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# Comparison of the static, dynamic and static-dynamic pressurised liquid extraction modes for the removal of nitrated polycyclic aromatic hydrocarbons from soil with on-line filtration-preconcentration

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**Abstract**

The three operational modes of pressurised liquid extraction (PLE) (namely, static, dynamic and static-dynamic) have been applied for the extraction of nitrated polycyclic aromatic hydrocarbons from both spiked and natural contaminated soils. A comparison of the three modes in terms of experimental set-up used, extraction time needed for total removal of the analytes and precision has been carried out. The use of a flow-injection manifold as interface between every pressurised extractor and a filtration-preconcentration system has allowed the partial automation of the proposed approaches. Efficiencies close to 100% have been provided by the three operational modes. However, the static-dynamic mode has been proved as the most suitable alternative providing the shortest extraction time (25 min) versus the static (30 min) and the dynamic (50–70 min) modes. Gas chromatography with MS–MS ion preparation mode has been used providing both high selectivity (no interferences were observed) and sensitivity (detection limits of low pg). The comparison of the proposed approaches with the reference method 3540 of the US Environmental Protection Agency (EPA) has shown that both methods provide similar efficiencies with an important shortening in the extraction time (25–70 min by PLE versus 24 h by the EPA method). The use of water as leaching agent has avoided the use of organic solvents providing an environmentally friendly method.


*Keywords:* Soil; Pressurised liquid extraction; Environmental analysis; Sample preparation; 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.

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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 identification 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, products from coal combustion, natural and waste waters, sediments, soils, aerosols from urban atmospheres, cigarette smoke and some foodstuffs [4–12].

Over the past few years, pressurised liquid extraction (PLE) has gradually emerged as an efficient means for increasing automatability, shortening process times and reducing the amounts of solvent required to leach solid samples [13–15]. There are three operational modes to carry out extraction with pressurised liquids: 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 nitro-PAHs from both spiked and natural contaminated soil samples, thus allowing their performance to be compared in terms of experimental set-up, extraction time and precision of the results they provide.

The use of liquid water at high pressure and temperature constitutes a promising alternative to other sample pre-treatments, as it is the ideal solvent for the establishment of environmental friendly methods. Water at high pressure and temperature has been traditionally named as subcritical water. The outstanding feature of subcritical water as a leaching agent is its capacity for altering its dielectric constant ($\varepsilon$). In fact, this parameter can be changed within a wide range by changing the temperature under moderate pressure, because it depends mainly on temperature and only slightly on pressure (the lower the pressure, the lower the $\varepsilon$). At ambient pressure and temperature, water has a dielectric constant of ca. 80; meanwhile at 250 °C (and pressure >40 atm to maintain the liquid state; 1 atm=101.325 Pa) is $\varepsilon$=27, which is between to those of ethanol ($\varepsilon$=24) and methanol ($\varepsilon$=33). This significant drop in the dielectric constant allows hydrophobic compounds, which have limited water solubility at room temperature, to be more readily dissolved in water.

In all cases, the use of a flow injection manifold as an interface between the extractor and a filtration-preconcentration system has allowed the development of partially automated methods. Gas chromatography–tandem mass spectrometry has been chosen as it has been shown as an excellent technique for simultaneous identification and quantification of nitro-PAHs [16].

## 2. Experimental

### 2.1. Reagents and samples

The nitro-PAHs 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–Aldrich (St. Quentin Fallavier, France). These compounds and the internal standard (4-nitro-*p*-terphenyl) were used to prepare stock standard solutions of 100 μg/ml by dissolving the required amount of each nitro-PAH in chromatographic-grade acetonitrile from Panreac (Barcelona, Spain).

Milli-Q water was used as extractant and chromatographic-grade acetonitrile (Panreac) was used for elution of the analytes after injection in the gas chromatograph. Analytical-grade dichloromethane (Panreac, Barcelona, Spain) was used as extractant in the reference EPA method 3540 [17].

A 300-g amount of clayey soil (1.2% 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 $g^{-1}$ 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 $N_2$ stream. Finally, 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. The soil did not show detectable levels of none of the analytes before spiking. The reference US Environmental Protection Agency (EPA) method 3540 was applied periodically to the spiked soil and no degradation of the analytes was observed during the time in which the research was performed.

