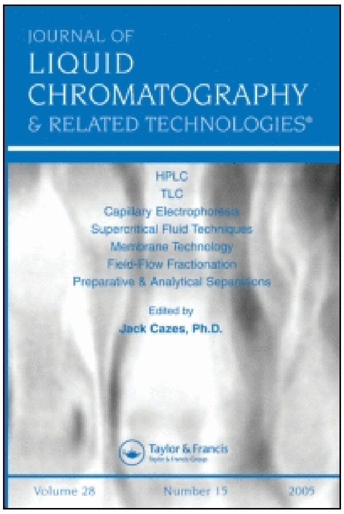

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BENEFITS OF A BINARY MODIFIER WITH BALANCED POLARITY FOR AN EFFICIENT SUPERCRITICAL FLUID EXTRACTION OF PAHs FROM SOLID SAMPLES, FOLLOWED BY HPLC

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed environmental contaminants. Their presence in soils indicates a possible contamination of ground water used for human purposes. Only with a rapid and efficient method could they be analysed in a routine setting.

Although there are a great number of publications on this subject, there are many differences between their ideal SFE conditions and related extraction recoveries.

With improvements on the extraction capacity of the supercritical fluid such as higher pressure, higher temperature and a

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stronger modifier effect, the developed method intends to be more robust and applicable to a larger range of matrices.

The optimal conditions consist on a 15 minutes extraction at 45 MPa pressure and 95°C. The supercritical fluid is a ternary mixture of CO2 modified with methanol/dichloromethane 5:1. The separation and quantitation was performed by HPLC coupled with a diode array detector in series with a fluorescence detector, which combines high sensitivity with the possibility to confirm the components' identity in a complex chromatogram. Three of the six studied PAHs show recoveries above 90% and the others, those of higher molecular weight, around 70 – 90%.

This modified SFE procedure was applied to three environmental samples and fulfils the requirements of rapidity, high extraction efficiency, and simple performance.

INTRODUCTION

Polycyclic aromatic hydrocarbons mainly result from incomplete combustion of organic materials, in particular fossil fuels, and in smaller amounts, naturally by forest fires and possibly microbiological synthesis. Other specific sources are railroad bed soils and street dust. As a result, they are widespread in soil, air, and water.(1,2)

Analysis of micropollutants is one of the most important applications of supercritical fluid extraction, applied here to extract PAHs from soils, according to 80/778/EEC Directive. Their natural degradation is difficult and dependent on their molecular weight as well as on environmental conditions. Therefore, they are persistent compounds in the environment due to their chemical stability and biodegradation resistance, showing a tendency to accumulate in different environmental compartments.(2,4)

Regardless of several studies published about supercritical fluid extraction, its application to a specific sample, and a given analyte always had to be optimised to get its better profits.(8) Supercritical fluids have physical properties, such as low viscosity, high solute diffusive power, and density-linked solvent strength that make extraction selectivity and automation feasible, thus, offering a promising alternative to traditional extraction methods.(9) Using the conventional extraction methods, the analyst is often confronted with complex extracts requiring clean up and fractionation procedures to isolate the target analyte from all co-extracted compounds.

Reduced extraction time (fast sample preparation), mild extraction temperatures, high selectivity, reduced solvent consumption and waste, and, sometimes, less concentration and fewer sample clean up procedures are considered important advantages of supercritical fluids techniques.(7,10,11)

Carbon dioxide is the most commonly used supercritical fluid, which has physical and chemical properties that allow it to solvate numerous compounds ranging in polarity from nonpolar to moderately polar. Regardless, one often cannot extract environmentally persistent pollutants without an organic modifier.(12)

At 40°C and 200 bar the density of CO_2 and its polarity are of the order of that of non-polar organic liquids, however, the viscosity being much lower and with diffusion coefficients similar to gases.(13) All these advantages make it an ideal candidate solvent for use in clean analytical methods.

PAHs are fairly soluble in supercritical CO_2, although the nature of the matrix has a major role on their efficient recovery from environmental solids. Often organic modifiers are required in conjunction with CO_2 to effectively remove the PAHs, which can vary substantially in their molecular weights.(15,16) Owing to its effectiveness, the most common modifier employed in SFE has been methanol because of its high polarity, its ability to access active sites in the matrix and to build hydrogen bonds with them, though frequently that is chosen in an empirical way.(11,12,17) Langenfeld et al.(12) studying the role of modifiers in SFE, concluded that dichloromethane was most efficient to extract high molecular weight PAHs.

