophobic 4–6 aromatic ring PAHs.

The validation of these four IS was performed by the analysis of certified reference materials prepared from real samples. The quality of analytical measurements for the determination of organic contaminants in the environment depends to a large extent on the availability and the use of certified reference materials (CRMs) to validate the analytical procedures and to demonstrate that the measurement process is under control.[7] However for several compounds there are no reference materials available, and then spiking with real samples is an appropriate validation method. The complex matrix and the certified results were the reasons for us to use the CRMs for PAHs with the new IS columns. Due to the known difficulties in determining PAHs in environmental samples, especially with regards to the clean-up procedures, a new methodology involving highly selective isolation by an anti-fluorene and anti-pyrene IS is proposed. This work follows previous work from our group on the use of IS columns for trace enrichment of environmental contaminants in water and sediment samples.[8,9]

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The objectives of the present work were: (*i*) to improve and extend the range of applications of a former method published by the authors[6] by using newly developed immunosorbents based either on anti-fluorene or anti-pyrene interactions for the selective isolation of 13 priority PAHs that are included in the EPA list using certified sediment reference materials; (*ii*) to quantify 13 PAHs and to compare the data obtained using IS with those obtained using conventional extraction and clean up procedures; (*iii*) to compare the recovery data and selectivity using four different immunosorbents; and (*iv*) to evaluate the performance of the IS for the determination of traces of PAHs in Aquacheck certified sludge and EQUATE sediment samples.

Experimental

Immunosorbent columns

Preconcentration of the water samples was carried out off-line using experimental cartridges prepacked with silica and anti-fluorene or anti-pyrene antibodies. Immunosorbents were obtained by binding antibodies covalently on this adsorbent. Polyclonal antibodies were supplied by Prof. Le Goffic (ENSCP, Paris, France) and they were obtained against fluorene or pyrene according to the procedure described in previous studies.[10,11] To induce an immunogenic response, the fluorene and pyrene were derivatized and linked to a carrier protein, that is, bovine serum albumin. The immunizing reagent thus obtained was injected into a rabbit, and the serum was collected 6–9 months after this immunization. From this serum, the G-type immunoglobulin (IgG) fraction which contains the active antibodies, was isolated and purified; 40 mg of purified IgG fraction was bound to 1 g of silica.[12] The main difference between the two antibodies 1 and 2 for the same compound is owing to animal variability. They were obtained using the same protocol for both anti-pyrene and for both the anti-fluorene antibodies but they were made in different laboratories and with different rabbits.

Chemicals

HPLC grade solvents acetonitrile, methanol and LC-grade water were purchased from Merck (Darmstadt, Germany). PAH standards were obtained from Promochem (Wesel, Germany) and deuterated PAH standards from Cambridge Isotopes (Cambridge, UK). Dichloromethane, hexane, isooctane, sodium phosphate, sodium chloride and sodium azide were obtained from Merck.

A stock solution of 10 μg mL^{-1} was used to spike LC-grade and Ebro delta river water at the μg L^{-1} level for the preconcentration through the cartridges and further determination of recoveries.

The phosphate-buffered saline (PBS) consisted of a 0.01 M sodium phosphate buffer containing 0.15 M NaCl (pH = 7.4).

Reference materials

(a) EQUATE sediment reference materials (European Union, Brussels, Belgium). EQUATE is a European Union project that had the objective of building institutional capacities in Central and Eastern European countries and to implement in these countries an infrastructure which supports and secures the quality of measurements for national and international water management. Within EQUATE, a program of interlaboratory studies has been developed. In this respect, the purpose was to provide the participating laboratories tools for diagnosis and improvement. Provision of reference materials and calibrators to the participants of EQUATE supports the process of improvement. In this way, reference river sediment samples were prepared by EQUATE for conducting interlaboratory studies. Certified sediment samples were obtained from the organization.

(b) Aquacheck sludges (WRc, Medmenham, Bucks., UK). The Aquacheck scheme provides proficiency testing samples for the determination of organic and inorganic chemicals and bacteria in saline, fresh and wastewater, sludges, sediments and soils and other biota. These services help laboratories to optimize their quality control and analytical performance for the benefit of their customers. The Aquacheck proficiency testing scheme provides independent proof of laboratory performance both for laboratory management and for their clients.

