



ELSEVIER

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Journal of Chromatography A, 994 (2003) 159–167

JOURNAL OF
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Automated fast extraction of nitrated polycyclic aromatic hydrocarbons from soil by focused microwave-assisted Soxhlet extraction prior to gas chromatography–electron-capture detection

F. Priego-Capote, J.L. Luque-García, M.D. Luque de Castro*

Department of Analytical Chemistry, Marie Curie Building, Annex C-3, Campus of Rabanales, University of Córdoba, E-14071 Córdoba, Spain

Received 20 December 2002; received in revised form 4 March 2003; accepted 6 March 2003

Abstract

An approach for the automated fast extraction of nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) from soil, using a focused microwave-assisted Soxhlet extractor, is proposed. The main factors affecting the extraction efficiency (namely: irradiation power, irradiation time, number of cycles and extractant volume) were optimised by using experimental design methodology. The reduction of the nitro-PAHs to amino-PAHs and the derivatisation of the reduced analytes with heptafluorobutyric anhydride was mandatory prior to the separation-determination step by gas chromatography–electron-capture detection. The proposed approach has allowed the extraction of these pollutants from spiked and “real” contaminated soils with extraction efficiencies similar to those provided by the US Environmental Protection Agency methods 3540-8091, but with a drastic reduction in both the extraction time and sample handling, and using less organic solvent, as 75–85% of it was recycled.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Focused microwave-assisted Soxhlet extraction; Extraction methods; Soil; Environmental analysis; Polynuclear aromatic hydrocarbons, nitrated

1. Introduction

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) represent an important category of pollutants since Jager [1] and Pitts et al. [2] discovered, nearly 25 years ago and independently, that PAHs can undergo atmospheric reactions with nitrogen oxides to form nitro derivatives. A number of them have

proved to be mutagenic and carcinogenic and the main portion of direct-acting mutagenicity of diesel and air particulates is associated with nitro-PAHs [3]. Some nitro-PAHs such as dinitropyrenes are among the most potent mutagenic compounds ever tested. According to the International Agency for Research and Cancer [4], some nitro-PAHs are possibly carcinogenic to humans. For this reason, considerable effort has been spent in the last two decades on finding out the sources and occurrence, studying their chemical and physical properties, investigating their mutagenic and carcinogenic activities and developing new analytical methods for the identifica-

*Corresponding author. Tel.: +34-957-218-615; fax: +34-957-218-615.

E-mail address: qallucam@uco.es (M.D. Luque de Castro).

tion and quantification of nitro-PAHs in the environment.

These compounds are directly emitted by diesel and petrol engines, and therefore, their concentrations are raised in cities with heavy traffic and they are further increased during smog episodes. So far, these compounds have also been found in carbon black and photocopier toners, fly ash, exhaust emissions from waste incineration plants, product from coal combustion, natural and waste waters, sediments, soils, aerosols from urban atmospheres, cigarette smoke and some foodstuffs [4–12].

Soxhlet extraction has for years been the most used technique for isolation of organic pollutants from environmental solid samples. However, the use of new extraction techniques that overcome the drawbacks associated with Soxhlet is, nowadays, one of the most active research lines in the field of solid sample preparation. The most significant drawbacks of Soxhlet extraction are the long time required for extraction and the large amount of organic solvent wasted, which is not only expensive to dispose off but which can cause environmental pollution itself. Moreover, the conventional device is not easy to automate. Different devices intending to overcome the main shortcomings of the conventional Soxhlet, but keeping its positive characteristics, have been developed [13].

The authors of the present research designed a focused microwave-assisted Soxhlet extractor the last prototype of which has solved the disadvantages of that designed previously by themselves (MIC II, SEV, Puebla, Mexico). Total automation of the extraction process and duality of the system, which allows the simultaneous processing of two samples, have been achieved in the last device [14].

Owing to the complexity of most environmental samples and the need for distinguishing among isomers of nitro-PAHs with different biological properties, chromatographic techniques have been mainly used for developing methods to determine these analytes. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been employed in more than 90% of the proposed methods [4]. The main advantage of GC is the highest separation efficiency, which allows the individual separation of a large number of compounds. Gas chromatography with an electron-capture de-

tection (ECD) system provides a selective and sensitive method for the quantification of nitro-PAHs, after derivatisation of the analytes to yield their fluorinated derivatives.

