Ultrasound-assisted extraction of nitropolycyclic aromatic hydrocarbons from soil prior to gas chromatography-mass detection

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Abstract

Continuous ultrasound-assisted extraction of nitropolycyclic aromatic hydrocarbons from soil prior to their individual separation and determination by gas chromatography (GC) with MS–MS detection is presented here. A multivariate optimisation of the variables affecting the continuous extraction step (namely, probe position, ultrasound radiation amplitude, percentage of duty cycle of ultrasonic exposure, sonication time, total extractant volume, extractant flow rate and temperature of the water-bath in which the extraction cell was placed) was performed. The method was compared with the reference EPA method 3540 using natural contaminated soils. Similar efficiencies were obtained but with a drastic reduction of both the extraction time (10 min versus 24 h) and the extractant volume (less than 10 ml versus 100 ml) by the proposed method. Detection limits of low picogram were obtained, with repeatability and reproducibility between 4.21–5.70 and 5.20–7.23%, respectively.
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1. Introduction

Nitrated polycyclic aromatic hydrocarbons (NPAHs) are widespread environmental pollutants. 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 NPAHs [1]. According to the International Agency for Research and Cancer [2], some NPAHs are possibly carcinogenic to humans. Thus, in the last years new analytical methods for the identification and quantification of NPAHs in the environment [3–7] have been developed.

Polycyclic aromatic hydrocarbons (PAHs) can undergo atmospheric reactions with nitrogen oxides to form nitro derivatives but NPAHs can also be directly emitted by diesel and petrol engines. These compounds have also been found in carbon black and photocopier toners, fly ash, exhaust emissions from waste incineration plants, products from coal combustion, natural and waste waters, sediments, cigarette smoke and some foodstuffs [8–11]. Recently, new extraction methodologies such as microwave-assisted extraction

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[12] and pressurised liquid extraction [13] have been used for the extraction of these compounds.

Ultrasonic radiation is a powerful means for acceleration of various steps of the analytical process in solid [14] and liquid samples [15]. This type of energy is of great help in the pre-treatment of solid samples as it facilitates and accelerates operations such as the extraction of organic and inorganic compounds [16–18].

Ultrasound-assisted leaching is an effective way of extracting a number of analytes from different types of samples. The influence of extremely high effective temperatures, which result in increased solubility and diffusivity, and pressures, which favour penetration and transport, at the interphase between an aqueous or organic solution subject to ultrasonic energy and a solid matrix, result in a high extractive power.

In many cases, ultrasound-assisted extraction is an expeditious, inexpensive and efficient alternative to conventional extraction techniques and in some cases, even to supercritical fluid and microwave-assisted extraction, as demonstrated by application to both organic and inorganic analytes in a wide variety of samples [19].

For the determination of NPAHs, gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been employed in more than 90% of all analyses [11]. The main advantage of GC is the higher separation efficiency, which allows the separation of a large number of compounds.

The method proposed here is based on the use of an ultrasound-assisted extractor for the leaching of NPAHs from spiked and natural contaminated soil prior to their individual separation and determination by GC–MS–MS.

2. Experimental

2.1. Instruments and apparatus

Ultrasonic irradiation was applied by means of a Branson 450 sonicator (20 kHz, 100 W) equipped with a cylindrical titanium alloy probe (1.5 cm diameter), which was immersed into a water-bath in which the sample cell was placed. An extraction chamber con-

sisting of a stainless-steel cylinder (10 cm × 10 mm i.d.) closed with screws at either end, which permitted the circulation of the leaching solvent through it, was used.

A Gilson (Middleton, WI, USA) Minipuls-3 low pressure peristaltic pump programmed for changing the rotation direction a preset intervals and PTFE tubing of 0.8 mm i.d. (Análisis Vínicos, Tomelloso, Spain) were used to build the flow manifold.

The extracts were analyzed using a Varian CP 3800 gas chromatograph coupled to a Saturn 2200 ion trap mass spectrometer (Sugar Land, TX, USA). Separations were carried out on a CP-SIL 24 CB-MS W cot fused-silica capillary column, 30 m × 0.25 mm i.d. × 0.25 μm (Varian, Sugar Land, TX, USA).