Aquacheck began as a commercial operation in 1985 with 27 participants from the UK water industry. At the end of 1998 there were over 400 participating laboratories from 30 countries. This truly international peer group coupled with the comprehensive range of matrices and determinands and rapid reporting of results makes Aquacheck the first choice in proficiency testing and benchmarking of laboratory performance in Europe.

Chromatographic conditions

LC-DAD analyses were performed with a Waters 600-MS solvent delivery unit with a 20 μl injection loop and a Waters 996 photodiode array detector (Waters, Millipore, MA, USA). Quantification was carried out with UV detection at 254 nm and 220 nm. The analytical column was 244 mm × 4 mm id packed with 3 μm octylsilica gel from Merck. The mobile phases used for the elution of the analytes were acetonitrile and water. The gradient elution was performed as follows: from 50% A (acetonitrile) and 50% B (LC-grade water) kept isocratic for 5 min, to 100% A and 0% B in 20 min and then isocratic for a further 5 min at 1 mL min^{-1}.

GC-MS analyses were performed with a Carlo Erba (Milan, Italy) GC8000 Series system coupled to a mass spectrometer (Fisons MD800; Loughborough, Leicestershire, UK). A 30 m HP-5 column (5% phenyl methyl silicone; 0.25 mm id and 0.25 μm film thickness) was used. The oven temperature program was from 90 to 120 °C at 10 °C min^{-1}, from 120 to 320 °C at 4 °C min^{-1} and then held at 320 °C for 10 min; injector and transfer line temperatures were 280 and 300 °C, respectively. Helium was the carrier gas (50 cm s^{-1}). Data were acquired in the electron impact mode (EI) with an electron energy of 70 eV and using selected ion monitoring (SIM). The injector was set in the splitless mode (1 μL injected), the split valve being closed for 48 s. Deuterated internal standards for quantification were pyrene-d_{10} and perylene-d_{12}.

Sample preparation

(a) Sediment extraction with alumina cleanup. Sediment and sludge reference samples, previously lyophilized, were extracted by sonication for 20 min with a mixture of dichloromethane–methanol (2 : 1) three times. The extracts were separated from the sediment by means of a centrifugation step. The three extracts were combined, evaporated to dryness and redissolved in a mixture of hexane–dichloromethane (19 : 1). Afterwards, the extracts were purified following a clean-up procedure with an alumina column.[3] Recoveries of the PAHs obtained by this method and using two internal standards, anthracene-d_{18} and benzo[*ghi*]perylene-d_{12}, varied between 70–90%. The elution step consisted of washing the column with

5 mL of hexane–dichloromethane (19:1) which removed the most hydrophobic impurities in the sample. Next, the column was eluted with 10 mL of hexane–dichloromethane (1:2) which removed the PAHs. This second fraction was preconcentrated in a rotary evaporator to 0.5 mL and then carefully evaporated to dryness with a gentle stream of nitrogen and brought up to a volume of 500 μL with isooctane. Two extracts were analyzed by GC-MS and one by LC-DAD.

(b) Sediment extraction with IS clean-up. Sediment and sludge samples, previously lyophilized, were extracted by sonication for 1 h with a mixture of dichloromethane–methanol (2:1).[13] The extracts obtained were preconcentrated in a rotary evaporator to 2 mL and then carefully evaporated to dryness with a gentle stream of nitrogen and brought up to a volume of 500 μL with acetonitrile. Afterwards, an aliquot of this extract was added to a volume of 100 mL of ground water in order to get the sample in an aqueous phase and be able to perform the preconcentration through the immunosorbent. The extract containing PAHs was dissolved in acetonitrile due to the low solubility of PAHs in water. The solubilities of the different PAHs in water (in mg L^{-1}) are: fluorene, 1.9; phenanthrene, 0.816; anthracene, 1.29; fluoranthene, 0.265; pyrene, 0.16; benzo[*a*]anthracene, 0.010; chrysene; 0.006; benzo[*b*]fluoranthene insoluble, i; benzo[*k*]fluoranthene, i; benzo[*a*]pyrene, 0.0038; dibenzo[*ah*]anthracene, 0.0005; benzo[*ghi*]perylene, 0.0002; indene[1,2,3-*cd*]pyrene, i. The sediment samples are thus treated as aqueous extracts and then follow the usual procedure established in the water sample preparation section.

