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Pressurized Hot Water Extraction with On-Line Fluorescence Monitoring: a Comparison of the Static, Dynamic, and Static−Dynamic Modes for the Removal of Polycyclic Aromatic Hydrocarbons from Environmental Solid Samples

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A comparison of the feasibility of the three operational modes of pressurized hot solvent extraction (PHSE) (namely, static, where a fixed extractant volume is used; dynamic, where the extractant continually flows through the sample; and static−dynamic mode, which consists of a combination of the two previous modes) for the extraction of polycyclic aromatic hydrocarbons (PAHs) from environmental solid samples (such as soil, sediment, trout, and sardine) has been performed. In all cases, a sodium dodecyl sulfate (SDS) aqueous solution was used as leaching agent. The use of a flow injection manifold between the extractor and a molecular fluorescence detector allowed real-time on-line fluorescence monitoring of the PAHs extracted from the samples, thus working as a screening system and providing qualitative and semiquantitative information on the target analytes extracted from both natural and spiked samples. The on-line monitoring option allowed the extraction kinetics to be monitored and the end of the leaching step to be determined independently of the sample matrix, thereby reducing extraction times. Efficiencies close to 100% have been provided by the three modes, which differ in the extraction time required for total removal of the target compounds. The time needed for the dynamic mode was shorter than that for the static mode. However, the establishment of a static extraction step prior to dynamic extraction was the key to shorten the time required for complete extraction. The method has been applied to a certified reference material (CRM 524, BCR, industrial soil/organics) for quality assurance/validation.

Polycyclic aromatic hydrocarbons (PAHs) are a group of compounds whose mutagenic or carcinogenic effects are well-known.[1] These substances can be produced in natural and anthropogenic processes, and they can be found in many kinds of samples, both biological (including humans) and environmental (air, water, and soil). Although the environmental concentrations of these organics are low, they tend to accumulate in organic tissues because of their lipophilic character and persistence to degradation,[2] which may eventually results in toxic concentration levels for organisms such as fish.[3] Their high toxicity and widespread occurrence has led the U.S. Environmental Protection Agency (EPA) to classify 16 of them as priority pollutants. For this reason, their detection and monitoring has become of concern.

The search for reducing both the use of organic solvents and the time needed for extracting organic pollutants from environmental matrixes has led to new extraction methods, including supercritical fluid extraction (SFE) and pressurized hot solvent extraction (PHSE). In the past few years, pressurized hot water (also called as high-temperature water, superheated water, subcritical water, or hot liquid water by different authors) has received increasing attention as an alternative extraction fluid[4−10] because it is environmentally friendly and also efficient under optimized conditions.

There are three operational modes to carry out extraction with pressurized hot water: the static mode, in which a fixed volume of extractant is used; the dynamic mode, in which the extractant flows continually through the sample; and the static−dynamic mode, which is a combination of the two previous modes. In the present work, these three operational modes were tested for the extraction of PAHs from environmental solid samples, thus allowing their performance to be compared in terms of experimental setup, dilution effect, extraction time and precision of the results they provide.

In all cases, the use of a flow injection manifold as an interface between the extractor and fluorescence detector allowed the real-time monitoring of the PAHs extracted. Nevertheless, this ap-

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(1) Jacob, J. *Pure Appl. Chem.* **1996**, *68*, 301−308.
(2) de Voogt, P.; Brinkman, U. In *Halogenated Biphenyls, Naphthalenes, Dibenzodioxins and Related Products*; Kimbrough, R. D., Jensen, A. A., Eds.; Elsevier Science: New York, 1989.
(3) Wilson, R.; Allen-Gil, S.; Griffin, D.; Landers, D. *Sci. Total Environ.* **1995**, *160/161*, 3.
(4) Hawthorne, S. B.; Yang, Y.; Miller, D. J. *Anal. Chem.* **1994**, *66*, 2912.
(5) Jiménez-Carmona, M. M.; Luque de Castro, M. D. *Anal. Chim. Acta* **1997**, *342*, 215.
(6) Rico, V. C. M.; de Castro, M. D. L. *J. Anal. At. Spectrom.* **1998**, *13*, 787.
(7) Crescenzi, C.; D'Ascenzo, G.; Samperi, R. *Anal. Chem.* **1999**, *71*, 2157.
(8) Ding, W.-H.; Fann, J. C. H. *J. Chromatogr. A.* **2000**, *866*, 79−85.
(9) Hawthorne, S. B.; Grabanski, C. B.; Martin, E.; Miller, D. J. *J. Chromatogr. A.* **2000**, *892*, 421−433.
(10) Alonso-Salces, R. M.; Korta, E.; Barranco, A.; Barrueta, L. A.; Gallo, B.; Vicente, F. *J. Chromatogr. A.* **2001**, *933*, 37−43.