The natural contaminated soils were provided by EMGRISA (Empresa para la Gestion de Residuos Industriales, Madrid, Spain), sieved to a size smaller than 0.5 mm, homogenised and stored at 4 °C in the dark until use.

### 2.2. Instruments and apparatus

The static pressurised liquid extraction was performed using the assembly in Fig. 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

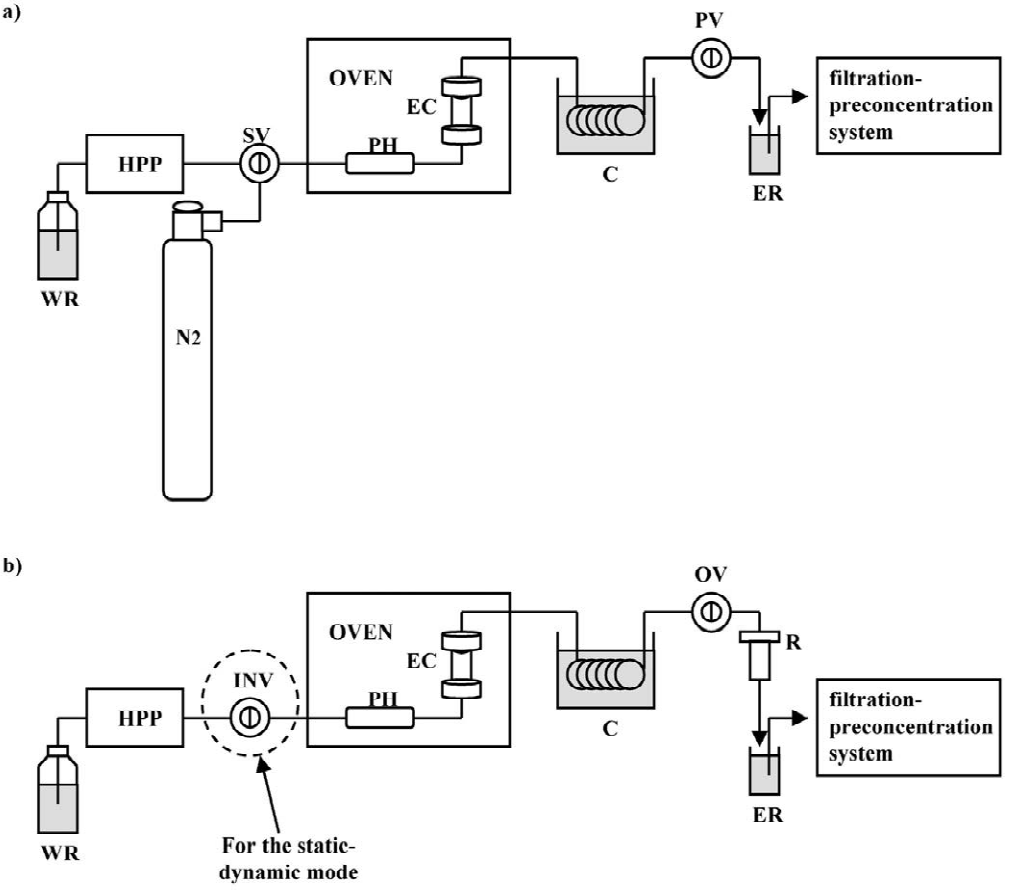


Fig. 1. Experimental setups. (a) Static mode; (b) dynamic and static-dynamic modes. WR, water reservoir; HPP, high pressure pump; SV, selecting valve; PH, preheater; EC, extraction cell; C, cooler system; PV, pressure valve; ER, extract reservoir; INV, inlet needle valve; OV, outlet needle valve; R, restrictor.

