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Selective immunoclean-up followed by liquid or gas chromatography for the monitoring of polycyclic aromatic hydrocarbons in urban waste water and sewage sludges used for soil amendment

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Abstract

A selective clean-up procedure using immunoaffinity solid-phase extraction was applied for the trace-level determination of polycyclic aromatic hydrocarbons (PAHs) in urban waste water and sewage sludges used for soil amendment. Anti-pyrene antibodies have been immobilized on a silica-based sorbent and the cross-reactivity of the antibodies towards structurally related compounds were allowed to extract the whole class of priority PAHs. The selectivity of the antibodies provided clean extracts from sludges and, therefore, the identification and quantification were shown to be easier using either liquid chromatography (LC) with UV diode array and fluorescence detection in series or gas chromatography–mass spectrometry (GC–MS), although some loss of up to 50% was observed for the clean-up. The identification of the PAHs by matching of UV and MS spectra was greatly improved. The procedure, including immunoclean-up and LC coupled to diode array and fluorescence detection, was validated using certified reference materials with native PAHs of concentrations in the range of 0.57–2.16 mg/kg (dry sludges).

Keywords: Soil; Sewage sludge; Environmental analysis; Water analysis; Extraction methods; Solid-phase extraction; Immunoextraction; Polynuclear aromatic hydrocarbons

1. Introduction

Sewage sludges are likely to contain some persistent and carcinogenic polycyclic aromatic hydrocarbons (PAHs) especially when run-off waters are collected with urban water in the waste water treatment plants (WWTPs), which occurs in many old facilities. Since a way of recycling sludges is to spread them on agricultural lands as fertilizers, there is a risk of soil contamination and that explains the recent regulation from the European Union for monitoring three PAHs in sludges used for soil amendments at concentrations between 2 and 5 mg/kg (dry sludges).

PAHs are commonly analyzed by liquid chromatography (LC) with fluorescence or UV detection (LC) or gas chromatography (GC) with mass spec-

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trometric detection (MS). LC–fluorescence allows quantification at very low levels and is easier to handle than GC–MS [1]. However, despite the fact that one fluorescence detector that had the capability of providing excitation or emission spectrum was recently commercialized, one major drawback of the use of LC with common fluorescence detectors is that analytes are identified by their retention factor alone. Identification can be reinforced by the UV spectrum using in series a diode array detector (UV DAD) [2,3]. Thanks to the selectivity and the high sensitivity of the fluorescence detection coupled to LC, quantification of PAHs in sludges at the mg/kg level was shown to be possible without any clean-up after simple supercritical fluid extraction of PAHs from sludges [2]. The method was applied to the monitoring of several types of sludges and quantification using fluorescence was not a problem in many samples. However, obtaining the UV spectrum was not always achieved with some sludges and then an additional clean-up was required. Moreover, while GC–MS is certainly the method of choice for confirmation, it cannot be used for any sludge without clean-up of the extracts. In addition, PAHs may be present as very complex mixtures, as shown by their analysis in many reference certified materials [1,4].

Common clean-up procedures use alumina, pure silica or Florisil and are laborious and time consuming [5–9]. Selective extraction sorbents, so-called immunosorbents (ISs), involve the highly selective antigen-antibody interactions and have been shown to eliminate the co-extraction of interfering compounds in complex matrices [10,11]. For aqueous samples, the extraction and clean-up are performed in the same step, whereas their application to the extracts from solid samples provides a very simple and efficient clean-up procedure. When the target molecules are small, the antibodies recognize not only the solute used to induce the immunogen response but also the compounds from the same structural family due to their cross-reactivity. The advantage of this cross-reactivity was exploited to develop class-selective ISs for several groups of pesticides [11–18], BTEX compounds (benzene, toluene, ethylbenzene and xylene) [19] and PAHs [19–23]. Recently, immunosorbents based on anti-fluorene and anti-pyrene antibodies have been developed [20–22]. Compared to the anti-fluorene IS, the anti-pyrene IS showed a higher affinity for the whole group of PAHs, and the on-line procedures were set up for the selective extraction of the PAHs in surface waters with concentrations as low as 0.02 μg/l, as required for the monitoring of surface water used for drinking purpose according to EU regulations [21].

The objective of this paper was to improve the determination of the PAHs by providing a simple immunoclean-up of extracts from waste water and WWTP sludges allowing the quantification and confirmation of the identity of an individual PAH using either LC coupled with fluorescence and UV diode array detections in series or GC–MS. The sixteen priority PAHs listed by the US Environmental Protection Agency (EPA) have been taken as model analytes. Special attention was given to the determination of fluoranthene, benzo[*b*]fluoranthene and benzo[*a*]pyrene, which are included in the EU regulation project with maximum allowed concentrations of 5, 2.5 and 2 mg/kg of dry sludges, respectively. Matrix effects were studied by comparing the results obtained with extracts from waste water, sludges and sediments. Certified reference matrices (sediments and sludges) were used for the validation of the whole procedure. Applications are given studying the partition of PAHs between the waste water and the sewage sludges in a treatment plant.