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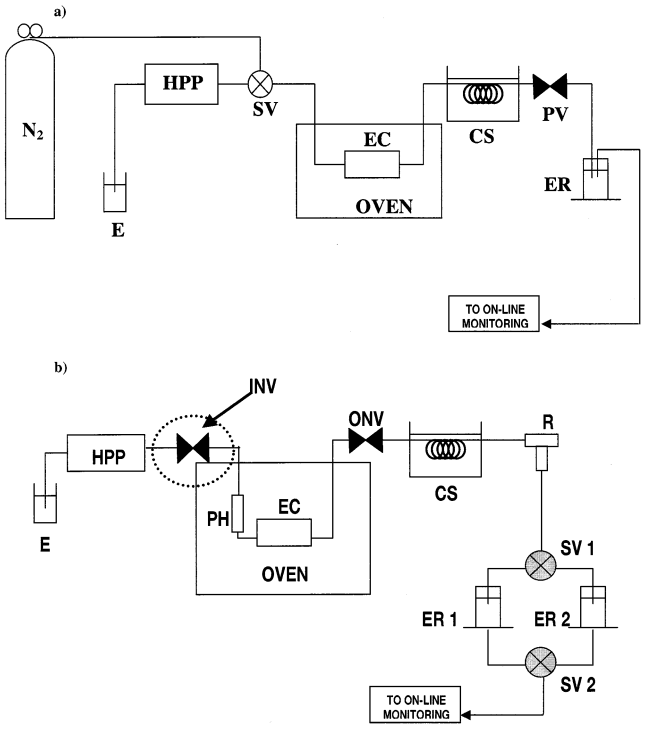

Figure 1. Schematic diagram of the extraction setup: (a) Static mode. E, extractant; HPP, high-pressure pump; SV, selecting valve; EC, extraction cell; CS, cooler system; PV, pressure valve; and ER, extract reservoir. (b) Dynamic and static−dynamic modes. E, extractant; HPP, high-pressure pump; INV, inlet needle valve; PH, preheater; EC, extraction cell; ONV, outlet needle valve; CS, cooler system; R, restrictor; SV, selecting valve; ER, extract reservoir.

proach allows monitoring only the overall content of fluorescent species, and it is not free from fluorescent interferences, so the use of chromatography is mandatory for the individual separation/quantification of the target PAHs.

EXPERIMENTAL SECTION

Instruments and Apparatus. The static pressurized hot water extraction was performed using the assembly shown in Figure 1a and consists of the following parts: (1) a Shimadzu (Tokyo, Japan) LC10AD pump with digital flow-rate and pressure readouts used to propel the extractant through the system; (2) an extraction chamber consisting of a stainless steel cylinder (100 × 10 mm i.d., 8 mL internal volume) (Análisis Vínicos, C. Real, Spain) closed with screws at either end, which permits the circulation of the leaching fluid through it; both screw caps contain stainless steel filter plates (2 μm in thickness and 0.25 in. i.d.) to ensure that the sample remains in the extraction chamber; (3) a gas chromatograph oven (HP 5720A, Hewlett-Packard, Avondale, PA) used as heating source into which the chamber is placed. (4) A cooler system (consisting of a loop made from a 1-m length of stainless steel tubing and cooled with water) used to cool the fluid from the oven temperature to ~25 °C; (5) an on/off pressure needle valve placed after the cooler to maintain the pressure during the static extraction; and (6) a valve located between the high-pressure pump and the oven, which allowed flushing of the system with N_2 after extraction.

The dynamic pressurized hot water extraction was performed using the previous assembly with the following modifications as shown in Figure 1b: (1) A stainless steel preheater located in the oven prior to the extraction cell was used to ensure that the solvent was at the required temperature when coming into contact with the sample. (2) An outlet valve located outside the oven was utilized to produce the overpressure required at the beginning of the extraction. (3) A restrictor (Análisis Vínicos, C. Real, Spain) was coupled to the outlet of the cooler (substituting the on/off valve) and used to maintain the pressure constant in the system during the dynamic extraction. (4) Two selecting valves: SV1 was used to collect alternately fractions of the extract in two reservoirs at preset intervals of 5 min; and SV2 was used to divert the different fractions of the collected extract to the monitoring system. The selecting valve located after the high-pressure pump and the N_2 stream were deleted, because no purging of the system was required in the dynamic extraction mode. The static−dynamic mode was performed using the same assembly described for the dynamic mode but included an inlet valve (see INV in Figure 1b) for developing the static extraction step.