The joint use of both the prototype of the focused microwave assisted Soxhlet extractor and a gas chromatograph equipped with an ECD system has enabled the development of the method for determining nitro-PAHs in soil here reported.

2. Experimental

2.1. Instruments and apparatus

The last prototype of the focused microwave-assisted Soxhlet extractor (MIC V, SEV), was used for the extraction step (see Fig. 1). The new device includes an optical sensor that is positioned at the desired siphon height to order the magnetron to start irradiation of the sample when the solvent reaches the sensor, thus enabling selection of the extractant volume. A solenoid valve in the bottom of the siphon is switched on at the end of the irradiation process to empty the sample vessel. Both devices are controlled by a microprocessor program which allows total automation of the extraction process. The new system also includes two identical extraction units, thus making possible the simultaneous processing of two samples or a replicate of the target sample. The device operates at microwave power between 120 and 300 W. A microprocessor programmer (SEV) was used to control the parameters involved in the microwave-assisted extraction, namely: microwave power, irradiation time, number of extraction cycles and unloading time [14].

A 50 ml Soxhlet extractor (Afora, Barcelona, Spain) was used to carry out the conventional Soxhlet extraction and a rotary-evaporator (Büchi R-200 with Heating Bath B-490, Switzerland) was used to evaporate the solvent after each extraction as required by the reference US Environmental Protection Agency (EPA) method 3540.

A Varian Star 3400 CX gas chromatograph (Walnut Creek, CA, USA) equipped with a ^{63}Ni electron-capture detector and a fused-silica capillary column (30 m \times 0.32 mm, 0.25 μm) coated with SGL-1