The aim of this work was to test the ability of a ternary supercritical fluid to efficiently extract PAHs from different environmental samples. The improvement and automation of sample preparation can lead to faster analysis time and to a greater precision of results.(5)

EXPERIMENTAL

Chemicals and Reagents

Chemicals used were acetonitrile, gradient-grade, from Panreac Quimica SA (Barcelona, Spain) and dichloromethane GR, methanol lichrosolv, and Water lichrosolv, all purchased from Merck (Darmstadt, Germany).

The pure compounds fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene were purchased from Supelco (Bellefont, PA, USA).

Sample Preparation

Sample sieving has to be standardised because this operation reduces the weight of the sample, resulting in the more contaminated part of the soil having a higher surface/weight ratio on small particles. A study using street dust reports the fraction with a particle size between 100-500 µm to be the one with major quantities of PAHs.(13) In the present work, the samples were sieved at 300 μm. All samples were also dried at 25°C during 48 hours, to assure low water content. Water usually hinders the extraction of apolar compounds by sheathing the surface of the matrix and acting as a barrier to CO_2 penetration.

The spiking process was as follows: an aliquot with 20 g of clean soil (soil A) was spiked with 500 μL from a mixture of the six PAHs in equal concentration, giving an overall amount of 4.5 mg/Kg (750 μg/Kg each). That small volume was first dissolved in 8 mL of acetonitrile to accomplish a good homogeneity of the soil. This soil was to be extracted the day after, which means ageing spiked samples for 17 hours.

Spiking techniques using an organic solvent to deposit the analytes onto the sample matrix, are not comparable with the deposition conditions experienced by the native analytes, although they are useful to test the solubility of the analytes.(20) As it is experimentally impossible to reproduce the environmental conditions that occur during deposition of pollutants in real-world samples, this seems to be one of the best approaches in developing the SFE conditions.(20)

The presence of PAHs was investigated in four samples with humidity and organic content as shown in Table 1: soil A was used for optimisation purposes, soil B had similar properties but contain native analytes, soil C was collected from a burned area due to forest fires, and burned bark of pine-tree was also analysed. For humidity and organic content determinations, a thermogravimetric technique was applied.(20)

Supercritical Fluid Extraction

All extractions were performed using an ISCO SFE System (ISCO Inc. Europe, Hengoed, UK) consisting of a Supercritical Fluid Extractor SFX 220 with dual-chamber, a Controller SFX 200, two Syringe pumps MODEL 260 D, and a Restrictor Temperature Controller associated with two Coaxially Heated Capillary Restrictors calibrated to a flow-rate of 1 mL/min. The extraction cham-

Table 1. Humidity and Organic Content of Soil A, Soil B, Soil C and Burned Bark After Sample Treatment

	% Humidity (wt/wt)	% Organic Content (wt/wt)
Soil A	2.0	10.5
Soil B	1.7	11.3
Soil C	2.6	15.9
Burned bark	8.4	81.5

ber was a stainless steel cartridge with 2.5 mL capacity, able to accommodate about 2.3 g of sample.

SFE was performed using industrial-grade carbon dioxide purchased from ArLiquido (Algés, Portugal), as was the nitrogen, Alphagaz.

In the method development, the following variables were considered: restrictor temperature, trapping solvent and its volume, pressure, temperature, % and composition of modifier, extraction time, and volume of supercritical fluid.

Sonication

A sample portion of 2.3 g was placed in a 40 mL glass bottle and to it was added 15 mL of dichloromethane. After 4 hours in an ultrasonic bath the solvent was filtered to an adequate glass vial, and another 15 mL of dichloromethane were added to the residue for sonication during 4 hours. At the end the solvent was, once again, filtered and the two fractions were combined.

Soxhlet Extraction

Soil B was Soxhlet extracted using the following procedure: three aliquots with 15 g each were placed in extraction thimbles. These aliquots were extracted during 18 hours with 100 mL of dichloromethane/n-hexane 3:1 at 60°C. This procedure was based on an internal, validated but not published, method.

HPLC Analysis

The extracts from the three extraction methods were evaporated with nitrogen to dryness. The evaporation process was studied and no differences were found between the rotation evaporator and nitrogen stream. The extracts were recovered afterwards with an appropriate volume of acetonitrile, and then 20 µL were analysed by HPLC using a C_{18} Reverse Phase Vydac column 501TP 54 (250 mm × 4.6 mm i.d., 5 µm) (Sigma-Aldrich, Hesperia, USA). A Rheodyne filter and a Supelguard LC 18 guard column (Supelco, Bellefont, PA, USA) were inserted before the analytical column.