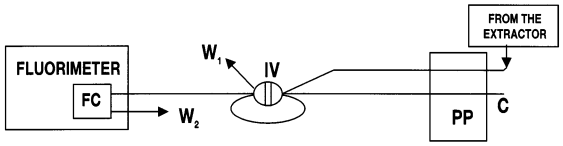

Figure 2. Schematic diagram of the on-line fluorescence monitoring setup: C, carrier; PP, peristaltic pump; IV, injection valve; FC, flow cell; and W, waste.

Dynamic on-line monitoring was performed by a flow injection (FI) manifold (Figure 2) constructed with a low-pressure peristaltic pump (Gilson, Worthington, OH), a Rheodyne 5041 low-pressure injection valve (Rheodyne, Cotati, CA) and PTFE tubing of 0.5 mm i.d. (Scharlau, Barcelona, Spain) connected to a Kontron model SFM 25 fluorometer (Kontron, Zurich, Swizerland) equipped with an 18-μL flow cell from Hellma (Jamaica, NY).

A Vac elut sps 24 (Varian) vacuum station incorporated to an Eye14 A-3S evaporator (Tokyo, Japan) and 500 mg C_{18} sorption cartridges from Análisis Vínicos were used for cleanup of the extracts when this step was necessary.

The individual chromatographic separation of the analytes was performed by an HP1100 liquid chromatograph (Hewlett-Packard, Avondale, PA) consisting of a G1311A high-pressure quaternary pump, a G1322A vacuum degasser, a Rheodyne 7725 high-pressure manual injector valve (20 μL injection loop), and a Hitachi, model F-1050 chromatographic fluorescence detector (Hitachi, Tokyo, Japan), equipped with a 12-μL flow cell and a D-2500 integrator (Hitachi). An Ultrabase C_{18} (250 × 4.6 mm; 5-μm particle size from Scharlau) was used as the analytical column.

Reagents. Ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA). Water modified with sodium dodecyl sulfate (SDS) (Merck, Darmstadt, Germany) as a micelle former was also used as extractant.

The PAHs [pyrene (Pyr), benzo[*a*]anthracene (b[*a*]ant), benzo[*ghi*]perylene (b[*ghi*]per), benzo[*a*]pyrene (b[*a*]pyr), and benzo[*k*]fluoranthene (b[*k*]flu)] were obtained from Aldrich (Milwaukee, WI). These compounds were used for preparing the stock standard solutions in HPLC grade acetonitrile (Merck). An N_2 stream (Carburos Metálicos, Barcelona, Spain) was used in the static mode for flushing the extract from the extraction chamber.

Ethyl ether (Panreac, Barcelona, Spain) was used for sample preparation.

Sample Preparation. Four types of matrixes, namely sandy soil (0.72% organic matter), sediment (1.24% organic matter), trout, and sardine, were selected for the study. The optimization of the extraction process was carried out using the sandy soil, because it did not provide fluorescence signals due to matrix components. A total of 400 g of air-dried sandy soil was sieved to a size smaller than 1 mm. Samples spiked with PAHs were prepared by adding 400 mL of ethyl ether (Panreac, Barcelona, Spain), containing the necessary volume of stock standard solution of the PAHs, to the soil in order to obtain a total concentration of 2.5 μg g^{-1} (0.5 μg g^{-1} of each PAH on a dry soil basis) within the expected concentration range of PAHs in natural samples. Then the slurry was shaken for 72 h and, after evaporation of the solvent, the soil was completely dried under a N_2 stream and then stored at −20 °C for 3 months until use in order to allow sorption equilibrium to be established. Sediment samples were prepared and spiked in the same way as sandy soil.

Fish samples were purchased in at local market in Córdoba, Spain, and analyzed in the raw state. Sampling was performed according to the protocol established by legislation.[11] The samples were washed with water, cut into pieces, and comminuted whole. Four grams of each sample was ground and spiked with PAHs by adding 20 μL of stock standard solution (100 μg g^{-1}), resulting in a final PAH concentration in the fish of 25 μg g^{-1} (0.5 μg g^{-1} of each PAH). Then the samples were refrigerated for 2 h at 4 °C before extraction in order to simulate the normal environmental contact between the fish and PAHs. This time was chosen as a result of a preliminary study that showed an increase of analyte retention in the spiked sample when the storage time (under refrigeration conditions) increased from 0 to 2 h, thus demonstrating the influence of this variable. Storage times between 2 and 24 h provided similar results, so 2 h was used in all the experiments. Neither of the samples had detectable levels of the target analytes before spiking.