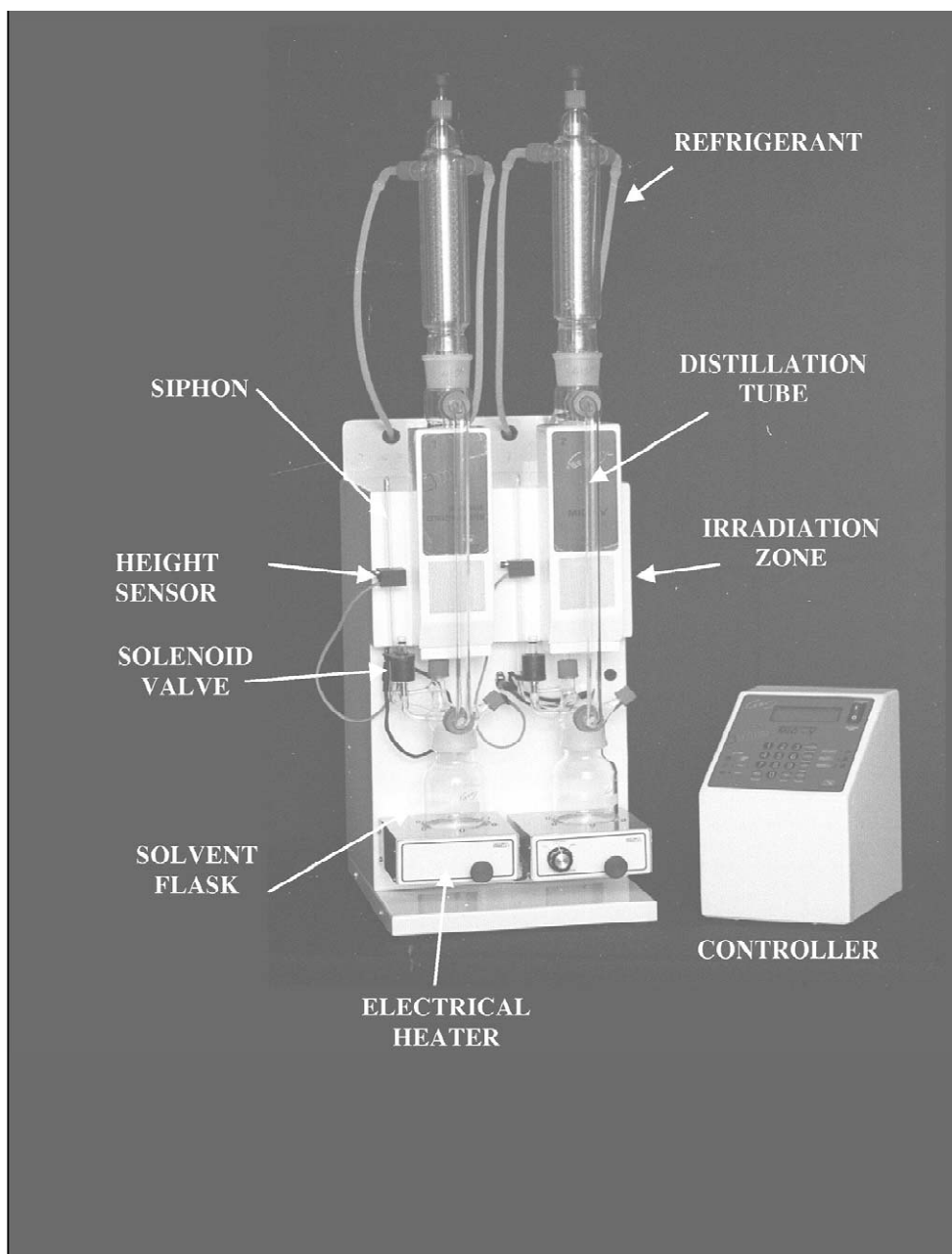


Fig. 1. Photograph of the dual automated focused microwave-assisted Soxhlet extractor.

(100% dimethylsilicon), provided by Sugelabor (Madrid, Spain), was used for the analysis of the extracts obtained.

2.2. Reagents and sample preparation

The nitro-PAHs 1-nitronaphthalene (1-NN), 3-ni-

trobiphenyl (3-NB), 2-nitrofluorene (2-NF), 3-nitrofluoranthene (3-NFa), 1-nitropyrene (1-NP) and 4-nitro-*p*-terphenyl were obtained from Sigma–Aldrich (St. Quentin Fallavier, France). These compounds were used together with the internal standard (4-nitro-*p*-terphenyl) to prepare stock standard solutions of 100 µg/ml—for minimising weighing errors—by dissolving the required amount of each nitro-PAH in chromatographic-grade acetonitrile from Panreac (Barcelona, Spain).

Reagent-grade dichloromethane from Panreac (Barcelona, Spain) was used as extractant and chromatographic-grade methanol (Panreac) was used for the reconstitution of the extract after evaporating the extractant.

A 0.05% (w/v) CuCl₂ solution, prepared using copper(II) chloride dihydrate (Panreac) in Milli-Q (Millipore, Bedford, MA, USA) water and sodium borohydride from Sigma–Aldrich (Steinheim, Germany) were used for the reduction of the nitro-PAHs to amino-PAHs.

Chromatographic-grade benzene from Panreac was used in the derivatisation step. Heptafluorobutyric anhydride (HFBA) from Aldrich (Milwaukee, WI, USA) was used as derivatisation reagent. A 5% (v/v) ammonia aqueous solution was prepared in Milli-Q water using 33% v/v analytical-grade ammonia from Panreac.

2.3. Sample preparation

Three-hundred grams of clayey soil (1.23% organic matter content) was sieved to a size smaller than 0.5 mm and spiked with nitro-PAHs by adding to the soil 300 ml of diethyl ether (Panreac) containing the necessary volume of the stock standard solution to obtain a final concentration in the dry soil of 0.25 µg/g in each nitro-PAH. Then, the slurry was shaken for 72 h and, after evaporation of the solvent, the soil was completely dried under an N₂ stream. Finally, the soil was put into a holder and stored under environmental conditions for 3 months in order to simulate natural conditions and, after this time, the soil was homogenised and stored at 4 °C in the dark until use.

The “real” contaminated soils (1.18% organic matter content) were provided by EMGRISA (Enterprise for Management of Industrial Residues

Public Limited Company), sieved to a size smaller than 0.5 mm, homogenised and stored at 4 °C in the dark until use.

2.4. Focused microwave-assisted Soxhlet extraction (FMASE) procedure

The device used for the FMASE is shown in Fig. 1. One gram of either the spiked or the real contaminated soil was weighed into a cellulose extraction cartridge, which was then capped with cotton wool and placed into the sample cartridge vessel located in the zone of microwave irradiation. Then, 150 ml of dichloromethane was poured into the distillation flask. This solvent was selected owing to it is the most common solvent for carrying out the extraction of nitro-PAHs using a reference method as extraction Soxhlet [4]. The extraction process consisted of a number of cycles that depended on the extraction kinetics of the target sample. Each cycle involved three steps

- (a) the extractant evaporated from the distillation flask, condensed in the refrigerant and dropped on the sample, filled the sample cartridge vessel. The volume of the extractant put into contact with the sample was controlled by the optical sensor height;
- (b) the magnetron started to irradiate the sample cartridge when the solvent reached the preset optical sensor height in the siphon, and
- (c) after irradiation, the solvent in the extraction vessel was unloaded to the distillation flask by automatic switching the solenoid valve.