The chromatographic determinations were carried out on a Waters high-performance liquid chromatography (HPLC) system including two HPLC pumps W. 515, a temperature control module connected to a HPLC oven from Waters, a photodiode array detector (PDA) W. 996, and a fluorescence detector (FD) W. 474. Data was collected by a personal computer using the chromatography manager Millennium 32. Quantitative measurements of PAHs performed with PDA were made using peak areas and a 6-point linear calibration curve from gravimetrically prepared standards, ranging from 0.05 to 2 mg/L. Trace levels of analytes, detected by fluorescence, were quantified using a 5-point linear calibration curve with standards ranging from 0.001 to 0.1 mg/L. The fluorescence detection time programme (time-min./λ excitation/λ emission/gain) was as follows: 0/288/462/100; 6/302/450/100; 9.3/308/428/10; 10.4/365/428/100; 13.0/300/460/100.

Figure 1 presents a FD chromatogram of a 0.01mg/L standard solution. Extraction blanks were obtained during the analytical procedure and no peaks of interest were found.

RESULTS AND DISCUSSION

The compounds of interest were recovered by decompression of the fluid into methanol held in a vial. Using methanol, the losses in volume during the extraction are smaller, compared with acetone, dichloromethane, or acetonitrile.

A 9-mL methanol volume was chosen as it gives a sufficient height on the collection vial to receive the depressurising stream with ideal interaction conditions with the gas, favouring the transference of analytes. At this step, the vial's shape can be important to give a good column height of solvent. The majority of losses that still exist with this method may be attributed to inefficient partitioning of the analytes from the depressurising extraction fluid, rather than to purging of trapped analytes from the collection solvent.(6) Thus, a high flow gas stream should be avoided.

Trapping of liquid solvents, compared to solid surfaces, offers the possibility of working with modifiers without major losses, although, further concentration steps are often needed to yield the volume needed to trap the analytes.

The restrictor outlet was held at 90°C protecting the capillary from blockage. During the rapid expansion of the supercritical fluid, cooling occurs, and heating the restrictor avoids plugging due to too rapid precipitation of analytes, resulting in the loss of solvation power.(11,23) This feature proved to be very useful when processing samples with high organic matter content. The temperature of the restrictor, to some extent, also influences the flow rate of the supercritical fluid.

After the collection method was established, the pressure was tested in the range of 30-50 MPa. The results can be seen in Figure 2. An elevation of the fluid pressure at a given temperature, results in an increase in the fluid density, thus, enhancing the capacity to solubilize PAHs, especially those of higher molecular weight. In the pressure range from 45 to 50 Mpa, a slight decrease of the recoveries was observed, possibly due to a decrease in the diffusion coefficients or trapping insufficiency at higher flow rates.