Procedures. *Static Pressurized Hot Water Extraction.* Four grams of sample was weighed and placed into the extraction cell in all experiments. After assembling the extraction cell in the oven, the unit was filled with extractant (2.5 10^{-2} M SDS aqueous solution) pumped at a flow rate of 1 mL min^{-1} [the selecting valve (SV) and the pressure valve (PV) remained in the required position]. After pressurization of the system, the oven was brought up to the working temperature (200 °C), and the extraction consisting of a number of 15-min static cycles was performed. The number of cycles depended on the extraction kinetics of the targeted compounds from the different matrixes. After each cycle, the pressure valve was opened, and fresh solvent (60% of the empty extraction cell volume) displaced the extract from the cell, which was then collected in the extract reservoir after being cooled to ∼25 °C. Then the pressure valve was closed, and a new extraction cycle was performed. After the last cycle, both the pressure and the selection valves were opened, and a nitrogen stream purged the system for collection of the final cycle extract.

Dynamic Pressurized Hot Water Extraction. This extraction mode involved the following steps: (1) the same amount of sample as the static mode (4 g) was placed in the extraction cell; (2) the unit was connected to the system and filled with 2.5 × 10^{-2} M SDS aqueous solution by closing the outlet valve in order to pressurize the system; (3) the oven was brought up to the working temperature (225 °C), and the outlet valve was then opened; (4) dynamic extraction was then performed by pumping the extractant through the oven at 1 mL min^{-1} using a restrictor to maintain constant pressure on the system (the extraction time depended on the sample matrix); (5) for kinetics experiments, the extract (cooled in the refrigerant at ∼25 °C) was collected at preset intervals of 5 min in two reservoirs, which were alternately exchanged.

Static−Dynamic Pressurized Hot Water Extraction. For this extraction mode, 4 g of soil was placed in the extraction cell, which was located in the oven. Then the system was filled with 2.5 × 10^{-2} M SDS aqueous solution by opening the inlet valve and closing the outlet valve (in this way, the system was pressurized), and the oven was brought up to the working temperature (225 °C). The static extraction step was developed for a preset time by closing the inlet valve. After completion of the static extraction, both the inlet and outlet valves were opened, and the high-pressure pump was switched on, driving the extractant through the system at a 1 mL min^{-1} flow rate during the variable dynamic extraction period, which depended on the sample matrix.

On-Line Fluorimetric Monitoring of the Extract. Static mode: after collection of the extract from a cycle, the peristaltic pump was activated, and the extract was aspirated to the FI manifold.

Dynamic and static−dynamic mode: After collection of the first fraction of the extract, the peristaltic pump was activated, and the fraction from the extract reservoir ER1 was aspirated to the FI manifold. Simultaneously, the position of the selecting valve SV1 was changed, and the second fraction of the extract was collected in the extract reservoir ER2. After monitoring and collection of the first and second fractions, (ER1 and ER2, respectively), the positions of both selecting valves (SV1 and SV 2) were changed, thus allowing collection of the third fraction of the extract in the reservoir ER1 and monitoring of the second fraction. The procedure was repeated until total removal of the target compounds was observed.

In all cases, the flow rate of the FI pump was set at 4 mL min^{-1}. The fluorescence of the extract was monitored in triplicate by making 500 μL injections of the extract into the carrier. The detector wavelengths were set at 300 and 400 nm for excitation and emission, respectively. The extraction was considered complete when the signal from the extract gave a value lower than 3.82 (fluorescence arbitrary unit), corresponding to the detection limit of the method.

Cleanup Step. When the individual quantification of the analytes was required, a volume of ∼5 mL of either the extract collected after each cycle (in the static mode) or the different fractions collected every 5 min (in the dynamic or static−dynamic modes) was passed through a C_{18} bonded column where the micellar phase was retained. Five mL of acetonitrile was then passed through the column for eluting the PAHs. A portion of the eluate (20 μL) was injected into the liquid chromatograph.

Chromatographic Determination. The HPLC separation of the PAHs was performed using a gradient elution program in which an 85:1.8:13.2 acetonitrile/methanol/water mixture was used as

(11) Community Directive 79/700/EEC; *Off. J. Eur. Commun.*, European Community: Brussels, 1979.