(c) Water samples. Water samples were filtered through a 0.45 μm filter (Millipore, Bedford, MA, USA) before use. Preconcentration of the samples was performed with an automated sample preparation system (ASPEC) XL. The (ASPEC) XL system, fitted with an external 306 LC pump for the dispensing of samples through the immunosorbent cartridge and with a 817 switching valve for the selection of samples, was a gift from Gilson (Villiviers-le-Bel, France).

The first step of the solid-phase extraction consisted of conditioning the immunosorbent (0.5 g of bonded silica) with 6 mL of PBS and then with 6 mL of LC-grade water. Afterwards, 10 mL of the sample were percolated through the immunosorbent at a flow-rate of 1 mL min^{-1}. The sample volume of 10 mL was chosen according to the results obtained in previous work.[6] Due to the high hydrophobicity of the PAHs, the addition of 10% of acetonitrile to the water samples before their extraction was necessary in order to avoid their adsorption to the flasks or connecting tubing. This modifier is usually an organic solvent (propan-2-ol, methanol, acetonitrile)[14–17] or a surfactant for example Brij 35.[14] The compounds trapped on the immunosorbent were eluted with 4 mL of a mixture containing 70% acetonitrile and 30% LC-grade water. Then, 10 ml of a mixture containing 30% PBS and 70% methanol were percolated through the immunosorbent for a washing step. Because PAHs are a very volatile class of compounds, the evaporation from this mixture containing acetonitrile and water could cause loss of the more volatile PAHs. For this reason, the water was eliminated from the sample, percolating this sample through anhydrous sulfate. Final extracts containing the PAHs in acetonitrile were evaporated with a stream of nitrogen up to 100 μL. Finally, a volume of 20 μL was injected into the LC-DAD system.

For recovery studies, 10 mL of Ebro delta river water sample spiked at 10 μg L^{-1} were percolated through the immunosorbent. This volume was chosen according to the results obtained in previous work.[6] Ebro delta river water was selected since the interaction between the water matrix (humic and fulvic acids) and the antibodies is low with no retention.

Validation of the immunosorbent was carried out by extracting two certified materials, a sewage sludge from Aquacheck and a sediment from EQUATE. When the immunosorbent was not in use, it was stored at 4 °C in a solution of PBS containing 0.2% sodium azide after a washing step using 70% methanol and 30% water (5 mL).

Results and discussion

Comparison of four immunosorbents

The selectivity of the IS for rapid determination of the PAHs in a complex matrix is shown in Fig. 1 presenting the LC-DAD chromatograms corresponding to the analysis of a reference sediment material extract after a clean-up step using the four immunosorbents. Many interfering compounds were eliminated and the resulting chromatograms show clear base-lines such as in the fourth chromatogram (d). It can be observed that it is easy to quantify and identify several of the PAHs at low concentration levels.

Fig. 2 shows the comparison between the GC-MS chromatograms corresponding to the analysis of a certified reference sludge from Aquacheck using two different clean-up steps on an alumina column (a) and an immunosorbent column (b), respectively, with two different temperature gradients.

As can be seen in chromatogram (b), the interferences corresponding to the matrix of the sediment sample are fewer than in chromatogram (a), thus indicating a high selectivity of the anti-pyrene 1 IS for the PAHs as compared with the conventional alumina clean-up.

Recoveries

In order to be able to evaluate the four immunosorbents studied, we first tested the extraction method using an aqueous spiked matrix. In previous studies performed in our group,[8,9] good extraction recoveries by immunosorbents of different compounds such as pesticides has been shown. For PAHs, the behaviour of an anti-fluorene immunosorbent was not so good as in the case of pesticides.[8] This is general behaviour observed for immunosorbents that show more difficulties in the determination of less polar analytes as compared with the more polar ones, like triazine pesticides.

