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Optimization and validation of solvent and supercritical-fluid extractions for the trace-determination of polycyclic aromatic hydrocarbons in sewage sludges by liquid chromatography coupled to diode-array and fluorescence detection

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Abstract

There is a need for a better characterization of sludges from wastewater treatment plants which are destined to be spread on agricultural land. Inorganic pollutants are regularly controlled, but organic pollutants have received little attention up to now. The main problem for trace analysis of organic pollutants comes from the complexity of the various matrices of sludges, which depends on their origins. Therefore, methods described for soils and sediments cannot be directly applied to sludges which contain high amount of lipids. This paper provides a method for trace-determination of polycyclic aromatic hydrocarbons in the range of mg/kg of dried sludge including an extraction step, and an analysis step by liquid chromatography with fluorescence detection for sensitive quantification and UV–diode-array detection for confirmation. The extraction step was optimized by a selection of the solvent used for both Soxhlet and supercritical-fluid extraction and by setting the experimental conditions (temperature, pressure, modifier, etc.) used for SFE. Extraction recoveries were found similar and quantitative for the two procedures. However supercritical-fluid extraction appears to be a good alternative to Soxhlet extraction because of the consumption of less time and solvent. Detection limits were obtained in the range of 0.1–1 mg/kg of dried sludges, with possible confirmation by UV spectra. The whole method (extraction/quantification/confirmation) was validated using sludges which have been certified by the Community Bureau of Reference from Brussels (CRM No. 088). Application to the determination of PAHs in urban sludges is presented.

Keywords: Extraction methods; Sewage sludge; Polynuclear aromatic hydrocarbons

1. Introduction

The application of sewage sludges to agricultural lands as fertilizer is an economical method of utilizing the huge amount of sludge generated by wastewater treatment plants. However, this utilization may cause the release of toxic substances in the environment. In contrast with inorganic compounds, – especially heavy metals and some bacteria – which are monitored on a routine basis, the characterization of organic pollutants in such a matrix have received little attention up to now. This can be explained by the fact that there is no established tolerance of limit values, so that no routine control exists. In France, the norm only includes inorganic compounds [1]. In Germany, concentration limits have been given for furans, dioxins, adsorbable halogenated organic compounds (AOX) and polychlorinated biphenyls

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(PCBs) [2]. In Switzerland, only AOX are controlled [3]. In 1986, the US Environmental Protection Agency (EPA) proposed a reglementation to decrease risks of agricultural land pollution by sludge application, and included concentration limits for organic pollutants such as trichloroethylene, benzo[*a*]pyrene, aldrin, dieldrin, chlordane, DDT, DDE, DDD, heptachlor, hexachlorobenzene, hexachlorobutadiene, lindane, toxaphene, PCBs and dimethylnitrosamine. However, only metallic compounds were taken into account in the final reglementation edited in 1993 [4] because of the lack of knowledge concerning the toxicity and the analysis of the organic compounds.

Polycyclic aromatic hydrocarbons (PAHs) are a relevant group of compounds which can be concentrated in sludges. Waste-water contains high amounts of PAHs coming from industrial waste and domestic sewage, atmospheric rainfall, precipitation, airborne pollutants, runoff of road surface, etc. Because of their low aqueous solubility and their high hydrophobicity properties, these PAHs are efficiently removed from sewage and adsorbed on solid particles during sedimentation in the wastewater treatment. This results in the formation of sewage sludges that may typically contain between 1 and 10 mg/kg of each individual PAH, as shown by several studies [5–11]. Using a tedious fractionation scheme, combined to gas chromatography–mass spectrometry (GC–MS), several PAHs including 2–6 ring hydrocarbons and their alkyl derivatives, nitroarenes, aminoarenes and azaarenes have been identified in Polish sludges [6]. Moreover, it is well known that their biodegradability in soil is low, and bacteria are only able to degrade the 2–3 ring PAHs. The weathering of PAHs in sewage sludges–amended soils was monitored over a 141-day period, showing thus that only low-molecular-mass PAHs were susceptible to abiotic and biotic loss processes [10].

Due to their high carcinogenic and mutagenic toxicity and their persistence, the EPA has included 16 PAHs in its priority pollutants list, and has developed methods for their monitoring in wastewater discharge [5]. In Europe, there is a list of six PAHs (fluoranthene, benzo[*a*]pyrene, benzo[*b*]-fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]-perylene, indeno[1,2,3-*cd*]pyrene) to be monitored in drinking and surface water used as a source for drinking water purpose. The sum of their concentrations must be lower than 0.2 μg/l and the maximal admissible concentration of benzo[*a*]pyrene is 0.02 μg/l [12].

The best available methods for monitoring PAHs are liquid chromatography (LC) with fluorescence detection or GC–MS [13]. The separation–detection steps are described as EPA Method 8310 using LC with fluorescence or UV detection and method 8100 using GC–MS. For the two methods, an extraction and concentration step is required and the main problem for trace analysis of organic pollutants comes from the complexity of the various matrices of sludge, which depends on their origins. Therefore, methods described for soils and sediments cannot be directly applied to sludges which contain high amount of lipids. Extraction procedures at ambient temperature with an aqueous phase to disperse particles of sludge and an organic phase to extract PAHs [9,11], ultrasonic [5,7,8] and Soxhlet [10] extractions have been described, and a clean-up of the extract is usually applied before column injection. All these studies show that there is a need for optimizing the extraction step.