Table 1. Optimization of the Methods

step	variable	tested range	optimum value
static leaching	temp, °C	100−250	200
	SDS concn, M	$2.5\ 10^{-2}$−$7.7\ 10^{-2}$	$2.5 10^{-2}$
	extraction time, min	5−15	15
dynamic leaching	temp, °C	100−300	225
	SDS conn M	$2.5\ 10^{-2}$−$7.7\ 10^{-2}$	$2.5 10^{-2}$
	flow rate, mL min^{-1}	1−3	1
static−dynamic leaching	extraction time, min	0−10	5
chromatographic separation	injection vol, μL		20
	Q mobile phase, mL min^{-1}	0.6−1.5	0.8
	elution; see text		
fluorometric detection	λ excitation−emission, nm		300−400

the initial mobile phase at a flow rate of 0.8 mL min^{-1}. The gradient program was as follows: (1) the initial mobile phase was held for 5 min, and then two linear gradients were established in order to reach, first, a 90:1.8:8.2 composition in 15 min and, second, a final 98.2:1.8:0 composition in 10 min more. Finally, 10 min was necessary for reestablishing the initial conditions. The injection volume of the sample was 20 μL. Fluorimetric detection was performed at 300 and 400 nm for the excitation and emission wavelengths, respectively. Quantification of the analytes was carried out by running five calibration curves (one for each analyte) using standard solutions having a concentration between 0.2 and 1.2 μg mL^{-1}.

RESULTS AND DISCUSSION

The order used in the optimization of the steps was as follows: first, the chromatographic separation and fluorometric detection of the target analytes were optimized for checking previous steps; second, the cleanup step, followed by a preliminary study of the extraction step in order to make possible the optimization of the monitoring step; and finally, the three extraction modes were studied in depth. The ranges over which the variables were studied and the optimum values are listed in Table 1.

Optimization of the Chromatographic Separation. The experimental variables were optimized in order to obtain appropriate separation of the analytes with respect to composition of the mobile phase, the flow rate, and the injection volume (see Table 1). An injection volume of 20 μL was selected in order to avoid saturation of the fluorimetric signal, which occurred with higher volumes.

Optimization of the Cleanup Step. A solid-phase extraction step was required because of the necessity for removing the micelles from the extracts, thus obtaining an appropriate phase that can be introduced into the chromatograph. In addition to removal of the surfactant, preconcentration of the target analytes is also achieved in this way. The study concerning this step consisted of the selection of both the best sorbent material in order to retain and separate the analytes from the extract and the solution composition required for their proper elution. Two types of sorbent were tested: C_{18} and C_{si}, and the best results were obtained with the former phase. After selection of the sorbent, different eluents were investigated in order to achieve elution of the PAHs from the solid phase in a minimum volume of elution solvent. Five milliliters of acetonitrile was found necessary for quantitative elution of the analytes.

Optimization of the Extract Monitoring. A flow rate of 4 mL min^{-1} was established for aspiration of the extract by the peristaltic pump. At higher flow rates, the sampling frequency was not enough for performing three replicates; lower flow rates were not assayed in order to not enlarge the monitoring step. This step started as soon as the extract from a static cycle (in the static mode) or a fraction of the extract (in the dynamic and static−dynamic modes) was loaded in the extract reservoir. A 500-μL loop of the injection valve was selected as optimum, because higher injection volumes on the order of 1 mL caused saturation of the fluorometric detector signal when the extract was concentrated with analytes; meanwhile, lower volumes on the order of 250 μL provided signals that were too small.

Features of the Extract Monitoring Method. A calibration graph was run with equal concentration mixtures of the five PAHs. Solutions of the mixture in acetonitrile were prepared in the range 0.1−2 μg mL^{-1}, that is, ranging between 0.02 and 0.4 μg mL^{-1} for each individual PAH. Each solution was injected in triplicate. The calibration graph was linear within the range studied and was used for calculation of the extraction recovery. When quantification of the target analytes is required, the use of chromatography is mandatory.

The relative detection limit was calculated by the equation $x_L = ks_{bl}/S$ where k is a constant; S, the sensitivity of the analytical method corresponding to the slope of the calibration line; and s_{bl}, the standard deviation of the blank responses obtained from the analyses of 10 sandy soil blanks. The value obtained was $x_{L(k=3)} = 0.022$ μg mL^{-1}.

Optimization of the Pressurized Hot Solvent Extraction Modes. Preliminary experiments were carried out using pure water and water modified with a surfactant (sodium dodecyl sulfate was used as recommended in the literature[10−12]) in order to obtain a micellar medium in which the PAHs could be more easily extracted. Better recoveries were obtained when water modified with SDS was used.

Static Pressurized Hot Solvent Extraction. The variables affecting this extraction mode (namely, temperature, SDS concentration, and extraction time) were studied in order to obtain the best recoveries consistent with performing a single extraction cycle. A full two-level factorial design involving an overall of $2^3 = 8$ experiments in addition to three center points was selected as the screening design.