After finishing the last cycle, the 75–85% of the extractant was recycled by a new cycle and condensed on the extraction chamber. So, the extract was evaporated to near-dryness and redissolved in 1 ml methanol. The next step was the reduction of the extracted nitro-PAHs to their amino-PAHs. 0.3 ml of 0.05% (w/v) aqueous solution of copper(II) chloride and 50 mg of NaBH₄ were added to the extract and the resulting mixture was shaken and allowed to stand at room temperature in the dark for 1 h. Then, 5 ml of water was added and, the amino-PAHs were extracted with three 5-ml portions of benzene. The organic layers were combined and the solvent was reduced to 5 ml with an N₂ stream. Derivatisation of the amino-PAHs in the benzene phase was performed

by adding 5 μ l of heptafluorobutyric anhydride and heating the sealed flask in a water bath at 50 °C for 15 min. The derivatisation provided a perfluorinated tag to the nitro-PAHs. The reaction mixture was cooled to room temperature and then 2 ml of 5% (v/v) aqueous ammonia solution was added to eliminate the excess of HFBA by shaken the mixture for 5 min. Finally, the organic and aqueous phases were allowed to separate and the benzene, containing the derivatised nitro-PAHs, was used for subsequent analysis [15].

2.5. EPA method procedure

The EPA method [16] consists of two steps: extraction and analysis.

- (a) EPA method 3540 for the extraction of semivolatile or nonvolatile analytes from solid samples: 1 g of spiked or real contaminated soil was placed in a cellulose thimble (25×88 mm, Albet, Barcelona, Spain). The overall Soxhlet glassware was fitted to a distillation flask containing 100 ml dichloromethane and 2–3 boiling glass regulators. After extraction for 24 h, the solvent was released by a rotary-evaporator.
- (b) EPA method 8091 for the determination of the concentration of nitroaromatics using GC–ECD: the conditions used to carry out the chromatographic analysis were those used in the proposed method. A derivatisation reaction was a previous step to determination of the analytes.

2.6. GC–ECD procedure

The GC separation of the nitro-PAHs was carried out using helium as carrier at a constant flow-rate of 1.8 ml/min. The column temperature program was 50 °C, held for 2 min, then increased at 20 °C/min to 120 °C and immediately increased at a rate of 5 °C/min to 250 °C and held for 1 min to this temperature. The injections (1 μ l injection volume) were of the splitless mode with the injector temperature set at 250 °C and the electron-capture detector at 300 °C.

3. Results and discussion

3.1. Chromatographic conditions

The experimental chromatographic variables were

optimised and the optimal working conditions thus obtained were those commented under Experimental. Complete separation of the analytes was achieved within 30 min, therefore the required time for a chromatogram was reduced in 9 min as compared with methods in the literature [15]. 4-NO₂-*p*-terphenyl was used as internal standard (IS) due to its physical and chemical behavior similar to that of the analytes (particularly in the derivatisation step). Furthermore, its retention time (29 min) was not far from that of the last analyte (27 min). In our study, each extract was spiked with 5 μ g of 4-nitro-*p*-terphenyl after extraction. According to the above study, 4-nitro-*p*-terphenyl is a suitable internal standard for this method.

The background of both spiked and natural samples was not significant.

3.2. Optimisation of the focused microwave-assisted Soxhlet extraction

A screening study of the influence of the main variables affecting the extraction step such as irradiation time, power of irradiation, extractant volume and number of cycles, was performed by means of a full two-level factorial 2⁴ type V resolution experimental design allowing three degrees of freedom and involving 16 randomized runs plus three centered points [17]. The upper and lower values given to each factor were selected from the available data and experience gathered in preliminary experiments. The tested and the optimum values obtained for each variable are shown in Table 1. For the optimisation study, 1-g portions of the spiked soil aged for 3 months was used.