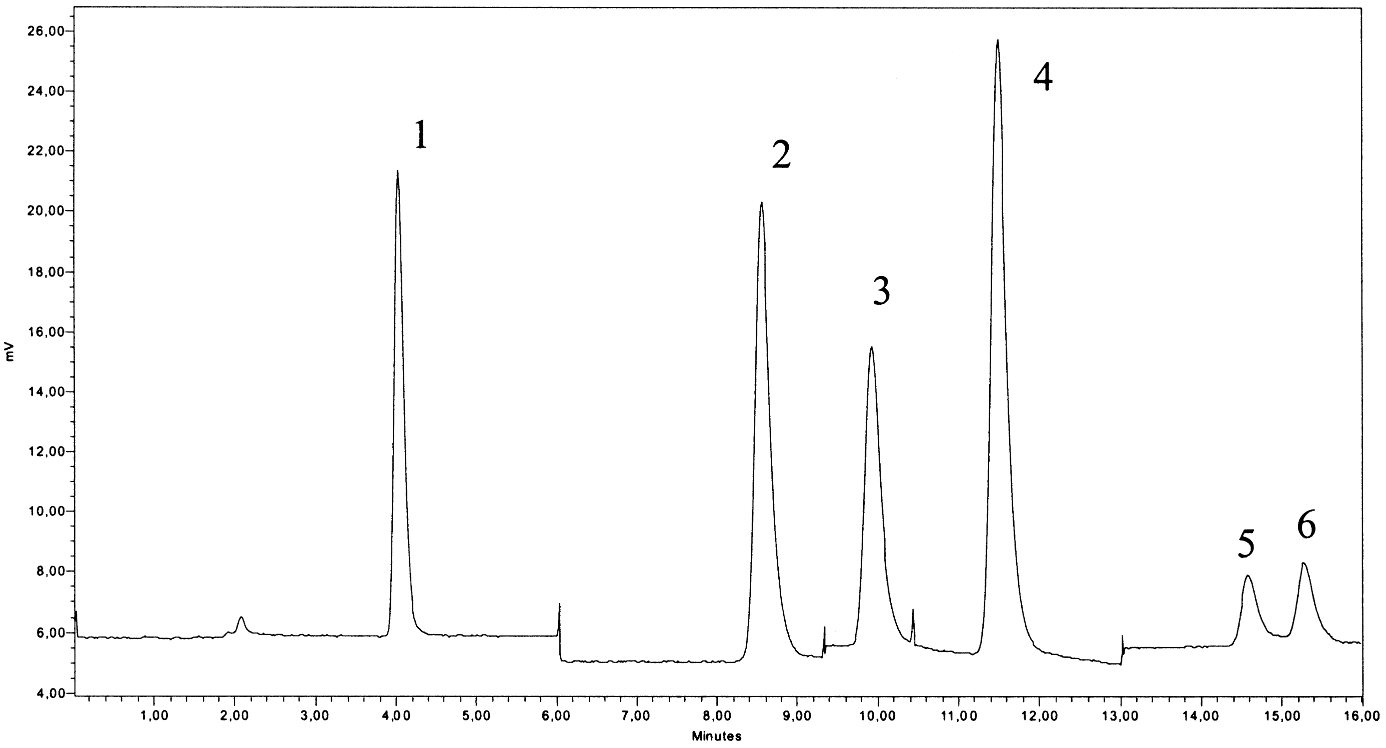

Figure 1. Representative fluorescence detection chromatogram of a 0.01 mg/L standard solution. Peaks and retention times: 1- fluoranthene-FLT (3.99 min.), 2- benzo[b]fluoranthene-BBFLT (8.48 min.), 3- benzo[k]fluoranthene-BKFLT (9.86 min.), 4- benzo[a]pyrene-BAPY (11.43 min.), 5- benzo[g,h,i]perylene-BGHIPER (14.52 min.) and 6- indeno[1,2,3-cd]pyrene-IP (15.21 min.).

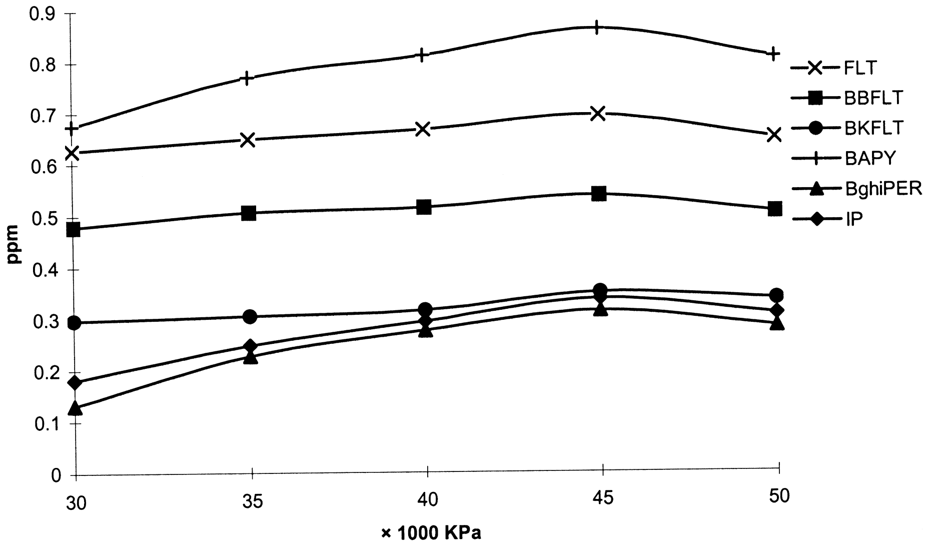

Figure 2. Influence of pressure in the extraction recovery of each compound maintaining the other variables as follows: 95% CO_2 + 5% methanol, 80°C, and 25 mL supercritical fluid (n=3).

Once the adequate pressure for analyte solubilization is achieved, temperature has been considered a more important variable than pressure, in order to release analytes having strong interactions with the sample matrix. Langenfeld et al.(24) suggested that raising the extraction temperature would be an alternative method to the use of modifiers. There are studies reporting good extraction recoveries of PAHs from an urban air particulate matter sample using only pure CO_2 at high temperatures such as 200°C, having results comparable to those of Soxhlet. As these temperatures are not attainable with the usual extractors, one can improve the recoveries using an organic modifier, but using only one pump to dispense the supercritical fluid would be a good alternative.