The aim of this study was to provide a method for routine analysis of PAHs in sludges with detection limits of individual PAHs at the mg/kg (dry sludges) level for each compound. Such a method should include a performent extraction step in order to avoid a clean-up step. In order to confirm identity of the analytes, the idea was to couple on-line LC with fluorescence detection (FL) for quantification and UV–diode-array detection (DAD) for confirmation because UV spectra of PAHs are very characteristic and different for each PAH. Soxhlet extraction and supercritical-fluid extraction (SFE) were investigated without a clean-up step because of the selectivity that can be obtained with this class of compounds when analysing by LC–FL. Certified sludges were used for the validation of the whole procedure and applications to urban sludges are presented.

2. Experimental

2.1. Apparatus

The HPLC system consisted of a Varian 9010 pump with Rheodyne 20 μl loop injector coupled

with a Varian 9070 fluorescence detector and a Varian 9065 photodiode-array detector (Varian, Les Ulis, France). Supercritical-fluid extractions were performed with a Star SFE Prepmaster apparatus in conjunction with a star SFE pump modifier and equipped with a Duraflow variable restrictor (Varian). A Croco–Cil oven (Touzart et Matignon, Les Ulis, France) was used for setting up the column temperature.

2.2. Chemicals

Acetonitrile of ''HPLC ultra'' grade was obtained from J.T. Baker–Mallinckrodt (Noisy le Sec, France). Dichloromethane, toluene and cyclohexane of ''spectroscopic'' grade were obtained from Merck (Nogent sur Marne, France). Deionized water was obtained from a Milli-Q water system (Millipore, St. Quentin en Yvelines, France). Glassbeads for SFE were obtained from Phase Sep (St. Quentin en Yvelines, France). CO_2 of ''SFC'' grade was obtained from Air Liquide (Paris, France). The standard mixture of the 16 priority PAHs in dichloromethane was obtained from J.T. Baker–Mallinckrodt at the following concentrations: naphthalene (No. 1): 1000 ppm; acenaphthylene (No. 2): 200 ppm; acenaphthene (No. 3): 100 ppm; fluorene (No. 4): 200 ppm; phenanthrene (No. 5): 100 ppm; anthracene (No. 6): 100 ppm; fluoranthene (No. 7): 200 ppm; pyrene (No. 8): 100 ppm; benz[*a*]anthracene (No. 9): 100 ppm; chrysene (No. 10): 100 ppm; benzo[*b*]fluoranthene (NO. 11): 200 ppm; benzo[*k*]fluoranthene (No. 12): 100 ppm; benzo[*a*]pyrene (No. 13): 100 ppm; dibenz[*ah*]anthracene (No. 14): 200 ppm; benzo[*ghi*]perylene (No. 15): 200 ppm; indeno[1,2,3-*cd*]pyrene (No. 16): 100 ppm.

Certified sludges (CRM No. 088) were prepared by the Community Bureau of Reference from Brussels and were obtained from Promochem (Molsheim, France). Certified concentrations of PAHs in these sludges are as follow (mass fractions of dry matter expressed as mg/kg): pyrene, 2.16; benz[*a*]anthracene, 0.93; benzo[*a*]pyrene, 0.91; benzo[*b*]fluoranthene, 1.17; benzo[*k*]fluoranthene, 0.57; indeno-(1,2,3-*cd*)pyrene, 0.81.

Sewage sludge samples were obtained from a urban sludge treatment plant and were collected at the end of the treatment, dehydrated to 52% of dry matter. Before extraction, sludges were dried in a ventilated oven for 24 h at 80°C and then ground and sieved to get rid of stones and pieces of wood.

2.3. Liquid chromatography procedure

A 250×3 mm Bakerbond PAH-16 Plus column including guard column was used (J.T. Baker–Mallinckrodt). Acetonitrile and water were used as eluent solvents at a flow-rate of 0.5 ml/min. The gradient elution program was 0–5 min: acetonitrile-(40:60) water; then a linear gradient elution from 40% acetonitrile at 5 min to 100% acetonitrile at 30 min, followed by isocratic elution with 100% acetonitrile for 5 min. The column temperature was maintained at 45°C. The fluorescence excitation and emission wavelengths were changed during the chromatographic separation in order to obtain better sensitivity. The excitation/emission wavelengths were set as follow: 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.

2.4. Extraction procedures

2.4.1. Spiking procedure

The PAH mixture was deposed with a micropipette directly onto the sludge sample. Once the analytes were spiked, the SFE cell and Soxhlet extractor were immediately sealed, and extractions were starting up to prevent the loss of volatile compounds.

2.4.2. Soxhlet extraction procedure

A 1-g amount of sludge sample (dry matter) was Soxhlet extracted with 80 ml of solvent (toluene, cyclohexane or dichloromethane) for 8 h. The extract was then evaporated with a rotary evaporator until 5 ml and under nitrogen flow to dryness. After that, the extract was redissolved in 3 ml of acetonitrile and 0.75 ml of water (acetonitrile–water, 80:20) for HPLC injection.

2.4.3. SFE procedure

The extraction cell having an internal volume of 3 ml was packed with a 1-g sample of dried sludge and glassbeads were added to fill the rest of the volume of the extraction cell. Pure or modified CO_2 (with 5% of toluene, dichloromethane or acetonitrile dur-

ing dynamic extraction step) was used as supercritical fluid. All extractions were performed at 500 atm and 150°C during 10 min in static mode and then 30 min in dynamic mode (1 atm=101 325 Pa). SFE flow-rate was controlled at 1 ml/min. All extracts were collected by inserting the outlet end of the restrictor into a vial containing 10 ml of solvent (toluene, dichloromethane or acetonitrile). The collecting solvent corresponds to the solvent used as modifier in order to avoid incomplete solubilization. The extract was then evaporated under nitrogen flow to dryness. After that, the extract was redissolved in 3 ml of acetonitrile and 0.75 ml of water (acetonitrile–water, 80:20) for HPLC injection.