When determining different compounds of the same chemical groups the affinity of the IS for aromatic compounds other than the antigen was achieved due to the similarity in the chemical structures. The matrix of Ebro delta river water is not retained at all in the IS. The recoveries of extraction of several PAHs from Ebro delta river water on the anti-fluorene and anti-pyrene immunosorbents using an off-line methodology are presented in Table 1. These recoveries were obtained after the extraction of 10 mL of a Ebro delta river water sample spiked at 10 μg L^{-1} with a mixture of 13 PAHs followed by LC-DAD. These recoveries ranged from 1 to 67% for all the compounds studied, indicating a certain affinity of the anti-fluorene and anti-pyrene immunosorbent for compounds other than the antigen. In previous work[6] it was seen that recoveries ranged from 7 to 56%. These recoveries are quite similar to the recoveries obtained in the present work. However, such recoveries are lower for the more hydrophobic compounds due to the difficulty of redissolving these hydrophobic compounds in water from a sludge or sediment. PAHs have a tendency to adsorb into the particles of these complex matrices and it is well-known that PAHs tend to stick everywhere. In this respect, there is a strong competition between all PAHs for their binding to the recognition sites of the antibodies. Therefore, the incomplete recovery observed with these immunosorbents could be due to different factors, such as overloading of the capacity of the column and/or breakthrough.

When antibodies against one single PAH have been carefully prepared, it is possible to trap many other PAHs. For this reason the IS anti-fluorene is able to trap fluoranthene or pyrene in addition to fluorene. Fluoranthene is a four-ring aromatic compound but is similar to fluorene. Depending on the hapten design, it may happen that the antibody may have a higher affinity for another compound than the target analyte used for its production. Due to this, the recovery of fluoranthene is higher than the recovery for fluorene. The IS anti-pyrene is used to retain the 4–6 ring compounds and we obtained low recoveries for this kind of PAH. We used 10% of acetonitrile in the water sample for solubilization of these compounds, since few of them are soluble in water (see solubility values in the Experimental section). In order to maintain the same conditions for the analysis of PAHs in this comparison of all IS, we used the same amount of acetonitrile in all samples. However highly hydrophobic compounds need more than 25%.[11] Moreover, by adding larger volumes of acetonitrile, the antibodies of the IS could be denatured because they lose their tridimensional conformation.

In Table 1, we show the comparison of extraction recoveries of several PAHs extracted from Ebro delta river water on four different IS. As can be seen in this table, the extraction recoveries are quite similar. There are no major differences in the recoveries between the different immunosorbent materials. Only anti-pyrene 1 had a much higher recovery for anthracene, but in all other cases the differences observed are not really significant.

Calibration curves and LOD

Calibration curves were constructed for all the compounds at concentrations ranging from 150 to 1000 μg L^{-1} (see Table 2). The limits of detection were calculated using a signal-to-noise ratio of 3 (the ratio between the peak intensity and the noise). Low detection limits in the ppb level can be obtained due to the high selectivity achieved by the immunosorbents and the high sensitivity encountered by DAD. The interaction of the matrix of water and the antibodies is low, thus leading to a high selectivity of the immunosorbent for the analytes studied. The LOD are affected by the recovery of extraction achieved by the immunosorbent and, therefore, depend on the affinity developed by the immobilized antibodies. The higher the affinity for a compound the lower is the detection limit. In addition, the LOD that are reported in Table 2 were calculated using an estuarine water matrix. These LOD can be somewhat higher when a complex sewage sludge or sediment sample is being analyzed, although the difference should be not be very much since in addition to the immunosorbent clean up there is change of solvent before the extract is re-dissolved in the water matrix.

Validation of the method

In this study it was important to compare the results of the selective extraction of PAHs from complex environmental samples such as sediments and sludges using the four IS. A way of validating the performance of a given IS is to apply it to the extraction of complex samples containing a known concentration of the target analytes. Quality control of analytical procedures aimed at determining PAHs in environmental samples requires not only the availability of PAH calibration standards of high purity but also of matrix materials in order to verify all steps of the analytical procedure. For this purpose, a sewage sludge from Aquacheck (Table 3) and two reference sediment materials from EQUATE (Tables 4 and 5) were extracted and analyzed following the methodology developed in this work and the results compared with those obtained with GC-MS. The aim was to apply the selective isolation made