Using a modifier, we found that a temperature of 95°C in the extraction chamber is high enough to obtain good recoveries in a short time (see Table 3). Modifiers can improve extraction efficiencies in two ways: by favouring the displacement of the analytes from the matrix and/or by increasing the ability of the fluid to solubilize and carry the compounds of interest.(11,26,27) However, they also enhance the co-extraction of contaminants to an extent equivalent to Soxhlet,(7) therefore, very good optimisation is needed. As can be seen in Figure 4, the high molecular weight PAHs showed a significant dependence on the modifier concentration. While the curves of the first three PAHs with low molecular weight are very similar, those of higher molecular weight (BAPY, BGHIPER and

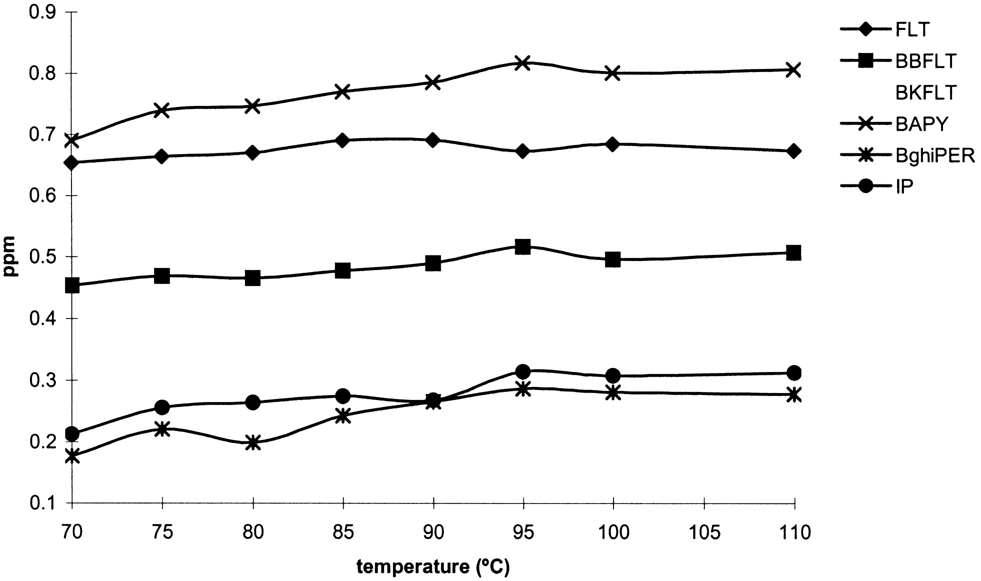

Figure 3. Influence of extraction temperature in the recovery of PAHs maintaining the other variables as follows: 95% CO2 + 5% methanol, 45 MPa, and 25 mL supercritical fluid (n=3). This parameter was tested in the range of 70-110°C with a 5°C step.

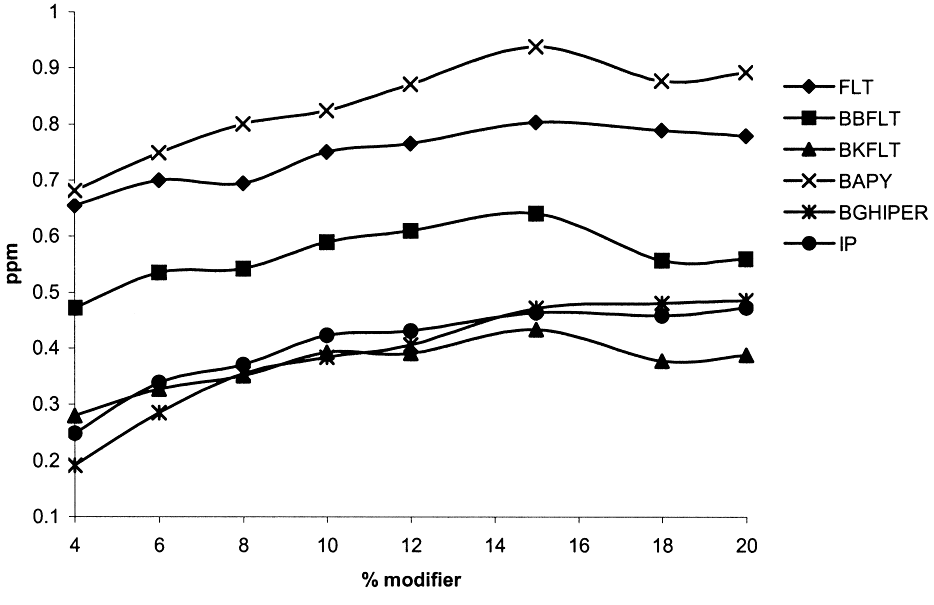

Figure 4. Influence of modifier percentage in the extraction recovery of each compound maintaining the other variables optimised at 95°C, 45 MPa and 25 mL supercritical fluid (n=3).