3. Results and discussion

3.1. Analytical separation of PAHs

Fluorescence and UV chromatograms obtained by direct injections of the 16 PAHs standard mixture are represented in Fig. 1. Separation of the 16 PAHs was carried out in 35 min. Only acenaphthylene does not appear in the fluorescence chromatogram because it does not fluoresce. HPLC with fluorescence and UV detectors provided a linear response from amount injected in the range of 0.1–50 ng. Detection limits, obtained by direct injection of the 16 PAHs standard mixture and calculated with a signal-to-noise ratio of three, are between 2 and 100 fold lower using fluorescence detection compared with UV detection, except for anthracene and chrysene, with similar UV and fluorescence limits of detection. With the experimental conditions of Fig. 1, fluorescence limits of detection are in the range of 0.04 ng injected for benzo[*b*]fluoranthene and benzo[*k*]fluoranthene to 1.3 ng for pyrene and chrysene, whereas detection limits with UV (254 nm) range from 0.3 ng for anthracene to 12 ng for naphthalene, acenaphthylene and acenaphthene.

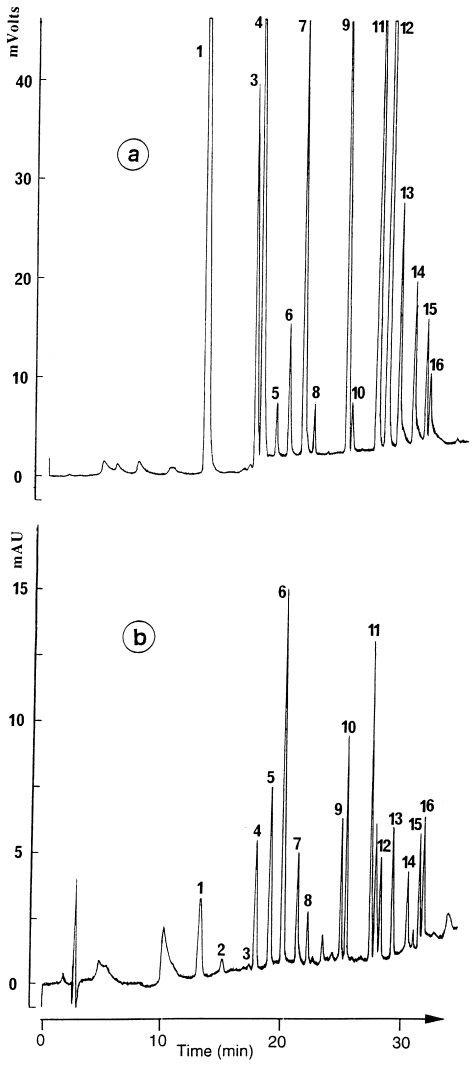

Fig. 1. Separation of the 16 PAH standard mixture, with detection by (a) fluorescence and (b) UV–DAD, represented at 254 nm. PAH numeration is as follows: naphthalene (1), acenaphthylene (2), acenaphthene (3), fluorene (4), phenanthrene (5), anthracene (6), fluoranthene (7), pyrene (8), benz[*a*]anthracene (9), chrysene (10), benzo[*b*]fluoranthene (11), benzo[*k*]fluoranthene (12), benzo[*a*]pyrene (13), dibenz[*ah*]anthracene (14), benzo[*ghi*]perylene (15), indeno[1,2,3-*cd*]pyrene (16). Injected amounts: 5 ng for PAH 3, 5, 6, 8, 9, 10, 12, 13, 16; 10 ng for PAH 2, 4, 7, 11, 14, 15; and 50 ng for PAH 1. See Section 2 for mobile phase gradient, flow-rate and fluorescence excitation/emission program.

3.2. Optimization of Soxhlet extraction conditions

It is well-known that recoveries obtained with spiked compounds may not be representative of those obtained with native compounds. Actually, spiked analytes on real-world samples are neither situated on the same binding sites as those of the native analytes nor adsorbed in the same manner. Spiked analytes are generally lightly coated on the

surface of the matrix whereas native ones can be strongly adsorbed inside the porous matrix. This can be explained by the diffusional and the kinetic limitations of the sorption process, and the several interactions which can have been simultaneously established between a native analyte and a complex matrix [8,14]. Therefore, spiked analytes are always less retained in the environmental matrices than the native ones and the use of spike recovery studies may overestimate the efficiencies of extraction methods with real samples. That is the reason why it is necessary to validate the extraction procedures with certified reference matrices when available.

However, due to the high price of the certified reference materials, the optimization was performed by spiking unknown urban sludges. Since a first screening indicated the occurrence of several PAHs in these sludges, non-spiked samples were systematically compared to spiked samples, and extraction recoveries have been calculated from the increase in peak areas or heights between chromatograms corresponding to the same non-spiked and spiked samples.

Toluene, cyclohexane and dichoromethane have been evaluated for Soxhlet extraction. This choice can be explained by the fact that (i) apolarity of toluene and cyclohexane is well adapted with apolarity of PAHs, (ii) electron donor–acceptor interactions may occur between toluene and PAHs. With regards to dichloromethane, it is often used in literature [5,8,10].