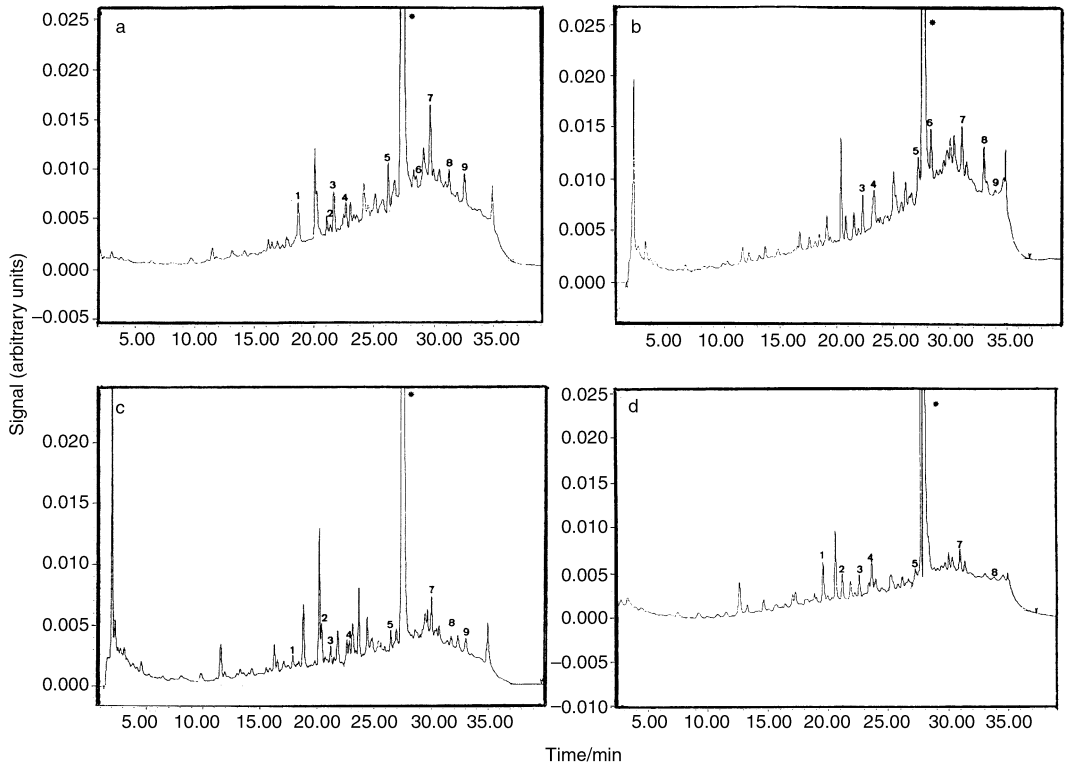

Fig. 1 LC-DAD chromatograms corresponding to the analysis of an EQUATE 96049 reference sediment material extract after a clean-up step using four different immunosorbents: (a) anti-fluorene IS 1; (b) antifluorene IS 2; (c) anti-pyrene IS 2; (d) anti-pyrene IS 1. Peaks: (1) phenanthrene, (2) anthracene, (3) fluoranthene, (4) pyrene, (5) benzo[*a*]anthracene, (6) chrysene (7) benzo[*b*]fluoranthene, (8) benzo[*k*]fluoranthene, (9) benzo[*a*]pyrene; * = interference caused by phthalates.

possible by the four immunosorbents to the extraction of PAHs in complex samples and to perform the comparison between IS and conventional alumina clean-ups.

PAHs were extracted from a sludge or sediment certified reference material using an ultrasonic methodology and were cleaned up on the IS. The results shown in Tables 3–5 are the mean concentrations calculated using the recoveries shown in Table 1. The recoveries of extraction were obtained after percolation of Ebro delta river water through the IS. In previous work,[6] we found that no matrix effect occurred and since the immunosorbents reported here are very similar to the previous study, we expected a similar behaviour. This allowed us to quantify the PAHs in the sludge and sediment extract by using the results calculated for Ebro delta river water.