The conclusions of this screening study were that the number of cycles was the most influential factor on the extraction efficiency for all the compounds, its increase had a positive effect on the analytes re-

Table 1
Optimisation of the focused microwave-assisted Soxhlet extraction step

Variable	Tested range	Optimum value
Irradiation power (%)	50–100	100
Irradiation time (s)	30–90	90
Extractant volume (ml)	25–45	45
Number of cycles	1–6	6

covery; so higher values should be tested. The power of irradiation was only a significant factor for two analytes (1-nitronaphthalene and 1-nitropyrene), so higher values should also be tested. However, the maximum value already tested was the maximum value provided by the equipment (300 W), so it was selected for further experiments. In the case of the irradiation time and the extractant volume, they were not significant variables within the range studied for any of the compounds, but their effects were positive in all cases, so the maximum values tested for both factors and provided by the extractor, 90 s and 45 ml, respectively, were selected for subsequent experiments. Although the analysis of the experimental design showed that more number of cycles ought to be performed, it was not necessary as six cycles (the maximum value tested) provided quantitative recoveries.

3.3. Features of the method

Calibration plots were run for the five analytes and by using the peak area as a function of the standard concentration of each compound. The linear dynamic ranges are shown in Table 2.

The limit of detection (LOD) for each analyte was expressed as the mass of analyte which gives a signal that is 3σ above the mean blank signal (where σ is the standard deviation of the blank signal). The LODs obtained ranged between 0.5 and 1.5 ng/g. The limits of quantification, expressed as the mass of analyte which gives a signal 10σ above the mean blank signal, ranged from 1.9 to 3.1 ng/g (Table 2). LODs and LOQs were estimated from standard solutions of these compounds.

In order to evaluate the precision of the proposed method, within-laboratory reproducibility and repeatability were evaluated in a single experimental

Table 3

Results obtained from the evaluation of the precision of the proposed method in terms of repeatability relative standard deviation (s_r) and within-laboratory reproducibility relative standard deviation (s_{WR}) obtained for each analyte

Parameter	1-NN	3-NB	2-NF	3-NFa	1-NP
% s_r	2.93	2.54	3.67	3.88	4.72
% s_{WR}	5.72	4.35	4.91	4.60	7.38

set-up with duplicates. The experiments were carried out using 1 g of the spiked clayey soil containing 0.25 $\mu\text{g/g}$ of each pollutant and the optimal conditions for all the steps. Two measurements of each analyte per day were performed on 7 days [18]. The results obtained are listed in Table 3. The repeatability, expressed as relative standard deviation, was from 2.54 to 4.72%; meanwhile, within-laboratory reproducibility ranged from 4.35 to 7.38%.

3.4. Validation of the proposed method

Due to the lack of certified reference materials containing nitro-PAHs, the proposed method was validated by comparison with the reference EPA method 3540-8091 in terms of extraction efficiency as the other steps are identical. The reference method is conventional Soxhlet for the determination of nitroaromatics in soils. Both the experimental conditions previously optimised and the Soxhlet method with a duration of 24 h were applied to 1-g portions of the spiked soil. Table 4 shows the average recoveries obtained by both methods for each analyte and the value of f (FMASE recovery/EPA method recovery). The values of average recoveries were within the experimental error and thus, the confidence interval or RSD related to the estimated f values were not significant in comparison with the f values. As can be seen, similar recoveries were

Table 2

Linear dynamic ranges, limits of detection (LOD), limits of quantification (LOQ) and regression coefficients (r) for the proposed method

Compound	Linear dynamic range ^a	LOD ^a	LOQ ^a	r
1-Nitronaphthalene	4–500	0.5	1.9	0.998
3-Nitrobiphenyl	4–400	1.5	3.1	0.998
2-Nitrofluorene	4–300	1.0	2.4	0.999
3-Nitrofluoranthene	4–400	0.8	2.2	0.997
1-Nitropyrene	4–500	0.6	2.1	0.998

^a Expressed as ng/g.

Table 4
Comparison of the FMASE and EPA methods for the spiked soil

Analyte	Recovery (expressed as percent)		f^a
	FMASE method	EPA method	
1-Nitronaphthalene	115.68	102.40	1.13
3-Nitrobiphenyl	104.55	106.53	0.98
2-Nitrofluorene	89.72	91.34	0.98
3-Nitrofluoranthene	112.38	96.54	1.16
1-Nitropyrene	116.53	109.32	1.07

^a FMASE recovery/EPA method recovery.

provided by both methods. For 1-nitronaphthalene, 3-nitrofluoranthene and 1-nitropyrene the f value was higher than 1.00; meanwhile, for 3-nitrobiphenyl and 2-nitrofluorene was 0.98.