2. Experimental

2.1. Apparatus

The LC system consisted of a 9012 pump with Rheodyne 20 μl and 50 μl loop injector coupled with a 9070 fluorescence detector and a 9065 photodiode-array detector (Varian, Les Ulis, France). A Croco-Cil oven (Touzart et Matignon, Les Ulis, France) was used for setting up the column temperature.

The GC system consisted of a Varian Star 340 CX equipped with a split/splitless injector. The GC system was coupled to a Varian Saturn 3 ion-trap mass spectrometer.

Focused microwave extractions were performed with a Soxwave 100 apparatus (Prolabo, Fontenay-sous-Bois, France).

2.2. Stationary phases and columns

A 250×3 mm Bakerbond PAH-16 Plus column including guard column was used (J.T. Baker–Mallinckrodt, Noisy le Sec, France). A DB-17 MS capillary column was used whose dimensions were 30 m×0.25 mm I.D. 0.25 mm film thickness (Chromoptic, Druyes-Les-Belles-Fontaines, France).

Disposable extraction cartridges containing 500 mg of C_{18} silica (Bakerbond) were obtained from J.T. Baker-Mallinckrodt.

The immunosorbents consisted of anti-pyrene antibodies bonded onto glutardialdehyde-activated silica particles of 50 nm pore size (from J.T. Baker–Mallinckrodt). Polyclonal antibodies were supplied by Professor F. Le Goffic (ENSCP, Laboratory of Bioorganic and Biotechnologies, Paris, France) and were immobilized on silica according to a procedure previously described [11]. For the off-line clean-up of the extracts, 250 mg of IS were packed in a disposable extraction cartridge.

2.3. Chemicals

Acetonitrile, methanol, isooctane and tetrahydrofuran (THF) of 'HPLC ultra' grade were obtained from J.T. Baker–Mallinckrodt. Toluene of 'spectroscopic' grade and acetone of 'chromatographic' grade were obtained from Merck (Nogent sur Marne, France). Ultrapure water was obtained from a Milli-Q water system (Millipore, St. Quentin en Yvelines, France). The standard mixture of the 16 priority PAHs in dichloromethane was obtained from J.T. Baker–Mallinckrodt at concentrations of 100 or 200 (mg/l) depending on the PAH. The four solutions of perdeuterated standards in dichloromethane ([$^2H_8$]naphthalene), [$^2H_{10}$]phenanthrene, [$^2H_{12}$]chrysene and [$^2H_{12}$]perylene) were obtained from CIL Cluzeau (Ste. Foy-La-Grande, France) each one at concentration of 2000 mg/l. The phosphate-buffered saline solution (PBS) used for the immunoaffinity clean-up experiments consisted of a 0.01 *M* sodium phosphate buffer containing 0.15 *M* NaCl (pH 7.4).

2.4. Environmental and certified samples

The certified reference sediment sample was a river sediment that was prepared and certified within a European Union project for interlaboratory studies (EQUATE No. 96050, a gift from D. Barcelo). The certified reference sludge sample (CRM No. 088) comes from an urban sewage sludge and was prepared by the Community Bureau of Reference (BCR) in Brussels. It was obtained from Promochem (Molsheim, France).

Waste waters were collected at the beginning of the process in a urban treatment plant just after the first decantation stage. Sewage sludge samples were obtained from the waste waters mentioned above and were collected at the end of their purification treatment. Sludges have been dehydrated to 52% of dry matter. Before extraction, they were dried in a ventilated oven for 24 h at 80°C, ground and then sieved to get rid of stones and pieces of wood.

Waste water samples (250 ml) containing 20% (v/v) of acetonitrile were percolated through the C_{18} cartridge and the elution was performed with 4 ml of THF. Sludge and sediment samples (1 g) were extracted using focused assisted microwave extraction with 60 ml of a mixture of toluene–acetone (50:50, v/v) for 20 min at 90 W. The final extracts were fractionated to be used in direct GC and LC analysis, and also for further clean-up using immunoextraction.