Table 3 indicates the certified values and the mean concentration after percolation of the Aquacheck sludge through the four immunosorbents using the methodology explained above. Some compounds were not detected due to the high hydrophobicity of the most non-polar compounds. The other compounds were well detected and the concentrations found did not differ significantly from the certified values. It is difficult to detect different contaminants in the sludge. Sludge is a very complex matrix containing high amounts of lipids and high concentrations of different contaminants. On the other hand the PAHs are structures without ramifications or functional groups and it is difficult to prepare a highly selective IS. For these reasons the aromatic contaminants other than the target PAHs compete for the binding sites of the immunosorbent, and as a consequence it is difficult to quantify the results for the benzo[*ghi*]perylene and indene[123-*cd*] pyrene, which are the most hydrophobic compounds in our list.

In Table 3, the comparison of the results obtained with four different IS can also be observed. In general, there is a good agreement between the determined and certified values. These results demonstrate that for some compounds these kind of immunosorbents are useful for the selective extraction of PAHs from environmental samples.

In Tables 4 and 5, can be seen the comparison between the results obtained after the analysis of two certified reference sediment from EQUATE using four different IS. We observed that some compounds were not detected due to the low concentrations at which they were present in the samples, such as dibenzo[*ah*]anthracene. Fluorene was not detected due to the coelution with an interference peak (phthalate) obtained when this methodology was used. Table 5 shows that we have overloaded the capacity[18] of the IS anti-fluorene 2, just the contrary to that observed in Table 4 where we have obtained good agreement with certified results. We have obtained these results because the PAH concentrations in the EQUATE 96050 sediment are higher than the PAH concentrations in EQUATE 96049 sediment. In Table 4, it has been demonstrated that most of the compounds present in complex sediments could be identified and quantified easily using immunosorbents.

Conclusions

The immunosorbent method has been shown to be an easy way of cleaning-up highly contaminated extracts such as sludge or sediment. The selectivity of the IS allows limitation of matrix effects and low levels of concentration were determined with