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.; 1 in.=2.54 cm) to ensure that the sample remains in the extraction chamber; (3) a gas chromatograph oven (HP 5720A, Hewlett-Packard, Avondale, PA, USA) used as heating source into which the chamber, together with a stainless steel pre-heater, is placed; (4) a cooler system (consisting of a loop made from 1-m length of stainless steel tubing, 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 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 pressurised liquid extraction was performed using the previous assembly with the following modifications as shown in Fig. 1b: (1) an outlet valve located outside the oven was utilized to produce the overpressure required at the beginning of the extraction; (2) a restrictor (Análisis Vínicos) was coupled to the outlet of the cooler (substituting the on/off valve) with the aim of maintaining the pressure constant in the system during the dynamic extraction; (3) the selecting valve located after the high pressure pump and the $N_2$ stream were deleted as 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 including an inlet valve (see INV in Fig. 1b) for developing the static extraction step.

Two Gilson Minipuls-3 low-pressure peristaltic pumps, three Rheodyne 5041 low-pressure injection valves, a laboratory-made column (5.0 cm in length and 4 mm I.D.) packed with $C_{18}$-Hydra, a 0.45-μm nylon filter (Technicon) and PTFE tubing of 0.8 mm I.D. were used to build the flow manifold shown in Fig. 2, where the filtration-preconcentration steps were carried out.

A 50-ml Soxhlet extractor (Probus, Barcelona, Spain) was used to carry out the reference EPA method 3540 and a rotary-evaporator (R-200, Büchi, Switzerland) was used to release the solvent after each Soxhlet extraction.

The extracts were analysed using a Varian CP 3800 gas chromatograph coupled to a Saturn 2200 ion trap mass spectrometer (Sugar Land, TX, USA). Separations were conducted on a CP-SIL 24 CB low bleed-MS wall-coated open tubular (WCOT) fused-silica capillary column, 30 m×0.25 mm I.D.×0.25 μm (Varian).

### 2.3. Procedures

#### 2.3.1. Static pressurised liquid extraction procedure

A 4-g amount of sample was weighed and placed into the extraction chamber in all experiments. After assembling the extraction chamber in the oven, the unit was filled with water pumped at a flow-rate of 2.5 ml/min (the selecting valve SV and the pressure valve PV remained in the required position). After pressurization of the system (150 bar), the oven was brought up to the working temperature (300 °C), and the extraction consisting of three static cycles of 10 min was performed. After each cycle, the pressure

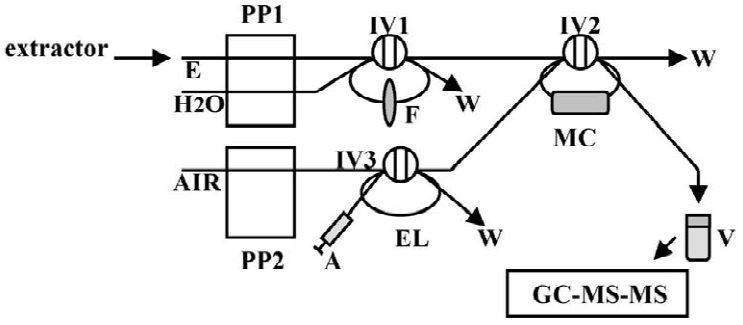


Fig. 2. Experimental setup for the on-line filtration-preconcentration step. E, extract; PP, peristaltic pump; IV, injection valve; F, filter; EL, elution loop; W, waste; MC, minicolumn; V, vial.

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 an $N_2$ stream purged the system for collection of the final cycle extract.

*2.3.2. Dynamic pressurised liquid extraction procedure*

This extraction mode involved the following steps: (1) the same amount of sample as the static mode (4 g) was placed in the extraction chamber; (2) the unit was connected to the system and filled with water pumped at 2.5 ml/min by closing the outlet valve in order to pressurise the system at 150 bar; (3) the oven was brought up to the working temperature (300 °C), and the outlet valve was then opened; (4) dynamic extraction was then performed by pumping the extractant through the oven at 0.5 ml $min^{-1}$ during 40 min using a restrictor to maintain constant pressure in the system.

*2.3.3. Static-dynamic pressurised liquid extraction procedure*

For this extraction mode, 4 g of soil were placed in the extraction chamber, which was located in the oven. Then, the system was filled with water by opening the inlet valve and closing the outlet valve (in this way, the system was pressurised at 150 bar), and the oven was brought up to 300 °C. The static extraction step was developed for 10 min by closing the inlet valve. After this time was elapsed, both the inlet and outlet valves were opened, and the high pressure pump was switched on, driving the extractant through the system at a 0.5 ml/min flow-rate for 12 min.