The effect of the organic solvent was studied by spiking sludges with rather high concentrations of PAHs (100 μl of the 16 PAHs standard mixture into 1 g of dry sewage sludge). Spiked quantities correspond to 10 mg/kg dry matter (concentrations are always reported per kg of dry matter) for PAHs No. 3, 5, 6, 8, 9, 10, 12, 13 and 16; 20 mg/kg for PAHs No. 2, 4, 7, 11, 14 and 15, and 100 mg/kg for PAH No. 1. When spiking ten times higher quantities than those usually found in such a matrix, the efficiency of each solvent to solubilize PAHs is easier to evaluate, even if PAH solubilization is not yet completely independent from the solubilization of the other unknown sludge compounds.

The recoveries obtained with the different solvents are given in Table 1. Cyclohexane and toluene provide the highest recoveries for each analyte which are above 70% except for naphthalene. Although widely used [5,10], dichloromethane provides lower recoveries and is not the best one. Some authors prefer to use acetonitrile as the extracting solvent for PAHs in order to avoid an evaporation to dryness step [5] before HPLC analysis. Whereas this permits to avoid losses of PAHs during the evaporation step, acetonitrile is not commonly used for the extraction

Table 1
Recoveries [a] for Soxhlet extractions with different solvents

PAH	Recovery (%)		
	Toluene	Cyclohexane	Dichloromethane
Naphthalene	63	60	67
Acenaphthene	80	75	68
Fluorene	83	78	73
Phenanthrene	96	100	83
Anthracene	113	110	82
Fluoranthene	87	81	63
Pyrene	84	80	56
Benz[*a*]anthracene	86	78	58
Chrysene	83	79	60
Benzo[*b*]fluoranthene	82	85	58
Benzo[*k*]fluoranthene	76	86	59
Benzo[*a*]pyrene	104	110	65
Dibenz[*ah*]anthracene	71	79	56
Benzo[*ghi*]perylene	73	79	50
Indeno[1,2,3-*cd*]pyrene	72	82	62

[a] RSD=5–20%, $n=3$.

Table 2
Recoveries [a] for supercritical-fluid extractions [b] with pure or modified CO_2 (5%, v/v)

PAH	Recovery (%)			
	Toluene	Dichoromethane	Acetonitrile	Pure CO_2
Naphthalene	57	58	60	71
Acenaphthene	72	65	62	64
Fluorene	78	68	62	64
Phenanthrene	95	95	73	91
Anthracene	101	110	96	118
Fluoranthene	76	76	63	73
Pyrene	76	70	57	68
Benz[*a*]anthracene	79	66	56	69
Chrysene	85	67	59	67
Benzo[*b*]fluoranthene	77	65	64	57
Benzo[*k*]fluoranthene	77	65	57	57
Benzo[*a*]pyrene	112	94	109	71
Dibenz[*ah*]anthracene	68	37	59	32
Benzo[*ghi*]perylene	71	29	65	20
Indeno[1,2,3-*cd*]pyrene	75	37	58	27

[a] RSD=5–20%, n=3.
[b] Temperature and pressure: 150°C and 500 atm.

of PAHs from sludges because of its relatively low solvent power.

With the non-spiked sludges, we have studied the ability of the three organic solvents to solubilize the unknown sludge compounds (matrix effect). We did not observe any difference between the three procedures in the corresponding UV and fluorescence chromatograms. The same compounds seem to have been extracted and detected with the three different solvents.

3.3. Optimization of SFE conditions

As for Soxhlet extraction, non-spiked samples were systematically compared to spiked samples, and extraction recoveries have been calculated from the

Table 3
Recoveries (R) and relative standard deviation (RSD) for Soxhlet and supercritical-fluid extractions of spiked sludges with toluene as solvent or modifier

PAH	Soxhlet extraction			SFE		
	R (%)	RSD [a] (%)	LOD (mg/kg)	R (%)	RSD [a] (%)	LOD (mg/kg)
Naphthalene	63	15	5	35	9	5
Acenaphthene	99	12	0.2	72	12	0.2
Fluorene	89	12	0.2	82	14	0.2
Anthracene	98	17	0.4	93	10	0.4
Fluoranthene	85	7	0.1	81	7	0.1
Pyrene	84	6	0.4	81	4	0.4
Benz[*a*]anthracene	80	6	0.05	83	8	0.05
Chrysene	94	19	0.4	95	19	0.6
Benzo[*b*]fluoranthene	70	8	0.09	72	6	0.09
Benzo[*k*]fluoranthene	72	8	0.05	74	7	0.04
Benzo[*a*]pyrene	82	22	0.2	81	20	0.2
Dibenz[*ah*]anthracene	66	6	0.9	72	6	0.4
Benzo[*ghi*]perylene	78	14	1.2	78	7	1.1
Indeno[123-*cd*]pyrene	73	7	0.6	71	8	0.8

[a] RSD for n=10.

increase in peak areas or heights between chromatograms corresponding to the same non-spiked and spiked samples.

Concerning the temperature and pressure conditions, we chose to work with a rather high temperature procedure as it is advised in the case of environmental samples with analytes strongly adsorbed inside the porous matrix [15]. A maximum temperature of 150°C combined with a pressure of 500 atm provided good results for the 16 PAHs extraction from sludges. Concerning the nature of the supercritical fluid, $CHClF_2$ is known to yield consistently higher recoveries than CO_2 [7], and this result seems to correlate with the high dipole moment of $CHClF_2$. But because $CHClF_2$ is responsible for ozone layer degradation and global warming potentials, extractions were performed with CO_2.