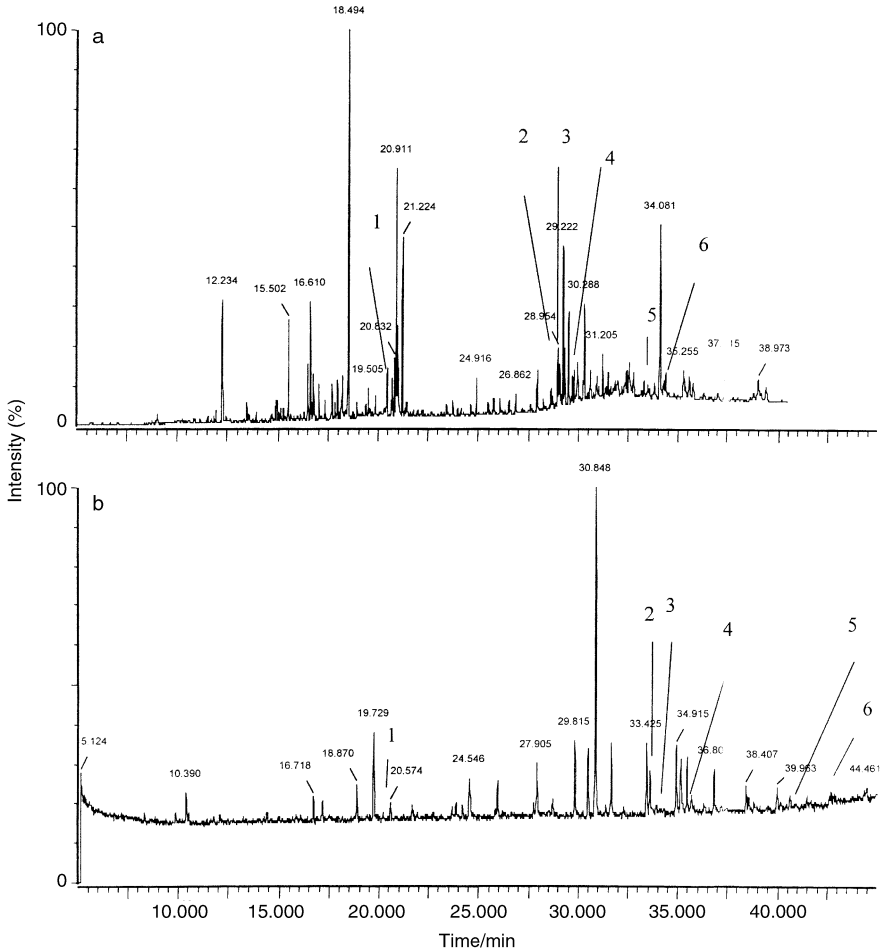

Fig. 2 GC-MS chromatograms corresponding to the analysis of a sewage sludge extract after a clean-up step using: (a) an alumina column; and (b) an immunosorbent column. respectively. Peaks: (1) fluoranthene, (2) benzo[*b*]fluoranthene, (3) benzo[*k*]fluoranthene, (4) benzo[*a*]pyrene, (5) benzo[*ghi*]perylene, (6) indene [1,2,3-*cd*]pyrene.

these four immunosorbents. For these reasons, we have obtained cleaner base-lines, so that compounds can be easily quantified and identified. The results obtained in this study for different anti-fluorene and anti-pyrene immunosorbents are similar to the results obtained for the only anti-fluorene used in previous work.[6] We can add that the four IS are suitable for the determination of 13 PAHs. It is also possible to trap many other PAHs with the exception of the more hydrophobic compounds giving results significantly similar to the certified values in several cases. These results show also that animal variability and hapten preparation are critical for the preparation of comparable antibodies. New research is needed along these lines to control and attain the most suitable concentration of immunoglobulins in order to achieve an immunopreconcentration with high recovery of PAHs.

Table 1 Extraction recoveries (%) obtained after the percolation of 10 mL of Ebro delta river water spiked at 10 μg L^{-1} with the mixture of PAHs through the four IS (n = 6)[a]

Compound	Anti-pyrene 1	Anti-pyrene 2	Anti-fluorene 1	Anti-fluorene 2
Fluorene	nq[b]	nq	nq	nq
Fenanthrene	2	1	28	2
Anthracene	56	14	18	9
Fluoranthene	49	53	67	48
Pyrene	38	45	36	30
Benzo[*a*]anthracene	20	38	32	18
Chrysene	20	40	28	19
Benzo[*b*]fluoranthene	13	26	18	4
Benzo[*k*]fluoranthene	10	16	14	5
Benzo[*a*]pyrene	7	11	6	4
Dibenzo[*ah*]anthracene	nq	25	nd	nd
Benzo[*ghi*]perylene	25	nd[c]	26	nd
Indene[1,2,3-*cd*]pyrene	nd	nd	nd	nd

[a] The relative standard deviation varied between 2 and 26%. [b] nq, Not quantified due to coelution. [c] nd, not detected.

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Table 2 Calibration data obtained with LC-DAD for the studied PAHs (n = 6)

Compound	Calibration equation	r^2	LOD/μg L^{-1}
Fluorene	$y = 118890x + 7321.8$	0.9932	9.6
Phenanthrene	$y = 315075x + 24521$	0.9914	3.5
Anthracene	$y = 704102x + 59314$	0.9911	1.6
Fluoranthene	$y = 74252x + 6229$	0.9913	13.5
Pyrene	$y = 73572x - 8078.4$	0.9986	19.3
Benzo[*a*]anthracene	$y = 189822x + 3936.3$	0.9973	5.9
Chrysene	$y = 278725x + 13181$	0.9908	4.2
Benzo[*b*]fluoranthene	$y = 203663x + 12063$	0.9926	6.1
Benzo[*k*]fluoranthene	$y = 144980x + 5893.4$	0.9923	10.4
Benzo[*a*]pyrene	$y = 184460x + 9182.3$	0.9922	8.0

Table 3 Mean concentration (n = 3) and target values of the PAHs (μg kg^{-1}) determined in a sewage sludge sample after treatment by four IS followed by LC-DAD