3.5. Study of the extraction kinetics: a comparison of the proposed method with the EPA method

One-gram portions of the spiked soil were used to compare the kinetics of the extraction of the proposed FMASE method with the EPA reference method. FMASE cycles from 1 to 6 were performed and the extract obtained in each cycle was removed from the distillation vessel and quantified by GC-ECD. Fig. 2a shows the focused microwave-assisted extraction kinetics of the nitro-PAHs from the spiked soils. Six cycles were sufficient for quantitative removal of the target compounds. Fig. 2b shows the conventional Soxhlet extraction kinetics of the nitro-PAHs from the spiked soils. As can be seen, the number of cycles needed in this case is much higher than using FMASE and thus, the time required for the extraction process—despite the total duration of each FMASE cycle was 8 min vs. 5 min corresponding to each conventional Soxhlet extraction cycle. The most interesting aspect is that the time necessary for total removal of the analytes by FMASE was 48 min vs. 20 h needed by the reference extractor (it was not necessary to lengthen the conventional extraction method to 24 h). This fact shows the high efficiency of microwaves for accelerating the extraction.

3.6. Application of the proposed method to real contaminated soil samples

The optimised method was applied to the ex-

traction of the analytes in soil samples naturally contaminated, provided by EMGRISA. The concentration of PAHs in these samples was known, but not that of nitro-PAHs. The results, summarised in Table 5, show that the analysed samples contained nitro-

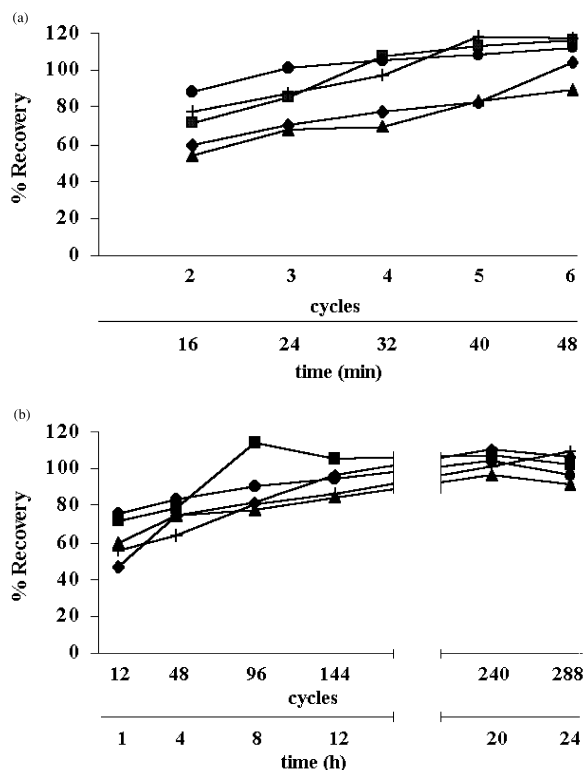


Fig. 2. Comparison of the extraction kinetics between FMASE and conventional Soxhlet extraction for the target analytes: (■) 1-nitronaphthalene, (◆) 3-nitrobiphenyl, (▲) 2-nitrofluorene, (●) 3-nitrofluoranthene and (+) 1-nitropyrene: (a) Recovery with FMASE vs. number of cycles and time of extraction. (b) Recovery with conventional Soxhlet extraction vs. number of cycles and time of extraction.

Table 5
Results obtained from two naturally contaminated soils

Analyte	Estimated soil concentration (expressed as ng/g)					
	Soil A			Soil B		
	FMASE method ^a	EPA method	<i>f</i> ^b	FMASE method ^a	EPA method	<i>f</i> ^b
1-Nitronaphthalene	9.18±1.14	8.13±0.81	1.13	6.46±0.80	5.68±0.56	1.13
3-Nitrobiphenyl	9.58±1.26	8.50±0.51	1.13	10.45±1.08	9.48±0.75	1.10
2-Nitrofluorene	97.30±7.20	91.39±6.12	1.06	105.40±7.30	107.30±8.20	0.98
3-Nitrofluoranthene	204.20±5.40	189.00±9.60	1.08	252.30±11.80	239.40±14.20	1.05
1-Nitropyrene	4.89±0.67	5.56±0.61	0.89	15.72±1.22	16.39±1.48	0.96

^a Number of cycles=6.