Extractions with pure CO_2 were first performed. Extraction recoveries have been reported in Table 2. The PAHs containing 2, 3 or 4 rings are well extracted, but recoveries of 5 and 6 rings PAHs decrease comparatively with recoveries obtained with a modifier. These results are in good concordance with the fact that the higher-molecular-

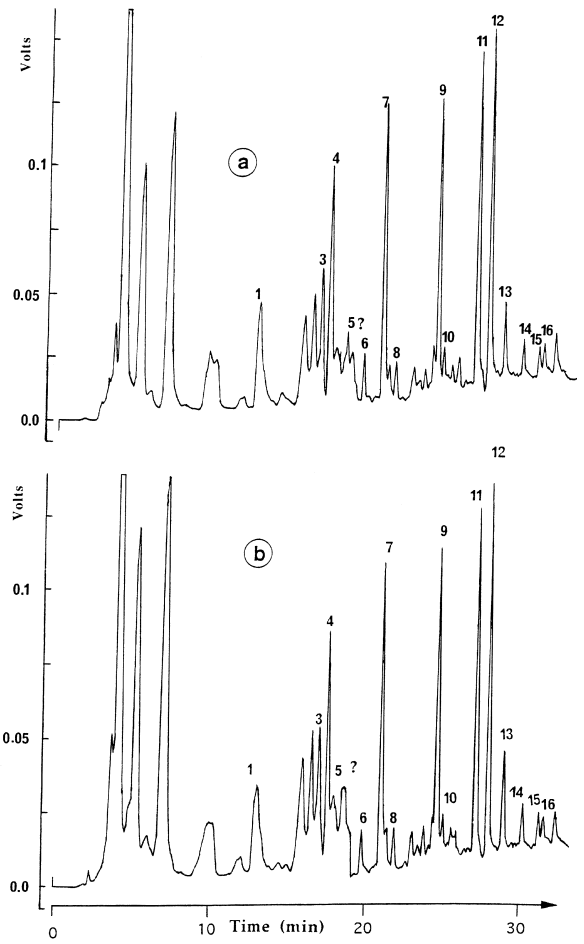

Fig. 2. Chromatograms corresponding to spiked sludges obtained by fluorescence detection and corresponding to (a) Soxhlet extract with toluene and (b) supercritical-fluid extract, with CO_2 and toluene as modifier. Experimental conditions for the separation as in Fig. 1.

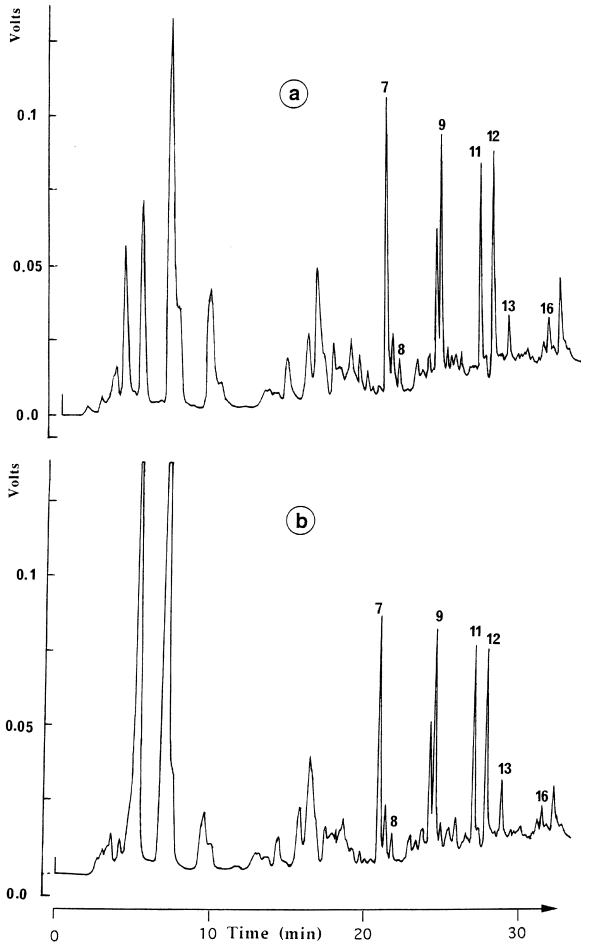

Fig. 3. Chromatograms corresponding to certified sludges obtained by fluorescence detection and corresponding to (a) Soxhlet extract with toluene and (b) supercritical-fluid extract, with CO_2 and toluene as modifier. Experimental conditions for the separation and peak numbering as in Fig. 1.

mass PAHs are the most strongly adsorbed on the sites of the matrix and with the fact that modifiers are known to facilitate the desorption processus by competing with the target analytes for the active sites on the sample matrix [7,16,17]. Because desorption is less efficient without modifier, differences of recoveries between with and without modifier would be exacerbated in the case of an extraction of a non spiked sample since analytes are more strongly adsorbed than in a spiked sample. That is why extractions are performed with modified CO_2.

Concerning the influence of modifiers, sludges were spiked at the same PAH concentrations as for Soxhlet extraction. Toluene, dichloromethane and acetonitrile have been evaluated as modifiers for the same reasons as with Soxhlet extraction concerning toluene and dichloromethane and because the use of acetonitrile has been described in the literature [5]. Extraction recoveries have been reported in Table 2. For the 4 ring PAHs, SFE is slightly more performent when toluene is added as CO_2 modifier. The advantage of using toluene as modifier over other dichloromethane and acetonitrile becomes very clear with 5 and 6 ring PAHs.