IP) show a good rise until 15% of modifier. Addition of methanol to carbon dioxide improves the recoveries of some PAHs, which cannot be efficiently extracted with pure CO_2.(2) Regardless, the larger compounds are still only partially extracted. By extending the extraction time the recovery of high molecular weight PAHs can be improved, but it can result in losses of the more volatile compounds.(9,16)

Experiences with dichloromethane as a static as well as a dynamic modifier, were also made but none showed better recoveries than the previous SFE conditions (see Table 2). Anyway, it was interesting to see that the low molecular weight PAHs were poorly worst recovered while, for the heavier ones, equal or better recoveries were observed. This can be explained by the similar polarity between dichloromethane (D 1.6) and those PAHs, while methanol (D 2.9) is capable of displacing the analytes and preferably carrying the ones with low molecular weight.

The advantages of each separate modifier were later combined in a binary modifier composed by MeOH/DCM 4:1. As shown in Table 2, the yields were improved for some compounds but decreased in the case of fluoranthene. In this sense, the DCM content in the modifier should be reduced.

The use of a ternary mixture composed of 85% CO_2 and 15% MeOH/DCM 5:1 and the other variables as described in Table 3 (conditions 3), gave the best results in this work. For the high molecular weight PAHs the modifier has to increase not only the strength on accessing the active sites but also the solubilizing power. Although, in a different way, Hollender et al.(8) refer to the use of ternary mixtures in extracting PAHs from lightly contaminated soils by using methanol added to strong acids or bases, like acetic acid or diethylamine.

Probably, in the same matrix active sites exit with different affinity to the analytes.(28) Thus, we may have different desorption rates which lead to a continuous release of analytes during the extraction time; the first extracted are those with weaker bonds to the matrix. The flow rate and the associated overall volume of supercritical fluid becomes increasingly important as the molecular weight increases, which might be expected, since the solubility of PAHs decreases dramatically with increasing molecular weight, and the ability of PAHs to adsorb to surfaces increases with molecular weight.(29)

Samples having strong contamination probably will have different extraction patterns from those with vestigial contaminants. In the first ones, we can predict that only a small part of the analytes are bonded, since they are the most adsorbed in a loose way, whereas, in the latter, most are firmly bonded to active sites in the matrix. An extraction of the first type will be almost complete within a few minutes, whereas, the latter will be lengthy. The time needed and the related supercritical fluid volume can vary greatly between these two situations. The recovery rate is, therefore, a function of both chemical natures of the solutes and of the matrix itself.

Table 2. Effect of Different Modifier/Extraction Mode Combinations on the SFE Efficiency

	% Recovery (RSD, %)				
Modifier	DCM 0,5 mLStatic 4 min. Dynamic 10 min (n=8)	DCM 15% Dynamic (n=8)	MeOH 15% Dynamic (n=8)	MeOH/DCM 4:1 15% Dynamic (n=15)	MeOH/DCM 5:1 15% Dynamic (n=20)
Fluoranthene	68.0 (17.9)	82.6 (12.2)	84.1 (8.7)[a]	81.7 (10.7)	91.8 (6.3)
Benzo[b]fluoranthene	61.2 (21.6)	84.4 (8.3)	86.5 (8.8)[a]	89.2 (5.6)	91.4 (6.2)
Benzo[k]fluoranthene	42.2 (23.1)	85.6 (9.7)	90.4 (8.7)[a]	90.4 (7.3)	93.2 (8.9)
Benzo[a]pyrene	81.8 (19.4)	77.8 (10.7)[a]	76.8 (6.3)	77.4 (6.3)	83.6 (7.3)
Benzo[g,h,i]perylene	43.6 (20.7)	73.5 (11.7)[a]	65.4 (13.1)	67.1 (11.3)	69.3 (13.8)
Indeno[1,2,3,-cd]pyrene	39.5 (15.0)	77.0 (16.4)[a]	68.7, (8.1)	75.2 (9.0)	76.5 (8.2)

The values marked ([a]) are the important ones when comparing the results obtained when using dichloromethane (DCM) and methanol (MeOH) at 15%.

Table 3. Percent Recovery Based on the Total Amount of Each PAH Spiked into the Samples, Using Different Extraction Conditions

	% Recovery (RSD, %)			
PAHs	Conditions 1 (n= 6) 95% CO$_2$ 100% CO$_2$ 25 mL SF 40 MPa 80 ºC	Conditions 2 (n=6) 85% CO2 5% MeOH 25 mL SF 40 MPa 80 ºC	Conditions 3 (n=20) 15% MeOH/DCM 5:1 15 min, 45 MPa 95 ºC	Conditions 3 vs Sonication (%) (n=4)
Fluoranthene	56.0 (11.5)	80.8 (7.4)	91.4 (6.2)	116.9
Benzo[k]fluoranthene	47.4 (12.5)	71.0 (9.1)	93.2 (8.9)	117.7
Benzo[a]pyrene	37.0 (10.2)	68.8 (8.9)	83.6 (7.3)	119.2
Benzo[g,h,i]perylene	43.2 (14.3)	34.2 (13.9)	69.3 (13.8)	106.6
Indeno[1,2,3-cd]pyrene	35.3 (12.2)	55.4 (8.7)	76.5 (8.2)	114.0

SF = supercritical fluid.
Comparison between SFE and ultrasonic extraction recoveries (%) based on 4 replicates (8 aliquots from the same spiked soil).