2.2. Reagents and solutions

The nitropolycyclic aromatic hydrocarbons 1-nitronaphthalene (1-NN), 3-nitrobiphenyl (3-NB), 2-nitrofluorene (2-NF), 3-nitrofluoranthene (3-Nfa), 1-nitropyrene (1-NP) and 4-nitro-p-terphenyl were obtained from Sigma (St. Quentin, Fallavier, France). These compounds were used to prepare the stock standard solutions and the internal standard solution (4-nitro-p-terphenyl) by dissolving the required amount of each NPAH in HPLC-grade acetonitrile (Panreac, Barcelona, Spain).

Reagent-grade dichloromethane (DCM) from Panreac was used as extractant and HPLC-grade acetonitrile (Panreac) was used to reconstitute the extract after evaporation of the extractant.

2.3. Samples

Three hundred grams of clayely soil (1.23% organic matter content), sieved to a size smaller than 0.5 mm, was spiked with NPAHs by adding to the soil 300 ml of ethyl ether (Panreac) containing the required volume of the stock standard solution to obtain a final concentration in the dry soil of 0.25 μg g^{-1} in each NPAH. The slurry was shaken for 72 h and, after solvent evaporation, the soil was completely dried under N$_2$ stream. Afterwards, the soil was put into a holder and stored at environmental conditions for 6 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. Before spiking the ref-
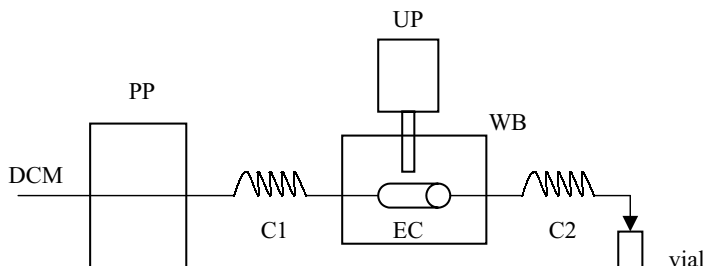
Fig. 1. Experimental set-up used for the extraction process. DCM, dichloromethane; PP, peristaltic pump; C1, C2, coils; UP, ultrasonic probe; WB, water-bath; EC, extraction chamber.

erence EPA method 3540 [20] was applied to the soil and no detectable levels of none of the analytes were found.

The natural contaminated soils were provided by EMGRISA (Enterprise for Management of Industrial Residues Public Limited Company, Madrid, Spain), sieved to a size smaller than 0.5 mm, homogenised and stored at 4 °C in the dark until use.

2.4. Extraction step

2.4.1. Proposed method

For the ultrasound-assisted extraction method, 4 g of soil was weighed and placed in the extraction cell of the experimental set-up in Fig. 1. The extraction chamber was immersed in the water-bath (20 °C) and, after closing, the system was filled with the extractant (dichloromethane) impelled by the peristaltic pump. The total volume of DCM for each extraction was 8 ml (optimum value). The extractant was circulated through the sample for 10 min under ultrasonic irradiation (duty cycle 0.6 s, output amplitude 30% of the nominal amplitude of the converter, applied power 100 W with the probe placed 1 cm from the upper surface of the extraction cell). During extraction, the direction of the extractant (at 2 ml min^{-1}) was changed each 90 s, thus minimising both dilution of the extractant and increased compactness of the sample in the extraction chamber that could cause overpressure of the system. After extraction, the solvent was released by a rotary-evaporator and the analytes were reconstituted with 0.5 ml of acetonitrile (containing 2 μg ml^{-1} of IS) and 2 μl was injected into the gas chromatograph.

2.4.2. Reference EPA method 3540

Four grams of spiked or real contaminated soil were weighed in a cellulose thimble (25 mm × 88 mm, Albet, Barcelona, Spain) which was placed into a distillation flask containing 100 ml dichloromethane and three boiling glass regulators. After Soxhlet extraction for 24 h, the solvent was released by a rotary-evaporator, the analytes were reconstituted with 0.5 ml of acetonitrile (containing 2 μg ml^{-1} of IS) and 2 μl was injected into the gas chromatograph.

2.5. GC–MS–MS analysis

For the GC–MS–MS analysis of the extracts helium was used as carrier gas at a constant pressure of 21 psi. The column temperature program was 50 °C, held for 2 min, then increased at 20 °C min^{-1} to 300 °C, held for 6 min. The injector was kept at 300 °C and the injection was performed in the splitless mode.