The recoveries are also dependent on the collection step. We have already mentioned that the collecting solvent corresponded to the solvent used as modifier in order to avoid incomplete solubilization. The collecting solvent can influence the recoveries of the extraction because of its solvent power to solute PAHs and the other sludge compounds. Moreover, its vapour pressure and volatility in the CO_2 outlet stream can sometimes decrease the recoveries of the analytes. In our case, we did not observe substantial losses of our three collecting solvents tested. According to the recoveries on Table 2, the collection step seems to have been well-performed. However, we can mention that when acetonitrile is used as solvent to collect analytes, a solid-phase is formed. It can probably be explained by the presence of compounds such as lipids that cannot be solubilized in acetonitrile.

For the following experiments, 5% (v/v) of toluene was used as CO_2 modifier during the dynamic extraction step.

3.4. Comparison of the performance of the two extractions methods applied to spiked sludges

Toluene was selected for the two methods and the application was performed using spiked sewage sludges at concentrations ten times lower than those applied for measuring recoveries in Tables 1 and 2, i.e. 1 or 2 mg/kg, except for naphthalene (10 mg/kg). These spiked concentrations better correspond to real samples since PAH concentrations detected in sludges were typically ranged from 1–10 mg/kg.

Recoveries, relative standard deviations (RSDs) and estimated limits of detection (LODs) for Soxhlet and supercritical-fluid extractions are reported in Table 3. Recoveries are similar and quantitative for both Soxhlet and SFE, except for naphthalene whose volatility is the cause of its loss with SFE during the collection step. Actually, the collection of the analytes was performed without any cooling system. Otherwise, a lower RSD is found using SFE. Recoveries of extractions are a little higher with the ten times smaller spiked amount. Limits of detection in spiked samples have been calculated on the fluorescence chromatograms. They are similar for the two

Table 4
Recoveries (*R*) and relative standard deviation (RSD) for Soxhlet and supercritical-fluid extractions of certified sludges

PAH	Certified quantities (mg/kg DM [b])	Soxhlet extraction		SFE	
		R (%)	RSD [a] (%)	*R* (%)	RSD [a] (%)
Pyrene (8)	2.16	113	9	108	6
Benz[*a*]anthracene (9)	0.93	114	6	100	8
Benzo[*b*]fluoranthene (11)	1.17	117	10	113	8
Benzo[*k*]fluoranthene (12)	0.57	118	13	111	11
Benzo[*a*]pyrene (13)	1.02	101	17	121	14
Indeno[123-*cd*]pyrene (16)	0.81	128	7	123	8

[a] RSD for $n=3$.
[b] DM=dry matter.

methods of extraction and lower than 1 mg/kg for the majority of individual PAHs except for naphthalene (No. 1) and phenanthrene (No. 5). In the sludge samples of interest, because of interfering compounds, detection and quantification seemed to be difficult for phenanthrene with lower concentrations than 5 mg/kg in sludges. This is shown in Fig. 2 where the chromatograms are presented which correspond to spiked extracts from the same sludges, obtained by Soxhlet extraction (Fig. 2a) and by SFE (Fig. 2b). Phenanthrene is not reported in Table 3 because it cannot be isolated from interfering compounds.

Finally, SFE appears to be a good alternative to Soxhlet extraction. Actually, SFE is as quantitative as Soxhlet extraction whereas it requires less solvent (11.5 ml instead of 80 ml) and less time (40 min instead of 8 h). Although the experimental conditions of the SFE process are more difficult to optimize, SFE is known to provide a more selective extraction,