2.5. Liquid chromatography procedure coupled with fluorescence and diode array detections in series

A gradient with LC-grade water and acetonitrile (flow rate of 0.5 ml/min) was applied for the separation of the PAHs: 40% acetonitrile from 0 to 5 min, up to 100% acetonitrile at 30 min and until 35 min. The column temperature was set at 45°C. The fluorescence excitation and emission wavelengths were programmed as follows: 280/340 nm at 0 min, 295/380 nm at 18.2 min, 280/430 nm at 19.3 min, 285/460 nm at 26 min until 35 min. Dry extracts

were reconstituted with a mixture of acetonitrile–water (80:20, v/v) before LC analysis.

2.6. Gas chromatography procedure coupled with mass spectrometric (ion trap) detection

The split/splitless injector was programmed as follows: purge delay for 0.75 min, purge flow for 25 ml/min, temperature at 280°C. The temperature program was 1 min at 90°C, then 5°C/min until 340°C and 2 min at 340°C. The carrier gas was helium at a constant flow rate of 1 ml/min. The ion-trap mass spectrometer was performed with electron impact ionization (70 eV, source temperature: 250°C) operated under full scan monitoring mode. The interface temperature was maintained at 250°C. Before the GC injections, the four perdeuterated standards were systematically added at the following concentrations: [$^2H_{10}$]phenanthrene and [$^2H_{12}$]chrysene at 75 μg/l, [$^2H_{12}$]]perylene at 150 μg/l and [$^2H_8$]naphthalene at 600 μg/l. Dry extracts for direct GC analysis were reconstituted in iso-octane.

2.7. Immunoselective clean-up procedure

The immunosorbent was first rinsed with 6 ml of PBS and with 6 ml of LC-grade water. An aliquot of the extract, diluted to 20 ml with water containing 25% of acetonitrile, was flushed through the IS at a flow-rate of about 2 ml/min. The 25% of acetonitrile had to be added to the sample in order to solubilize PAHs and to prevent the losses due to adsorption on the vessels. As shown in previous studies dealing with various immunosorbents, desorption requires a high proportion of organic solvent and cannot be obtained by a pH change for these small size molecules of interest [10,20]. The elution of the IS was achieved by percolating 5 ml of a mixture of acetonitrile–water (70:30) [21]. A pure organic solvent, such as THF or acetonitrile, was not used because ISs are still at the laboratory study stage and regeneration was necessary. Mixtures of acetonitrile or methanol and water are then preferred because they ensure easy regeneration after use, but they have the drawback of requiring long evaporation times with the risk of partial losses of some PAHs. Therefore, the eluate was next percolated on a C_{18} cartridge, which had been previously conditioned, and the elution from the C_{18} cartridge was achieved with THF, which is easier to evaporate. The THF extract was then fractionated in two extracts that were prepared to be used for LC and GC injections, respectively. After use, the IS was washed with 20 ml of an acetonitrile–water (70:30) mixture to prevent any memory effect. The IS was then regenerated with 10 ml of water and stored at 4°C in a solution of PBS containing 0.2% of azide.

3. Results and discussion

The on-line coupling of the extraction step with the LC analysis was shown to be the most appropriate method for monitoring the PAHs in water samples because it does not contain any evaporation step and, consequently, avoids losses of some of the more volatile PAHs [3]. Using the selectivity of the immunoextraction by packing the on-line precolumn with the anti-pyrene immunosorbent, LC with fluorescence detection and UV DAD in series could be used for both the quantification and confirmation in one step, because silica-based immunosorbents are pressure-resistant and can be regenerated for optimal use in on-line techniques [20]. However, some waste water are very complex and further confirmation using GC–MS (or LC–MS) is required. Therefore off-line analyses of aqueous samples were used because of the possibility for analyzing the extracts with several methods when necessary. Moreover, off-line extraction of the solid-matrix is unavoidable.

3.1. Extraction of PAHs from aqueous and solid matrices

Waste water samples were extracted using disposable C_{18} solid-phase extraction cartridges eluted with THF. Since 2–3 ring PAHs are volatile, the THF evaporation is known to originate losses, which have been estimated by spiking 4 ml of THF with the 16 PAHs and were found to be important from naphthalene up to phenanthrene and anthracene. Losses for fluoranthene could be avoided using mild conditions. No losses were observed for the other ones. The extraction recoveries after the SPE sequence have been measured with ultrapure and waste water

spiked at concentrations of 0.075–0.15 μg/l, depending on the PAHs. Except for the four more volatile ones, recoveries were found to be above 70% for all the analytes. The average recoveries were in the range 85–90% for ultrapure and tap water and were slightly lower with surface and waste water (75–80%), indicating a small effect of the matrix, certainly due to the adsorption of the PAHs on the small particulate matter remaining in the filtered samples of waste water.