We found that a 15 minute extraction is a good compromise between completeness and required extraction time. It represents about 18 mL of supercritical fluid, measured at the pump, which is much more than five times the cell volume reported to be the mean volume to achieve quantitative extractions.(8) For samples with a difficult desorption step, the initial rate of extraction is often fast, followed by a very slow extraction rate for the remaining analytes. Therefore, the relative recoveries/extraction times could be decisive in routine settings where reproducibility could be more important than quantitative extraction.(28)

Additionally, it's a recent trend to give more importance, in a method, to the extraction of environmentally relevant pollutant molecules (i.e., less tightly bound and available to organisms and transport), rather than to the complete extraction of molecules, regardless of how tightly bound they are to the matrix.(30)

With the set of conditions used, the restrictor gives a flow-rate of ~1.3 mL/min of supercritical fluid through the extraction cell, measured at the pump. Some authors report great influence of the flow rate used for SFE on extraction efficiencies of some samples, while little or no effect can be found on extraction yields of other samples.(5,6,9,11,28)

Table 3 presents the results of important stages from the beginning to the end of the optimisation process.

Benzo[g,h,i]perylene is the most difficult to extract from soils, as was stated in other works.(20) The recovery for benzo[b]fluoranthene is in good agreement with those studies, regarding the same spiking method. Low molecular weight PAHs are best extracted with modifiers such as methanol and acetonitrile. Conversely, the high molecular weight PAHs require modifiers capable of dipole-dipole interactions and π-π interactions, such as dichloromethane and toluene at high percentages. Larger PAH molecules have a more expanded π-electron system, which can have a greater number, or better, interactions with active sites in the matrix.(11,12,16,29) Some authors agree that the great difference in the recoveries between spiked and native PAHs is found for the low weight compounds, whereas, those with higher molecular weights than phenanthrene have a similar extraction behaviour to the native PAHs,(20) which we have confirmed.

The behaviour of these six PAHs is between these two extremes: for fluoranthene, conditions 2 (Table 3) recovers 24% more than conditions 1, whereas, conditions 3 recovers only 11.8% more; for indeno[1,2,3-cd]pyrene, conditions 2 extracts 20.1% more, while the rise with conditions 3 is 21.1%.

To confirm the performance of the SFE method on real samples, soil B was also submitted to a Soxhlet procedure (see Table 4). As expected, the recoveries obtained were slightly lower than in spiked samples, but generally in good agreement. This means that correcting the results with the recoveries found for the SFE method, we can estimate the amount of PAHs present in a given sample.

Two more real samples (soil C and burned bark) with quite different matrix properties were also extracted. It was possible to identify some PAHs at the concentrations given in Table 4. The contamination of these two samples was not high, which may be an indication that the formation of PAHs resulting from

Table 4. SFE Recovery Based on the Amount of PAHs Extracted by Soxhlet from a Real Sample (Soil B)

	Soil B			Soil C	Burned Bark
PAHs	Soxhlet Extraction (n=3)	SFE (n=6)	SFE Recovery, %	SFE (n=7)	SFE (n=8)
Fluoranthene	12.5 (6.5)[a]	10.9 (17.5)[a]	87.2	14.3 (23.9)[a]	50.9 (5.8)[a]
Benzo[b]fluoranthene	7.4 (4.8)	6.6 (12.0)	89.6	4.4 (26.1)	15.4 (4.8)
Benzo[k]fluoranthene	3.1 (5.6)	2.8 (14.3)	87.9	0.3 (37.2)	9.0 (6.9)
Benzo[a]pyrene	6.6 (8.4)	4.9 (14.8)	73.1	0.6 (35.4)	25.5 (6.7)
Benzo[g,h,i]perylene	12.9 (4.7)	8.8 (15.5)	68.3	11.7 (52.1)	35.4 (9.5)
Indeno[1,2,3-cd]pyrene	4.4 (4.9)	3.6 (10.1)	82.8	27.7 (26.5)	11.9 (17.1)

[a] Amount (μg/Kg), (RSD, %).

Amount of PAHs present in Soil C and Burned bark with the determination carried out by SFE.