The study showed that the temperature and the extraction time are the key variables, and the SDS concentration appears to be

Table 2. HPLC−Fluorometry Results from the Spiked Soil Extracts

		Pyr		b[*a*]ant		b[*k*]flu		b[*a*]pyr		b[*ghi*]per	
sample	extraction mode	recovery %	RSD %	recovery %	RSD %	recovery %	RSD %	recovery %	RSD %	recovery %	RSD %
	static	99.9	3.5	100	2.8	98	2.9	98.6	4.7	100.1	5.1
sandy soil	dynamic	100.5	2.6	103	4.1	98.6	1.9	97.7	3.1	102.3	2.3
	static−dynamic	99.8	1.3	99.6	2.3	101.2	3.7	99	3.7	99.8	3.4
	static	100.2	4.3	102.1	6.2	99.2	2.2	100.1	4.5	94.4	3.9
river sediments	dynamic	102.5	2.6	96.6	2.4	100.1	1.3	98.8	2.9	98.3	2.8
	static−dynamic	99.7	5.4	100.3	5.2	98.2	3.2	101.5	4.8	101.3	6.1
	static	102.3	5.8	99.8	2.4	100.4	3.9	104.4	3.2	100.3	2.8
trout	dynamic	99.4	4.3	99.7	1.9	99.4	1.1	99.5	1.7	97.6	6.3
	static−dynamic	100.2	2.1	101.3	3.6	103.5	0.9	99.7	2.3	104.5	1.7
	static	100.1	0.9	98.1	5.4	103	6.8	100	4.8	99.7	1.8
sardine	dynamic	101.3	1.9	99.8	2.1	104.6	2.4	98.6	3.3	99.2	0.9
	static−dynamic	97.6	2.8	101.2	2.4	99.3	4.5	101.7	5.2	100.7	3.2

not significant when working above the critical micellar concentration; thus, the lowest SDS concentration tested, 2.5×10^{-2} M, was selected for subsequent experiments, since higher concentrations provided a gell-like extract that was difficult to handle. Concerning both temperature and extraction time, higher values should be tested, because their effects on the recovery were positive. However, when temperatures higher than 200 °C were used, the extracts obtained had the appearance of a dense gel and caused blockage of the system. Therefore, 200 °C was selected for further experiments. Extraction times from 15 to 30 min were tested in 5-min increments. The results showed that the extraction efficiency decreased with the increase of the extraction time, probably as a result of micelle degradation. Thus, 15 min was selected for subsequent experiments.

Finally, the overall optimized procedure was applied to different samples (namely, sediment, trout, and sardine) in order to check the influence of the sample matrix on the number of cycles needed for quantitative recovery. The kinetics of the extraction for each analyte strongly depended on the type of matrix; nevertheless, four cycles was necessary for total removal of the analytes in these matrixes, but only three cycles was necessary for the sandy soil.

Dynamic Pressurized Hot Solvent Extraction. The variables affecting this extraction mode (namely, temperature, SDS concentration, and flow-rate) were also studied. A full two-level factorial design involving an overall of $2^3 = 8$ experiments in addition to three centered points was selected as the screening design.

The study showed that the temperature is the key variable. The SDS concentration and the flow-rate were not significant; thus, the lowest values tested for each one, 2.5×10^{-2} M and 1 mL min^{-1}, respectively, were selected for further experiments. Concerning the temperature, values higher than 200 °C should be tested, because the effect of this variable on the recovery was positive. Temperatures from 200 to 300 °C were tested at 25 °C intervals. The results showed that the extraction efficiency decreased with an increase in extraction temperature over 225 °C, probably as a result of micelle degradation. Thus, 225 °C was considered as the optimum. Problems of gell-like extract formation were not found in the range of temperatures studied.

The optimized procedure was also applied to different matrixes in order to check the extraction time needed for quantitative recovery. The kinetics of the extraction strongly depended on the sample matrix. Twenty-five minutes was necessary for total removal of the analytes from the sandy soil; meanwhile, 45 min was necessary for river sediments, and 35 min for both trout and sardine samples.

Table 3. HPLC−Fluorometry Results from the CRM Extracts (mg Kg^{-1})

	Pyr	b[*a*]ant	b[*k*]flu	b[*a*]pyr
certified value	173 ± 11	22.5 ± 1.8	6.2 ± 0.6	8.6 ± 0.5
static	172.4 ± 10	23.1 ± 1.2	6.5 ± 0.2	8.5 ± 0.4
dynamic	171.2 ± 8.9	22.9 ± 0.9	5.9 ± 0.2	8.6 ± 0.4
static−dynamic	175.6 ± 7.2	21.1 ± 0.3	6.6 ± 0.3	8.2 ± 0.4

Static−Dynamic Pressurized Hot Solvent Extraction. To shorten the total extraction time needed for quantitative recovery of the target compounds, a combination of both the static and the dynamic extraction modes was performed. Static extraction times ranging from 0 to 10 min combined with dynamic extraction times between 10 and 35 min were tested in order to achieve a total extraction time shorter than those used in the two previous studied modes.