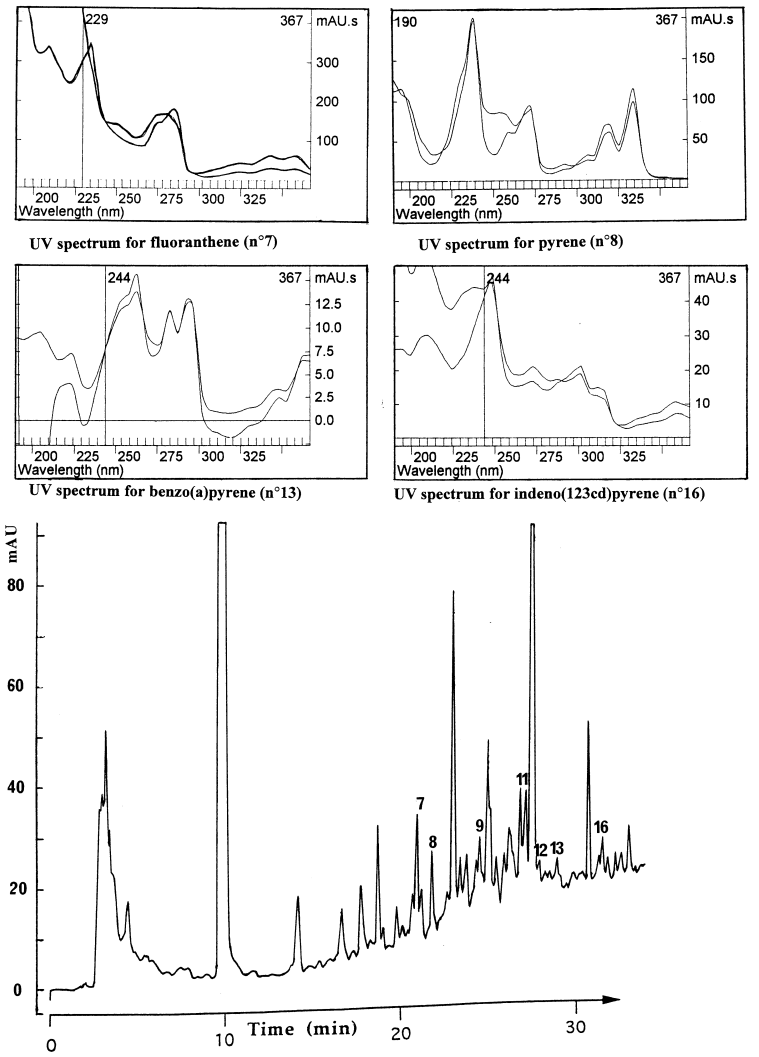

Fig. 4. Chromatograms obtained by UV detection at 254 nm corresponding to a SFE extract of the certified sludge sample. In inserts, the match between the UV spectrum in the DAD library and the peaks 7, 8, 13 and 16 in certified sludges. Experimental conditions for the separation and peak numbering as in Fig. 1.

thus leading to cleaner extracts. The chromatograms corresponding to the two procedures were not really different, so that the difference in selectivity was not obvious. This is shown in Fig. 2: in addition to the spiked PAHs, the compounds extracted from the matrix are the same. However, despite the similarity of the two chromatograms, we have observed that in contrast with dry supercritical-fluid extracts, dry Soxhlet extracts presented a bulky aspect. Moreover, it was impossible to redissolve the dry Soxhlet extract totally in pure acetonitrile and we can suppose that various extracted compounds remained adsorbed on the solid persistent phase. This could explain why selectivity of the SFE over the Soxhlet extraction is not observed on the chromatograms.

3.5. Validation of the two extraction procedures using certified sludges

Chromatograms of certified sludges are given in Fig. 3. Soxhlet and supercritical-fluid chromatograms are similar. Otherwise, by comparing Fig. 3 with Fig. 2, matrix effects in certified sludges seem to be the same as those observed in our sludge samples.

Recoveries and RSDs are reported in Table 4. Quantitative recoveries are obtained for each PAH even for the heaviest ones that are supposed to be more strongly adsorbed on solid matrices. These results allow us to validate our two extraction methods in combination with LC–FL as performent analytical methods to quantify PAHs in sludges with detection limits at the low mg/kg level. No clean-up is required to reach these detection limits.

In the method described above, identification of compounds just occurs by a retention time and a fluorescence signal. Up to now, there was no fluorescence detector able to provide a fluorescence signal with a wide range of wavelengths in the same time as UV–diode-array detectors do with UV spectra. Therefore, in order to confirm identity of compounds, although less sensitive, we have investigated the UV–DAD response. The UV chromatogram corresponding to the SFE extract is given in Fig. 4 with some UV spectra. As can be seen, there is a good agreement when comparing the UV spectra with those referenced in the UV–DAD library for pyrene, (peak No. 8), benzo[a]pyrene (No. 13) or indeno[1,2,3-*cd*]pyrene (No. 16). These results obtained with certified sludges show the great interest in coupling LC with both fluorescence and UV–DAD. Fluorescence provides good quantification and UV–DAD allows confirmation of identity thanks to the very characteristic UV spectra for each PAH. This identification can be made at concentrations in the low mg/kg levels.

Although not certified in sludge, peak No. 7 corresponds to fluoranthene, as shown by the correspondence of the UV spectrum in the UV–DAD library with the one of the certified sludge (Fig. 4). Its concentration is estimated at 3.4 mg/kg. This

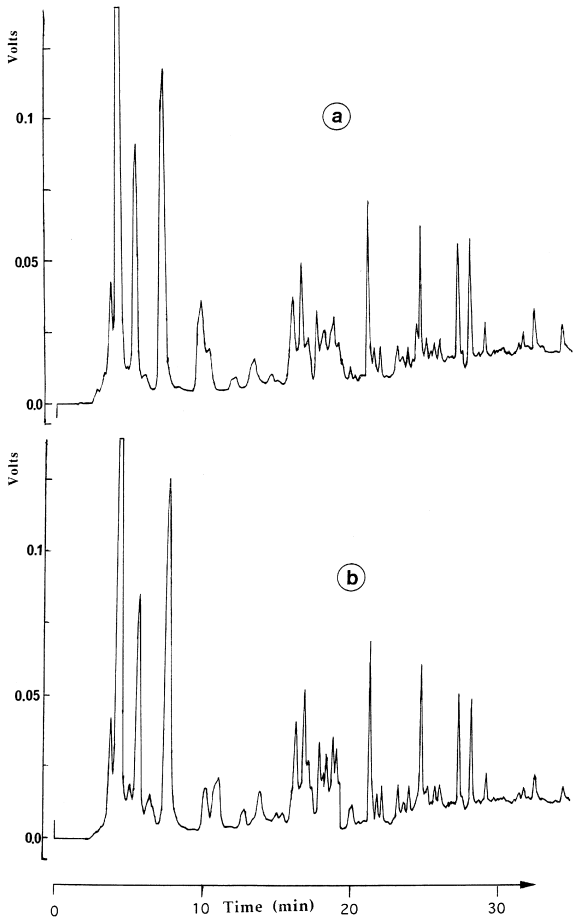

Fig. 5. Chromatograms corresponding to unknown sludges sample obtained by fluorescence detection and corresponding to (a) Soxhlet extract with toluene and (b) supercritical-fluid extract, with CO_2 and toluene as modifier. Experimental conditions for the separation and peak numbering as in Fig. 1.

measurement was made on three replicates and their RSD was evaluated at 8%.