					Aquacheck (distribution 150) reference values (Mean of WRc)	
Compound	Anti-pyrene 1	Anti-pyrene 2	Anti-fluorene 1	Anti-fluorene 2	All labs	Spiked
Fluoranthene	278.0 (10.8)[a]	278.9 (41.2)	179.5 (6.5)	214.0 (10.0)	319.6	255.0
Benzo[*b*]fluoranthene	635.0 (3.6)	nq[b]	711.5 (18.6)	680.7 (4.4)	608.3	714.0
Benzo[*k*]fluoranthene	269.1 (11.5)	nq	nq	nq	221.4	284.0
Benzo[*a*]pyrene	259.4 (2.5)	268.1 (5.9)	643.5 (8.2)	nq	446.1	656.0
Benzo[*ghi*]perylene	nd[c]	nd	nd	nd	682.4	708.0
Indene[1,2,3-*cd*]pyrene	nd	nd	nd	nd	520.8	629.0

[a] RSD (%). [b] nq, Not quantified due to coelution. [c] nd, Not detected.

Table 4 Mean concentration (n = 3) and target values of the PAHs (μg kg^{-1}) determined in sediment Equate reference sample after treatment by four IS followed by LC-DAD

	EQUATE 96049/μg kg^{-1}				
	Mean value				
Compound	Anti-pyrene 1	Anti-pyrene 2	Anti-fluorene 1	Anti-fluorene 2	Certified value
Fluorene	nq[a]	81	nq	nq	57 (11)
Phenanthrene	370 (5)[b]	182 (7)	154 (9)	107 (26)	201 (8)
Anthracene	41 (2)	52 (2)	28 (37)	65 (8)	56 (10)
Fluoranthene	176 (3)	235 (56)	208 (8)	328 (6)	326 (11)
Pyrene	321 (9)	348 (47)	411 (11)	322 (1)	347 (9)
Benzo[*a*]anthracene	87 (4)	64 (7)	119 (6)	104 (5)	155 (11)
Chrysene	91 (13)	nq	183 (1)	229 (57)	175 (9)
Benzo[*b*]fluoranthene	145 (4)	349 (73)	260 (3)	nq	212 (12)
Benzo[*k*]fluoranthene	72 (8)	nq	164 (36)	198 (21)	138 (8)
Benzo[*a*]pyrene	110 (6)	67	230	111 (14)	142 (12)
Dibenzo[*ah*]anthracene	nd[c]	nd	nd	nd	34 (11)
Benzo[*ghi*]perylene	nd	nd	nd	nd	107 (14)
Indene[1,2,3-*cd*]pyrene	nd	nd	nd	nd	133 (14)

[a] nq, Not quantified due to coelution. [b] RSD (%). [c] nd, not detected.

Table 5 Mean concentration (n = 3) and target values of the PAHs (μg kg^{-1}) determined in sediment Equate reference sample after treatment by four IS followed by LC-DAD

	EQUATE 96050/μg kg^{-1}				
	Mean value				
Compound	Anti-pyrene 1	Anti-pyrene 2	Anti-fluorene 1	Anti-fluorene 2	Certified value
Fluorene	nq[a]	90	nq	nq	199 (1)
Phenanthrene	508 (17)[b]	nq	509 (7)	nq	537 (12)
Anthracene	277 (4)	nq	348 (8)	nq	342 (9)
Fluoranthene	846 (10)	1107 (2)	719 (13)	nq	953 (13)
Pyrene	931 (5)	850 (4)	809 (19)	999 (11)	837 (11)
Benzo[*a*]anthracene	574 (2)	438 (3)	531 (1)	1082 (9)	556 (11)
Chrysene	543 (7)	665 (8)	826 (4)	nq	549 (12)
Benzo[*b*]fluoranthene	729 (6)	971 (3)	783 (9)	nq	696 (12)
Benzo[*k*]fluoranthene	652 (5)	477 (8)	540 (10)	nq	397 (10)
Benzo[*a*]pyrene	nq	446 (69)	431 (28)	317 (4)	408 (13)
Dibenzo[*ah*]anthracene	nd[c]	nd	nd	nd	84 (12)
Benzo[*ghi*]perylene	nd	nd	nd	nd	407 (12)
Indene[1,2,3-*cd*]pyrene	nd	nd	nd	nd	133 (14)

[a] nq, Not quantified due to coelution. [b] RSD (%). [c] nd, not detected.

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