In most of the matrixes tested, the influence of the performance of static extraction on the extraction time needed for quantitative removal of the target analytes depended on the sample matrix. Thus, for sandy soil, the extraction time was shortened from 45 and 25 min (for the static and dynamic extraction modes, respectively) to 15 min (5 min static plus 10 min dynamic), for river sediments from 60 and 45 min (for the static and dynamic extraction modes, respectively) to 20 min (5 min static plus 15 min dynamic). However, a static step prior to the dynamic one did not shorten the time needed for quantitative removal of the PAHs from both trout and sardine.

HPLC-fluorometric quantification of extracts from the spiked samples, obtained under the optimal extraction conditions, was performed in order to check the precision of the proposed methods. The results of this study, as shown in Table 2, allow one to conclude that the method provides satisfactory results in terms of both recovery and precision.

Application of the Methods to a Certified Reference Material. The use of a CRM had a double objective: first, to demonstrate the efficiency of the method using micelle formation for the extraction of PAHs in natural samples, and second, to show

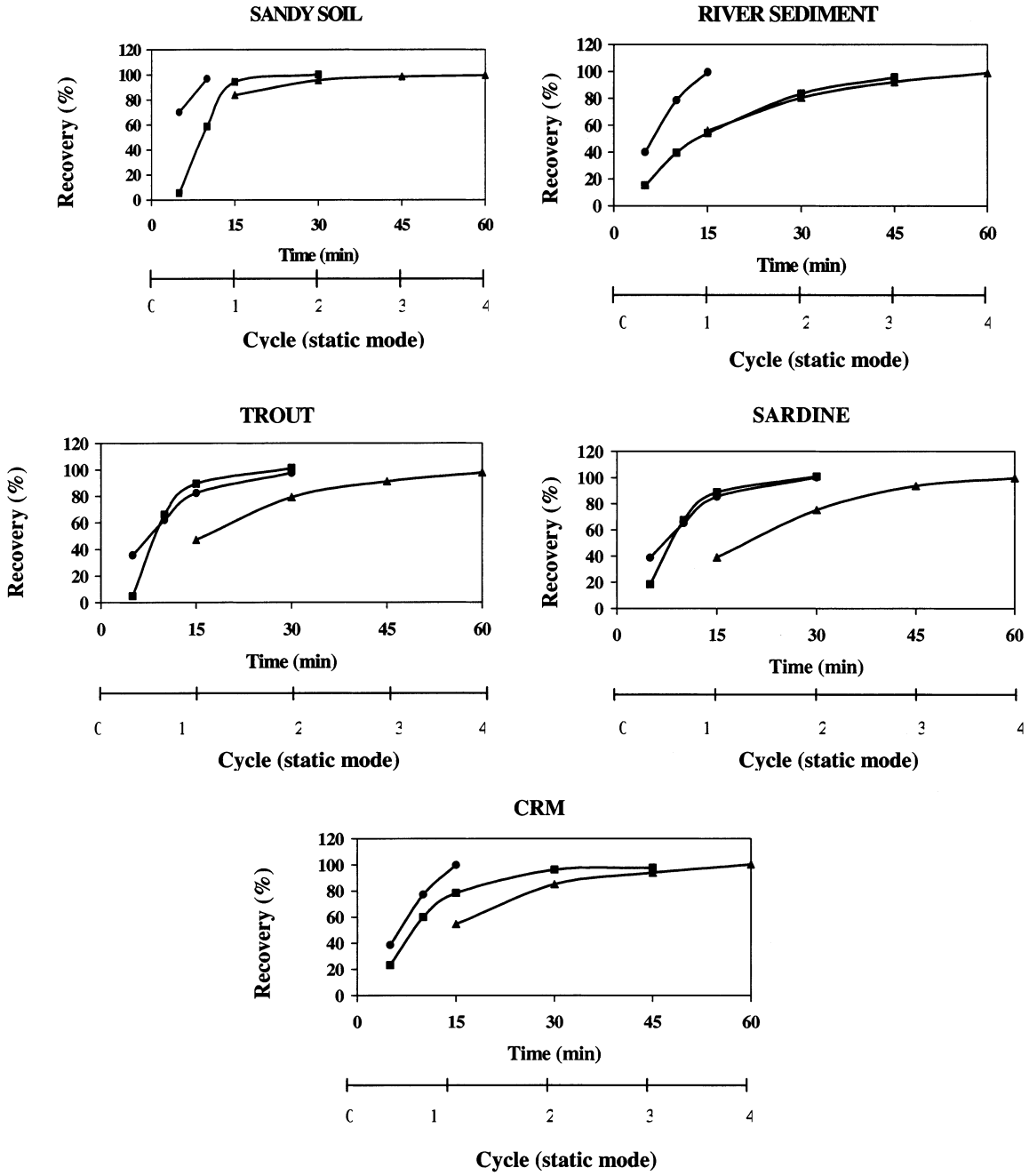

Figure 3. Overall recovery of the analytes from the different matrixes by the different operational modes: ▲, static mode; ■, dynamic mode; ●, static−dynamic mode.