^b FMASE extraction efficiency/EPA method extraction efficiency.

PAHs in different concentration. As can be seen, the concentration of 3-nitrofluoranthene and 2-nitrofluorene was much higher than that of the other analytes. Similar estimated soil concentrations were provided by the EPA method and the proposed method. The *f* factor was higher than 1.00, except for 1-nitropyrene, which was 0.89 and 0.96 for soil A and B, respectively.

4. Conclusions

The last prototype of FMASE has overcome shortcomings of previous designs allowing both the simultaneous processing of two samples and fully automated performance with a drastic reduction in sample handling.

The feasibility of this new extractor (MIC V) has been proved in this study for leaching nitro-PAHs (namely, 1-nitronaphthalene, 3-nitrobiphenyl, 2-nitrofluorene, 3-nitrofluoranthene and 1-nitropyrene) from soils as a prior step to derivatisation and GC–ECD determination. The use of FMASE allowed to obtain similar results in a time shorter than that required by EPA 3540 method (1 h vs. 24 h) with only six extraction cycles and recycling 75–85% of the extractant solvent. A within-laboratory reproducibility between 4.35 and 7.38% and repeatability from 2.54 to 4.72%, both expressed as relative standard deviation, have demonstrated the suitability of the method for the analysis of these pollutants from soils.

Acknowledgements

Spain's Comisión Interministerial de Ciencia y Tecnología (CICYT) is gratefully acknowledged for financial support (project No.BQU-2002-01333). EMGRISA is also thanked for providing the contaminated samples.

References

- [1] J. Jager, *J. Chromatogr.* 152 (1978) 575.
- [2] J.N. Pitts Jr., K.A. Van Cauwenberghe, D. Grosjean, J.P. Schmid, D.R. Fitz, W.L. Belsler, G.B. Knudson, P.M. Hynds, *Science* 202 (1978) 515.
- [3] B.J. Finlayson-Pitts, J.N. Pitts Jr., *Science* 276 (1997) 1045.
- [4] J. Cvacka, J. Barek, A.G. Fogg, J.C. Moreira, J. Zima, *Analyst* 123 (1998) 9R.
- [5] M. Wilhelm, G. Matuschek, A. Kettrup, *J. Chromatogr. A* 878 (2000) 171.
- [6] J.C. Moreira, J. Barek, *Quím. Nova* 18 (1995) 362.
- [7] J. Jacob, W. Karcher, J.J. Belliardo, R. Dumler, A. Boenke, *Fresenius J. Anal. Chem.* 340 (1991) 755.
- [8] J.C. Moreira, G.S. Kuriyama, J. Barek, Proceedings of the National Conference on Polycyclic Aromatic Hydrocarbons in General and Industrial Environment, Garga, 1996, p. 63.
- [9] J. Barek, J. Cvacka, J.C. Moreira, J. Zima, *Chem Listy* 90 (1996) 805.
- [10] M. Barzegar, A. Daneshfar, M. Ashraf-Khorassani, *Anal. Chim. Acta* 349 (1997) 245.
- [11] M. Murayama, P. Dasgupta, *Anal. Chem.* 68 (1996) 1226.
- [12] C.T. Kuo, H.W. Chen, *J. Chromatogr. A* 897 (2000) 393.
- [13] J.L. Luque-García, M.D. Luque de Castro, *Anal. Chem.* 73 (2001) 5903.
- [14] J.L. Luque-García, M.D. Luque de Castro, *Analyst A* (2003) in press.
- [15] X. Jinhui, F.S.C. Lee, *Anal. Chim. Acta* 416 (2000) 111.

- [16] EPA Method 3540, Extraction of solid samples of the semivolatile or nonvolatile analytes and EPA method 8091 for the determination of the concentration of nitroaromatics using GC–ECD, EPA, Washington, DC, 1994.
- [17] C. Mate-Jiménez, General Course on Statgraphics, Vols. I and II, UPCO Publishers, Madrid, 1995.
- [18] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics, Part A, Elsevier, Amsterdam, 1997.