Focused microwave-assisted extraction (FMWE) was applied to sediment and sewage sludges. Since the extraction solvent was a mixture of toluene with acetone, losses by evaporation for the 2–3 ring PAHs were much lower than those observed when evaporating THF for water extracts, which can be explained by the higher volatility of THF and the difference in solubility of the PAHs in these solvents. The extraction recoveries for sludges spiked with 1–2 mg of each PAH per kg of dry sludges were found to be above 75% except for benzo[*b*]fluoranthene (70%), benzo[*k*]fluoranthene (74%) and dibenzo[*a,h*]anthracene (65%).

3.2. Immunoclean-up of extracts

The dry extracts obtained after the non-selective extraction of waste water and solid samples were dissolved in 20 ml of pure water containing 25% acetonitrile and then percolated through the anti-pyrene IS before elution as described in Section 2. The addition of 25% acetonitrile was shown to be necessary for the solubilization of the more hydrophobic PAHs, but it has the drawback of decreasing the extraction recoveries on the anti-pyrene IS, due to a lower affinity between antibodies and analytes in media containing organic solvent. These immunoextraction recoveries were first measured by percolation of 20 ml of pure water containing 25% acetonitrile and spiked with 20 ng of each PAH. Recoveries are in the range of 45–60% as shown in the first column of Table 1. The matrix effect was studied by dissolving extracts from spiked waste water and from the non-spiked sediment and sludge in 20 ml of water containing 25% acetonitrile. The extracts have been divided in two parts, one being analyzed directly using LC–fluorescence for quantification, whereas the clean-up was applied to the second part before LC. Therefore, it was possible to obtain the recovery due to the immunoclean-up step alone and to assess the matrix effect for the real (non-spiked) solid samples. The results are also reported in Table 1 and show that the extraction recoveries due to the immunoclean-up are similar for the three extracts.

Table 1
Extraction recoveries (%) and standard deviation ($n=3$) of the immunoclean-up step

PAH	Recoveries from[a] ultrapure water	Recoveries from extracts of liquid[b] or soil sample		
		Waste water	Sediment	Sewage sludge
Anthracene (6)	38±8	45±10	47±9	nd[c]
Fluoranthene (7)	53±6	47±6	31±4	nd
Pyrene (8)	56±8	63±10	46±7	50±6
Benz[*a*]anthracene (9)	49±2	65±8	47±8	45±10
Chrysene (10)	47±4	61±5	49±7	nd
Benzo[*b*]fluoranthene (11)	55±5	66±5	54±7	46±6
Benzo[*k*]fluoranthene (12)	56±5	62±4	56±8	57±10
Benzo[*a*]pyrene (13)	44±4	55±7	52±10	44±11
Dibenz[*a,h*]anthracene (14)	48±5	58±5	nd	nd
Benzo[*ghi*]perylene (15)	50±3	51±3	60±7	nd
Indeno[1,2,3-*cd*]pyrene (16)	48±4	45±3	48±8	42±8

[a] Water (20 ml, spiked with 20 ng of each PAH) directly percolated through the IS cartridge.
[b] Environmental sample extracts from C_{18} cartridge (water) or from FMW extraction (certified sediments or urban sludges) divided into two parts. See text for recovery calculation.
[c] nd: Not detected.

3.3. Performances and validation of the total procedure

The recoveries corresponding to the whole procedure are reported in Table 2. They were calculated by taking into account the recoveries for both the extraction steps and the immunoclean-up step, respectively, using the experimental values obtained with the spiked pure water (first column of Table 1). These recoveries were around 40%. However, as shown below, the loss in recoveries was accompanied by a gain in selectivity and identification of analytes.

Using GC-ion trap MS with electron impact (EI), the chromatograms corresponding to extracts from 200 ml of waste water spiked with 0.075–0.15 μg/l of each PAH were compared. In order to compare chromatograms from the same sample, the extracts were divided in four equal parts. Identification was performed using the characteristic ions which correspond to the molecular ion $M^{+\cdot}$, so that ions selected for identification were those corresponding to the molecular mass, except for acenaphthene and fluorene with two abundant ions at $M^{+\cdot}$ and $(M\text{-}1)^{+\cdot}$. Without the clean-up, as expected, identification was not possible using the simple spectrum match, whereas after the clean-up, identification and quantification using characteristic ions was achieved for phenanthrene, fluoranthene, pyrene, benzo[*a*]anthracene and benzo[*a*]pyrene using the extract corresponding in fact to the waste water spiked at concentrations ranging from 20 to 40 ng/l. Identification of remaining PAHs was possible using the whole extract. These results confirmed that immunoclean-up is as efficient than that obtained using a more conventional clean-up procedure with a silica or an alumina column. Quantitative analysis was made using the four deuterated internal standards and the limits of identification in the waste water was estimated to 20 ng/l for fluoranthene and benz-[*a*]anthracene to 150 ng/l for indeno[1,2,3-*cd*]pyrene from an extract of 250 ml after clean-up. With the extract from 1 g of dry sludge, the limit of identification was estimated as 0.5 mg/kg for fluoranthene and as 1 mg/kg for benzo[*b*]fluoranthene and benzo-[*a*]pyrene.