the independence of the system from the sample matrix effect as a result of its ability to provide a real-time monitoring of the extraction. The certified reference material used was CRM 524 (Community Bureau of Reference, BCR, Brussels, Belgium), an industrial soil in which eight PAHs are the certified compounds.

The optimized conditions for each extraction mode were applied to 0.1 g of the CRM. The on-line monitored fluorescence was not quantifiable after 4 cycles (60 min) for the static mode, 45 min for the dynamic mode, and 20 min for the static−dynamic mode (5 min static plus 15 min dynamic), thus showing that the combination of the static and dynamic extraction modes was the best alternative. The individual PAHs were fluorometrically quantified after HPLC separation, and extraction efficiencies close to 100% for the three operational modes under the optimized conditions were obtained in all cases. The within-laboratory reproducibility of the proposed methods was studied by seven replicates of the CRM on different days. Depending on the analyte, relative standard deviations between 4.3 and 5.8, 3.9 and 5.2, and 4.1 and 5.9% for the static, dynamic, and static−dynamic modes, respectively, were obtained. As can be seen by the results shown in Table 3, the CRM extraction can be considered quantitative.

COMPARISON OF THE STATIC, DYNAMIC, AND STATIC−DYNAMIC OPERATIONAL MODES

The three extraction modes were compared in terms of experimental setup, dilution effect, extraction time and precision.

The experimental setup and procedure of the three extraction modes have many common elements. The most salient differences

are as follows: in the static mode, the system must be purged with a gas stream after the last extraction cycle. In the dynamic and static−dynamic modes, the extraction cell is preceded by a preheater used to ensure that the extractant comes into contact with the sample at the oven temperature, and a restrictor is placed at the end of the system to maintain the pressure needed in the system during dynamic extraction. The only difference between dynamic and static−dynamic modes is the inlet valve used to carry out the static step in the static−dynamic mode.

A higher dilution of the extract is produced in the extractions involving a dynamic step as higher extractant volumes are used. However, the static mode has the disadvantage that the partition equilibrium of the analytes between the extract and the sample matrix is established in each cycle, since during each cycle, there is not fresh solvent flowing through the sample. Thus, the extraction efficiency of each cycle strongly depends on the partition equilibrium constant. In the dynamic and static−dynamic modes, the equilibrium is displaced to completeness as fresh solvent is continuously pumped through the sample.

As commented previously, the extraction time needed for total removal of the PAHs using the three operational modes strongly depends on the sample matrix; however, the dynamic mode was faster than the static mode for all of the matrixes checked. In the static−dynamic mode, total removal of the analytes was achieved in a time shorter than that required by the dynamic mode in the case of soil samples, but similar times were necessary for trout and sardine samples. A comparison of the extraction kinetics for the three extraction modes applied to the different matrixes is shown in Figure 3.

The within-laboratory reproducibility study shows that the three operational modes are similar, thus demonstrating the suitability of them for the analysis of PAHs in environmental solid samples.

CONCLUSIONS

A comparison of the three pressurized hot water extraction operational modes (namely, static, dynamic, and static−dynamic) with on-line real-time fluorescence monitoring for the extraction of PAHs from solid samples has been performed. Efficiencies close to 100% have been obtained for the three modes, which differ in the extraction time required for total removal of the target compounds. The time needed for the dynamic mode was shorter than that for the static mode. However, the establishment of a static extraction step prior to dynamic extraction was the key to shorten the time required for complete extraction.

The results obtained demonstrated that fluorometric monitoring of pressurized hot solvent extraction constitutes an approach as efficient as the conventional Soxhlet method for the extraction of PAHs from solid samples but with the following positive features: (a) the drastic reduction of the extraction time, because the extraction kinetics can be monitored and, thus, the end of the leaching step determined independently of the sample matrix; (b) the use of modified water as leaching agent avoids the use of organic solvents, thus reducing costs and providing an environmentally friendly method; (c) the approach can work as a screening system; and (d) the overall content of the analytes (global fluorescence) can be determined.

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