All the experiments were performed under automatic gain control (AGC) with a target value of 5000 for GC–MS–MS. The filament emission current was 80 μA and the electron multiplier voltage offset of +200 V. The modulation amplitude was set at 4.0 V using perfluorotributylamine (FC-43) as a reference gas. The ion trap mass spectrometer was operated at 200 °C, while the interface and manifold temperatures were maintained at 280 and 50 °C, respectively. For the NPAHs determination, the optimum excitation voltage yielding maximum production of fragment ions was determined by series of multi-step experiments in which collision induced dissociation (CID) voltage (resonant and no resonant) was adjusted in 10 sequential steps.

Table 1
Conditions for the GC–MS–MS determination of the NPAHs

Analyte	Parent ion (m/z)	CID[a] voltage (V)	ESL[b] (m/z)	Product ion (m/z)	Rt[c] (min)
1-Nitronaphthalene	173	59	76.0	129	11.41
3-Nitrobiphenyl	199	71	87.6	153	12.56
2-Nitrofluorene	211	98	92.9	165	14.45
3-Nitrofluoranthene	247	72	99.0	217	17.93
1-Nitropyrene	247	73	99.0	217	18.85

[a] Collision induced dissociation.
[b] Excitation storage level.
[c] Retention time.

3. Results and discussion

3.1. Optimisation of MS–MS conditions

Both resonant and non-resonant CID were used to determine the best conditions for each compound. Non-resonant wave form was selected for all the analytes. Table 1 shows the optimised MS–MS parameters. At the excitation voltage selected for each analyte, approximately 25% of the molecular ion signal remained and the product ion signal was maximal. These parameters were used to write the six ion preparation method files used for the analysis of the NPAHs of interest.

3.2. Optimisation of the extraction step

Firstly, some preliminary experiments were performed in order to choose the best extractant and the optimum volume of acetonitrile to reconstitute the analytes. Water, acetonitrile, dichloromethane and n-hexane were tested and the highest signals were obtained with dichloromethane. The results of these experiments showed that the optimum volume for reconstituting the analytes was 0.5 ml.

A multivariate design was used to optimise the extraction step. The variables optimised were the probe position, ultrasound radiation amplitude, percentage of duty cycle of ultrasonic exposure, sonication time, total extractant volume, extractant flow rate and temperature of the water-bath in which the extraction cell was placed (Table 2). The probe position was measured as the distance between the tip horn of the ultrasonic probe and the surface of the extraction cell.

A Placket–Burman design allowing seven degrees of freedom and involving 12 randomized runs plus three centred points was built for a screening study of the behaviour of the main factors affecting the extraction step. The upper and lower values given to each factor were selected from data gathered in the preliminary experiments.

The conclusion of this first screening study were that the probe position, the percentage of the duty cycle of ultrasonic exposure, the extraction time and the temperature of the water-bath were statistically not influential factors in the ranges under study. However, the results showed better recoveries with minimum distance between the extraction cell and the tip horn of ultrasonic exposure and with the minimum temperature of the water-bath. Thus, the lower values tested for the probe position (1 cm) and temperature (20 °C) and medium values tested for the duty cycle (60%) and the extraction time (10 min) were selected for subsequent experiments.

The other variables, namely radiation amplitude, extractant flow rate and extractant volume, were

Table 2
Optimisation of the extraction step

Variable	Tested range	Optimum value
Screening study (Plackett–Burman)		
Probe position (cm)	1–3	1
Radiation amplitude (%)	3–9	–
Duty cycle (%)	30–90	60
Sonication time (min)	5–15	10
Total extractant volume (ml)	5–8	–
Extractant flow rate (ml min^{-1})	3–5	–
Temperature (°C)	20–40	20
Full factorial design		
Radiation amplitude (%)	1–3	3
Extractant volume (ml)	8–10	8
Extractant flow rate (ml min^{-1})	1–3	2

Table 3
Features of the method

Analyte	Calibration equation	r^2	Linear range ($ng\,ml^{-1}$)	Detection limit (pg)
1-NN	$y = 4.82 + 0.33x$	0.9987	30–2200	30.0
3-NB	$y = 143.79 + 3.74x$	0.9897	10–2200	6.1
2-NF	$y = 40.72 + 1.10x$	0.9963	10–2200	9.2
3-Nfa	$y = 240.10 + 3.47x$	0.9965	30–2200	7.3
1-NP	$y = 86.29 + 1.22x$	0.9961	10–2200	17.6

Table 4
Results of the precision study

Parameter	1-NN	3-NB	2-NF	3-NFa	1-NP
S_r (%)	5.15	4.21	5.70	4.60	5.34
S_{WR} (%)	7.23	5.53	6.43	5.20	7.05

S_r (%), repeatability relative standard deviation; S_{WR} (%), within-laboratory relative standard deviation.

mean blank signal, ranged between 6 pg for 3-NB and 30 pg for 1-NN.