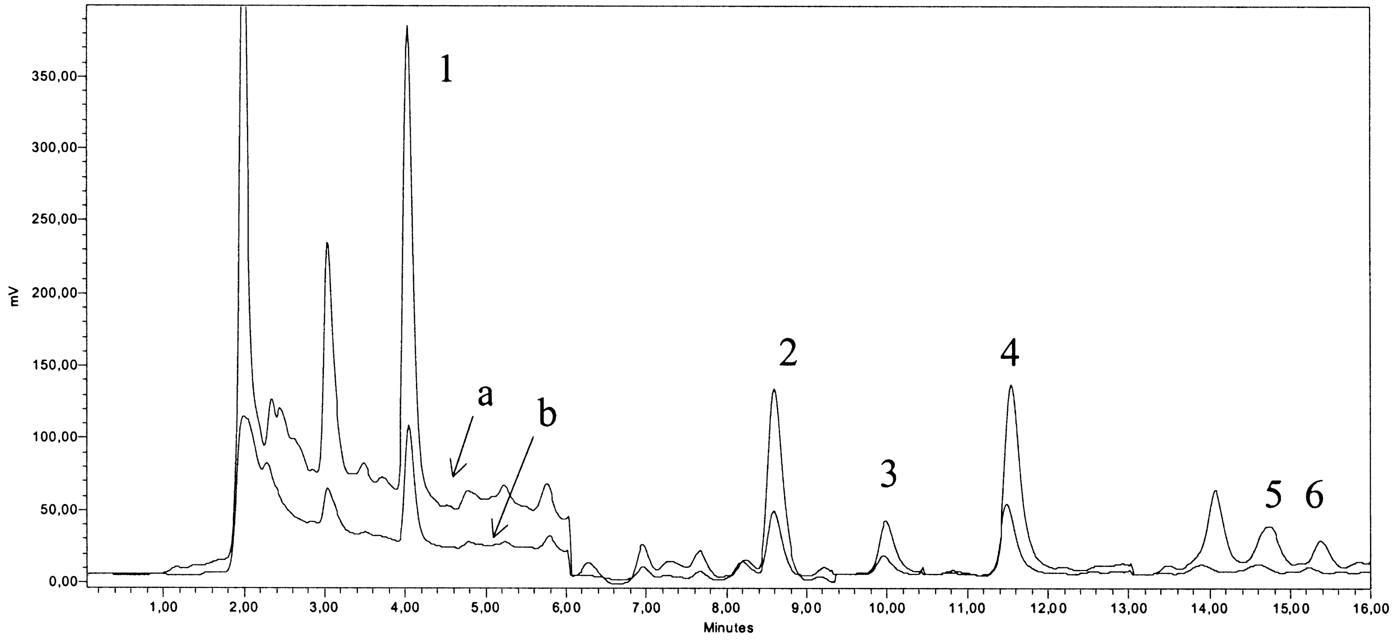

Figure 5. Representative chromatograms of soil B extracted by: a- Soxhlet; b- SFE. Note that the amount of sample extracted by these techniques was not the same. For peak assignment refer to Figure 1.

organic matter combustion wasn't very intense. Representative chromatograms can be seen in Figure 5.

The comparison between supercritical fluid extraction, sonication, and Soxhlet procedures (Tables 3 and 4) reveals that SFE can give better or equal results, respectively, with significantly faster and easier procedure.

The increasing SFE/sonication recoveries for the lower molecular weights confirms the important role of methanol in good extraction efficiencies for the low molecular weight PAHs, since the sonication solvent was only dichloromethane. The ternary SFE mixture that we propose (Table 3, conditions 3) has balanced polarity properties and strength enough to achieve good extraction yields over a broad range of PAHs and matrices.

CONCLUSIONS

Due to strong matrix-analyte interactions and deficient solubility of PAHs in the supercritical fluid, aggressive conditions (namely temperature and % modifier) have an important role in compensating CO_2 insufficiencies. An important discrimination was noted against the high molecular weight hydrocarbons for which complete recovery is very difficult to achieve. The SFE conditions have to overcome matrix-analyte interactions of greater strength when dealing with environmental samples. These conditions have to displace the analytes maintaining certain selectivity.

The ternary supercritical fluid was used to enhance extraction yields by improving the modifier-matrix interactions and reducing the analyte-matrix interactions. Also, it became more suitable to carry the analytes. Its importance was clearly pointed out.

Supercritical fluid extraction, as a rapid and accurate technique for sample preparation, fulfils the requirements for a routine setting for the analysis of PAH-contaminated soils. The fact that SFE extracts could be analysed without further time-consuming clean up steps represents a gain in time and analyte recovery. In this work, good extraction yields with good reproducibilities (RSD <9% for 5 compounds) were achieved.

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