The effect of the immunoclean-up was also studied using LC with fluorescence and UV DAD in series. Table 3 reports the detection limits defined with a ratio signal-to-noise of 3 and measured with the spiked waste water. These limits were in the range of 1–25 ng/l, thus allowing an efficient monitoring of waste water.

The detection limits were also estimated for urban sludges using the certified samples containing six certified PAHs at concentrations ranging between 0.57 and 2.16 mg/kg. Using these certified sludges was also a means for validating the whole procedure. Quantification was made using the recoveries reported in Table 2. The values have been reported in Table 4 as well as the certified values and the corresponding RSDs. The agreement between the measured values and the certified ones is good, taking into account the RSDs. Quantification was

Table 2
Extraction recoveries (%) and standard deviation ($n=3$) corresponding to the whole procedure including extraction through C_{18} cartridges for liquid samples (200 ml) and FMW extraction for sludge samples (1 g) followed by the immunoclean-up and analysis by LC–fluorescence detection

PAH	Waste water sample	Sludge sample
Anthracene (6)	13±9	36±10
Fluoranthene (7)	39±8	42±6
Pyrene (8)	38±6	45±7
Benz[*a*]anthracene (9)	36±5	38±5
Chrysene (10)	35±6	39±9
Benzo[*b*]fluoranthene (11)	41±8	39±6
Benzo[*k*]fluoranthene (12)	43±7	41±5
Benzo[*a*]pyrene (13)	36±5	36±6
Dibenz[*a,h*]anthracene (14)	34±5	31±4
Benzo[*ghi*]perylene (15)	37±3	40±4
Indeno[1,2,3-*cd*]pyrene (16)	38±4	36±4

Table 3
Limits of detection using the immunoextraction clean-up and LC with fluorescence detection

PAH	Waste water (ng/l)[a]	Sewage sludge (mg/kg)[b]
Fluoranthene (7)	2	0.2
Pyrene (8)	25	0.6
Benz[*a*]anthracene (9)	3	0.2
Chrysene (10)	25	nd
Benzo[*b*]fluoranthene (11)	2	0.2
Benzo[*k*]fluoranthene (12)	1	0.1
Benzo[*a*]pyrene (13)	1	0.4
Dibenz[*a,h*]anthracene (14)	10	nd
Benzo[*ghi*]perylene (15)	10	nd
Indeno[123-*cd*]pyrene (16)	10	0.8

[a] Waste water (200 ml) was mixed with 50 ml acetonitrile, extracted on C_{18} silica followed by the immunoclean-up and analyzed by LC with fluorescence detection.
[b] Sewage sludge (1g) was extracted using focused microwaves followed by the immunoclean-up and analyzed by LC with fluorescence detection.

easily performed using fluorescence, except for indeno[1,2,3-*cd*]pyrene. But quantification was difficult using the UV DAD because the levels were too close to the detection limits. However, the identity of the certified PAHs could be confirmed with their UV spectrum, except for PAHs 11 and 12. We also identified and quantified the fluoranthene compound, which is not certified by the BCR. The detection limits were measured in sludges using 1 g of certified sample and are been reported in Table 3. They were found in the range of 0.1 mg/kg for benzo[*k*]fluoranthene–0.8 mg/kg for indeno[1,2,3-*cd*]pyrene. The concentrations of benzo[*b*]fluoranthene and benzo[*a*]pyrene were around 1 mg/kg, which is the half of the regulatory level (2.5 and 2 mg/kg, respectively). These detection limits allow a good monitoring of the sludges in WWTPs.

The extract from 1 g of the certified river sediment containing 11 PAHs at concentrations below mg/kg was also analyzed using the whole LC procedure,

Table 4
Mean values and certified values in mg/kg (of dry materials) measured for various PAHs in certified sediment and certified sludge samples after FMW extraction and immunoclean-up on the anti-pyrene immunosorbent