3.6. Application to real samples

Soxhlet and supercritical-fluid extractions were performed with non-spiked sewage sludge samples from an urban treatment plant. Fluorescence chromatograms obtained with Soxhlet and supercritical-fluid extracts (Fig. 5) are very similar. Fig. 6 represents the chromatogram corresponding to the SFE extract obtained with UV–DAD at 254 nm. PAHs No. 7, 8, 9, 10, 13, 15 and 16 could be identified by their retention time and their UV spectra at concentration based on 100% recoveries of extraction, as follows (expressed in mg/kg): fluoranthene (No. 7), 2.2; pyrene (No. 8), 2.4; benz[*a*]anthracene (No. 9), 0.6; chrysene (No. 10), 1.4; benzo[*b*]fluoranthene (No. 11), 0.7; benzo[*k*]fluoranthene (No. 12), 0.3; benzo[*a*]pyrene (No. 13), 0.7; ben-

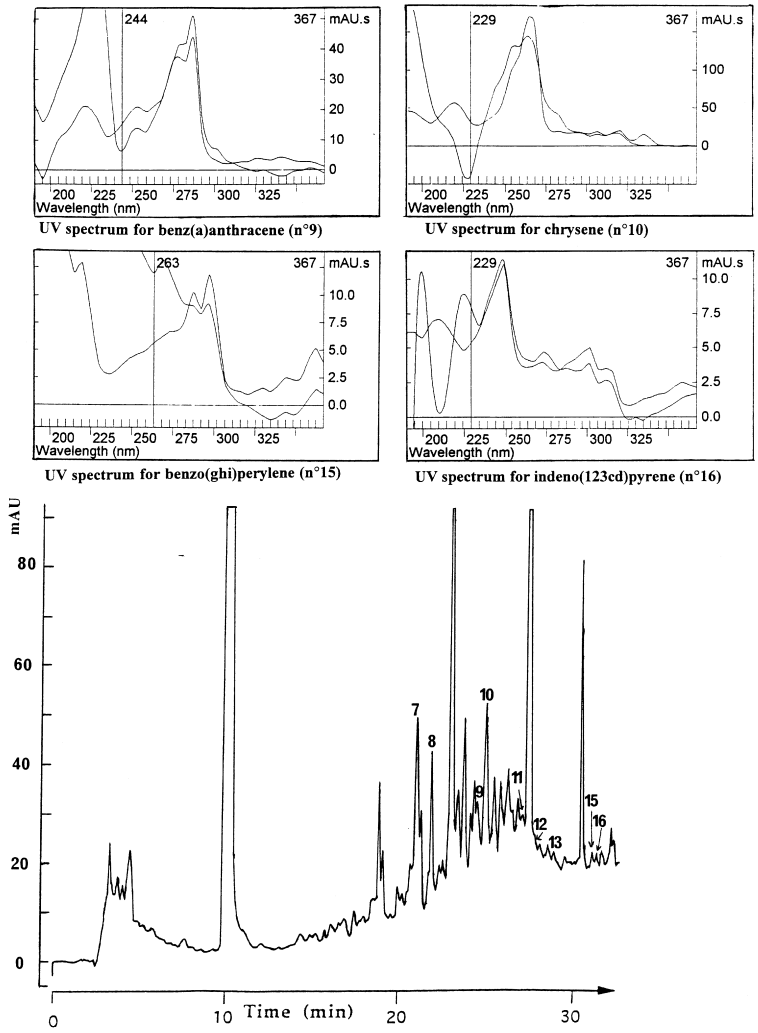

Fig. 6. Chromatograms obtained by UV detection at 254 nm corresponding to a SFE extract of unknown sludge sample. In inserts, the match between the UV spectrum in the DAD library and the peaks 19, 10, 15, 16 in sludge sample. Experimental conditions for the separation and peak numbering as in Fig. 1.

zo[*ghi*]perylene (No. 15), 0.4; indeno[123-*cd*]pyrene (No. 16), 0.8. Relative standard deviation (RSD) ranged from 7–18% ($n=3$).

UV spectra of PAHs No. 9, 10, 15 and 16 are given in Fig. 6. Identification is more difficult for benzo[*b*]fluoranthene (No. 11) and benzo[*k*]fluoranthene (No. 12) because of interfering compounds. A clean-up with immunosorbents could be helpful to better identify these two compounds.

4. Conclusion

SFE is a good alternative to Soxhlet extraction because of the same quantitative recoveries and the use of less time (40 min instead of 8 h) and solvents (11.5 ml instead of 80 ml) with SFE. According to the results obtained with certified sludges, we have validated SFE–LC–UV–DAD/fluorescence detection as a good method for the quantification and identification of PAHs with detection limits in the range of 0.1–1 mg/kg of dried sludge except for the naphthalene and the phenanthrene. Perhaps, an additional clean-up with immunosorbents could permit one to decrease detection limits under 5 mg/kg of dried sludge for the phenanthrene. It also could permit one to better identify benzo[*b*]fluoranthene and benzo[*k*]fluoranthene with UV spectra.

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