3.4. Evaluation of the precision of the method

In order to evaluate the precision of the proposed method, within-laboratory reproducibility and repeatability were estimated in a single experimental set-up with duplicates [21]. The experiments were carried out using 4 g of soil. In all experiments, the optimal values obtained for the variables were used. Two extractions and measurements of the target analytes per day were carried out on 7 days.

To determine the variance due to the between-day effect, Eqs. (1) and (2) were used:

$$S_{between}^2 = \frac{MS_{between} - MS_{within}}{n_j} \tag{1}$$

where n_j is the number of replicates per day. The within-laboratory reproducibility, S_{WR}^2, is equal to:

$$S_{WR}^2 = S_r^2 + S_{between}^2 \tag{2}$$

As shown in Table 4, the repeatability, expressed as relative standard deviation, ranged between 4.21% for 3-NB and 5.70% for 2-NF; meanwhile the within-laboratory reproducibility ranged between 5.20% for 3-NFa and 7.23% for 1-NN.

influential factors. Lower values for both the radiation amplitude and extractant flow rate and higher values of extractant volume were studied using a two-level full factor design, involving eight randomised runs plus three centred points. In this case, radiation amplitude had a positive effect for all the analytes and was statistically influential for NB, NFT and NP so the highest value (30% of the nominal value of the converter) was selected. The extractant volume had a negative effect for all the analytes and was statistically influential for NB and NN; thus, the lowest value (8 ml) was selected. The extractant flow rate was not an influential factor for none of the analytes so, an intermediate value ($2\,ml\,min^{-1}$) was chosen.

3.3. Features of the method

Calibration curves for each analyte were plotted using standard solutions of the analytes in chromatographic grade acetonitrile. The equations of the calibration plots, the correlation coefficients, the linear range and the detection limit for each analyte are summarized in Table 3. The detection limits, expressed in picograms entering on-column and reaching the detector, which gives a signal that is 3σ above the

Table 5
Validation of the method

Analyte	Soil 1		Soil 2	
	UAE method	EPA method	UAE method	EPA method
1-NN	8.72 ± 1.20	8.63 ± 0.95	5.57 ± 1.55	5.65 ± 0.77
3-NB	9.35 ± 1.58	8.75 ± 0.53	10.72 ± 2.26	10.22 ± 0.65
2-NF	89.20 ± 3.50	91.22 ± 3.03	103.01 ± 6.20	105.41 ± 3.05
3-Nfa	200.10 ± 7.35	195.56 ± 9.27	245.21 ± 8.37	243.04 ± 10.30
1-NP	4.97 ± 2.30	5.11 ± 2.03	16.87 ± 3.15	17.05 ± 1.27

Soil concentration expressed as $ng\,g^{-1}$; $n = 3$.

3.5. Validation of the method

In order to evaluate the accuracy of the proposed method, and because there were not commercially available certified reference materials, it was compared with the reference EPA method 3540 to extract NPAHs from soil. Both methods were applied to two soil samples naturally contaminated, which were provided by EMGRISA. As can be seen in Table 5, the results obtained by the proposed method are in good agreement with those provided by the reference method, which shows the applicability of the proposed approach to extract this type of compounds from soil.

4. Conclusions

A fast and simple method for the extraction of NPAHs from soil prior to GC–MS–MS determination is proposed. This is the first time that an ultrasonic probe has been used for accelerating the extraction of NPAHs from soil. The use of a dynamic approach allowed the extraction of the analytes in 10 min versus 24 h for the reference EPA method 3540 and the use of less than 10 ml of extractant versus 100 ml with the reference method. Moreover, similar extraction efficiency for all the analytes in natural contaminated soils was obtained by the proposed method in comparison to the reference method. The precision, expressed as within-laboratory reproducibility relative standard deviation, provided by this approach ranged between 5.20 and 7.23%.

The use of GC–MS–MS for the determination of the target analytes allows to achieving a high selectivity (no interferences were observed) and a high sensitivity, with detection limits at the low picogram levels.

Acknowledgements

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