PAH	Sediment		Sewage sludge	
	Certified values (and RSD[a])	Calculated values (and RSD[b])	Certified values (and RSD[a])	Calculated values (and RSD[b])
Anthracene (6)	0.34(9)	0.38(28)	nc[c]	nd[d]
Fluoranthene (7)	0.95(13)	0.68(15)	nc	3.4(13)
Pyrene (8)	0.84(11)	1.03(17)	2.16(4)	1.91(13)
Benz[*a*]anthracene (9)	0.56(11)	0.50(19)	0.93(10)	0.72(21)
Chrysene (10)	0.55(12)	0.42(22)	nc	nd
Benzo[*b*]fluoranthene (11)	0.70(13)	0.89(17)	1.17(7)	1.04(18)
Benzo[*k*]fluoranthene (12)	0.40(10)	0.38(17)	0.57(9)	0.49(20)
Benzo[*a*]pyrene (13)	0.41(13)	0.57(22)	0.91(10)	0.73(24)
Dibenz[*a,h*]anthracene (14)	0.08(14)	nd	nc	nd
Benzo[*ghi*]perylene (15)	0.42(13)	0.45(15)	nc	nd
Indeno[1,2,3-*cd*]pyrene (16)	0.41(15)	0.51(18)	0.81(7)	0.75(22)[e]

[a] RSD (%) for $n=5$.
[b] RSD (%) for $n=3$.
[c] nc: Not certified.
[d] nd: Not detected.
[e] Close to detection limit.

showing that quantification was possible at concentrations from 0.08 to 0.95 mg/kg (see Table 4). Because the sediment matrix was much cleaner than that of the sludges, quantification was achieved using both the fluorescence and the absorbance UV detectors in series, except for PAHs 11 and 12 with UV. The identities of the PAHs were confirmed by the UV spectra given by the UV diode array detector.

3.4. Application to the monitoring of waste water and sewage sludges in a treatment plant

Waste waters were collected at the beginning of the treatment plant, just after being submitted to the first decantation process. Sludges were collected at the end of their treatment, ready to be applied on soil.

The analysis of the waste water extracts using GC–MS and LC with fluorescence and UV DAD detections has shown that no PAH was present in the waste water after the first decantation stage at concentrations higher than the limits of detection (LODs), as reported in Table 3. This result is not surprising due to the hydrophobicity and low water solubility of PAHs, one can expect them to be adsorbed on particulates at the entry of the WWTP and to be quantitatively recovered on sludges. In principle, the first decantation process removes around 50% of the particulate matter and the analytes associated to them.

UV and fluorescence chromatograms of sludge extracts after or before clean-up on the anti-pyrene IS are presented on Fig. 1A–D. The comparison of the chromatograms on Fig. 2A–D obtained with or without the immunoclean-up shows that a great amount of interfering compounds have been eliminated, especially the early eluted compounds that correspond to polar and moderately polar analytes. Nine PAHs could be determined in these urban sludges by their retention times on the fluorescence chromatogram, and their identity was confirmed by their UV spectrum. Quantification was performed using the recovery values in Table 2. The nine PAHs (and their respective concentrations in mg/kg) include: fluoranthene (2.21), pyrene (2.45), benz[*a*]anthracene (0.62), chrysene (1.25), benzo[*b*]fluoranthene (0.71), benzo[*k*]fluoranthene (0.32), benzo[*a*]pyrene (0.62), benzo[*ghi*]perylene (0.34) and indeno[1,2,3-*cd*]pyrene (0.86). The relative standard deviation (RSD) ranged from 12 to 23% ($n=3$). As regards to the regulatory levels, the concentrations of the three targeted PAHs were three to four times lower than the regulatory level.

The occurrence of these PAHs was confirmed using GC–MS. The total ion chromatograms obtained by GC–MS are given Fig. 2A and B respectively after or before the immunoclean-up. The comparison of these two figures allows the efficiency of the clean-up to be pointed out since many interfering compounds have been eliminated. No analyte could be identified by MS without the clean-up step. In this regard, LC with fluorescence and UV DAD detections are more efficient because the quantification and identification of some PAHs can be made without clean-up. With the immunoclean-up, the PAHs that have been identified by LC–UV DAD could be confirmed by GC–MS using their retention times and their mass spectra, but required the analysis of the total extract from 1 g of sludges. The examples of anthracene, fluoranthene and pyrene are given in inserts in Fig. 2A. Phenanthrene was also identified and confirmed by MS, whereas it could not be detected with UV DAD.

Sludges are known to contain high amounts of organic matter especially lipidic substances, which explains the complexity of the chromatogram without clean-up. In Fig. 2B, several lipids could be identified by the mass spectra referenced in library. Moreover, the complexity of the various matrices of sludges depends on their origins. If the waste waters contain industrial sewage in addition to domestic sewage, then sludges are likely to contain various industrial compounds (polymers or other synthesized compounds). The analysis by GC–MS of sludge extracts is, therefore, impossible without any clean-up. Conventional clean-up methods are based on a fractionation based on the polarity of the analytes, which is difficult to optimize when the matrix components are unknown. The advantage of the immunoclean-up is that it is based on structure recognition, so that unknown analytes are eliminated. In contrast with other clean-up procedures, the identity of analytes is reinforced by the selective extraction.

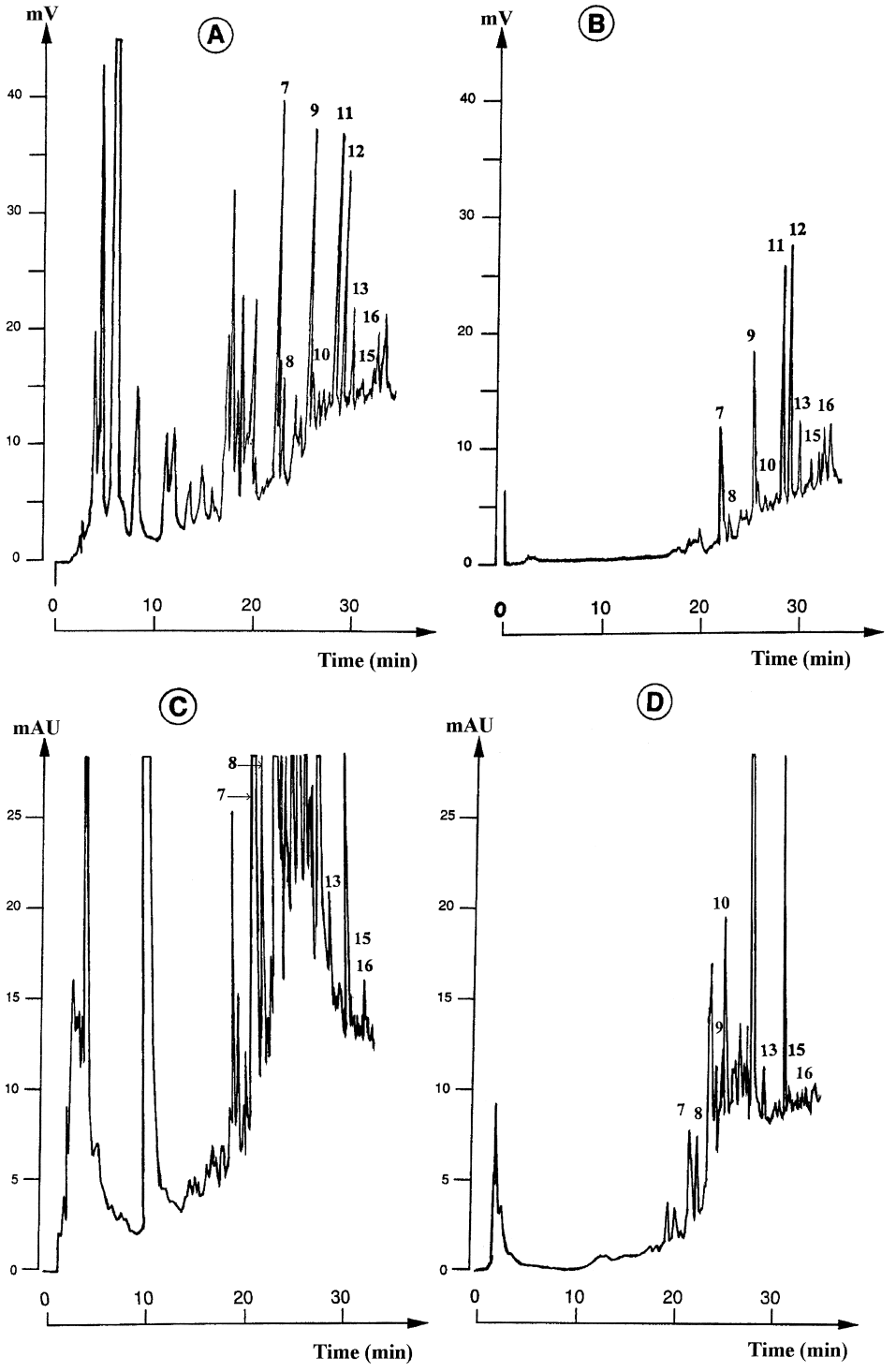

Fig. 1. Chromatograms of an urban sludge extract obtained: (A) before a clean-up and by LC–fluorescence, (B) after a clean-up and by LC–fluorescence, (C) before a clean-up and by LC–UV DAD (254 nm) and (D) after a clean-up and by LC–UV DAD (254 nm). See Section 2 for the preparation of the sludge extracts and the LC conditions.

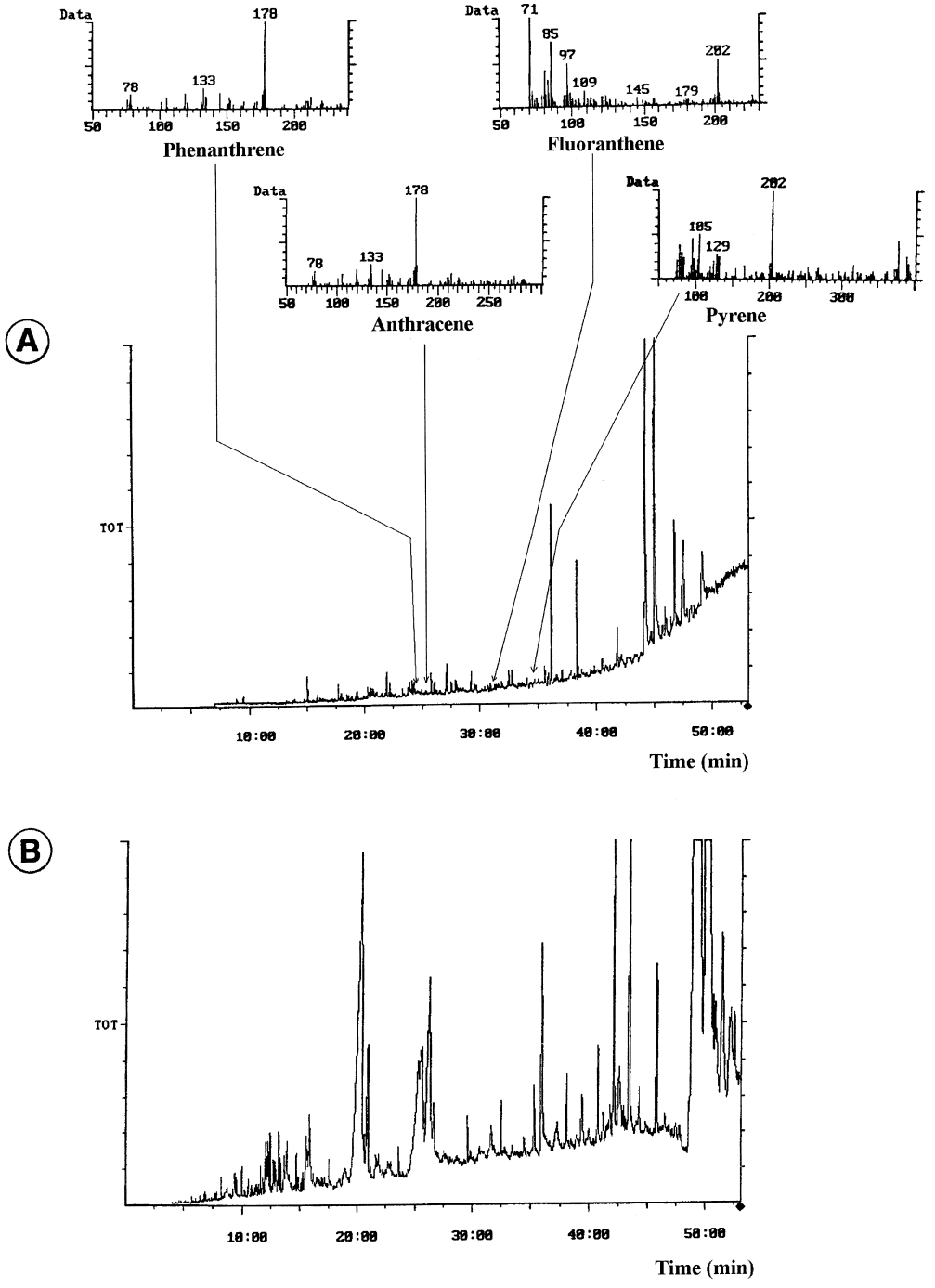

Fig. 2. Total ion chromatograms of a urban sludge extract: (A) after or (B) before a clean-up on the ISs. In inserts, the mass spectra of PAHs 5, 6, 7 and 8. See Section 2 for the preparation of the sludge extracts and the GC conditions.

4. Conclusion

The clean-up of extracts using a selective anti-pyrene immunosorbent is very efficient in improving the characterization of PAHs from fluoranthene to indenopyrene in sewage sludge. Many interfering compounds were eliminated and the resulting chromatograms show clearer base-lines, so that compounds can be easily quantified and identified, even if the extraction recoveries for the immunoextraction step are about 50%. The whole procedure involving focused-microwave extraction and immunosorbent clean-up followed by LC analysis coupled with UV and fluorescence detections in series was validated. The detection limits from the handling of only 1 g of sample are in the range of 0.1–0.8 mg/kg of dry sludge, which is lower than the regulatory level. This new approach for the sample handling of these complex sewage sludge samples can be of great interest for studying the fate of toxic chemicals during the treatment process.

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