*2.3.4. On-line filtration-preconcentration procedure*

The extract from each static extraction cycle was firstly cleaned from particles by passage through a filter (F in Fig. 2), which was located in the loop of the injection valve (IV1). The filtrate was driven at 3 ml/min to a sorption column where the analytes were retained. The column was also located in the loop of an injection valve (IV2), thus, enabling elution in the direction opposite to retention. After the extract from the last extraction cycle was passed through the sorption column and before elution, an air stream was passed through the column at 5 ml/min for 1 min in order to remove residual aqueous phase from the column and connections. Meanwhile, the loop of the elution valve (IV3) was filled with 500 μl of acetonitrile (containing 2 μg/ml of 4-nitro-*p*-terphenyl as internal standard, I.S.) by using a syringe that aspirated the eluent from a bottle which was tightly stopped to avoid concentration changes of the I.S. On switching the elution valve, the analytes were eluted at 1 ml/min flow-rate and the eluate from the column collected in a glass vial containing anhydrous sodium sulphate. A 2-μl aliquot was injected into the gas chromatograph. Between samples, the filter was cleaned by passing water in the direction opposite to filtration at a high flow-rate, and the sorbent in the column was conditioned by circulating methanol and water through it.

*2.3.5. Reference EPA method 3540 procedure*

Four grams of spiked or real contaminated soil were 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 two to three boiling glass regulators. After extraction for 24 h, the solvent was released by a rotary-evaporator, the extract recompose with 0.5 ml of acetonitrile (containing 2 μg $ml^{-1}$ of I.S.) and a 2-μl aliquot injected into the gas chromatograph.

*2.3.6. GC–MS–MS analysis*

The analysis of the extracts was carried out using helium as a carrier gas at a constant pressure of 21 p.s.i. The column temperature program was 50 °C, held for 2.0 min, then increased at 20 °C/min 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 +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 the manifold temperatures were maintained at 280 and 50 °C, respectively. For nitro-PAHs 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 non-resonant) was adjusted in 10 sequential steps.

## 3. Results and discussion

### 3.1. Optimisation of MS–MS conditions

Both non-resonant and resonant CID were used to determine the best conditions for each compound. Non-resonant wave form was selected for all the analytes. The optimised MS–MS parameters are listed in Table 1. 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 utilised for the analysis of the nitro-PAHs of interest.

### 3.2. Optimisation of the on-line filtration/preconcentration

#### 3.2.1. Filtration

Solid particles, both those passing through the screw caps of the extraction chamber and those formed in cooling the extract, attained the preconcentration minicolumn and caused deterioration of the packed sorbent. For this reason, a continuous filtration system, similar to that reported previously [18], was located in-line between the extractor and the preconcentration minicolumn (see Section 2.3). The location of the filter in the loop of an auxiliary injection valve, IV1 in Fig. 1, allowed cleaning of the filter after use by passage of distilled water at high flow-rate in the direction opposite to filtration. The inclusion of the filter in the dynamic approach dramatically enlarged the sorbent minicolumn life, which was not deteriorated during all the subsequent experiments.

#### 3.2.2. Preconcentration

The study of the preconcentration step was performed using 10 ml of a standard solution containing 1 μg of each analyte. The order used for optimising this step was as follows:

##### 3.2.2.1. Sorbent and eluent

Different sorbents (silica, $C_{18}$ and $C_{18}$-Hydra) and eluents (acetonitrile, *n*-hexane, cyclohexane, dichloromethane and methanol) were tested. For the silica sorbent, the best eluent was dichloromethane; meanwhile for the $C_{18}$ sorbent, was methanol. However, better recoveries were observed using $C_{18}$-Hydra as sorbent and acetonitrile as eluent. Good recoveries were also obtained using methanol as eluent so mixtures of both solvents in different proportion were tested; however, none of the mixtures provided better recoveries than pure acetonitrile. Thus, $C_{18}$-Hydra and acetonitrile were selected

Table 1
Main parameters for the GC–MS–MS analysis of the nitro-PAHs extracted

| Compound | Parent ion (*m*/*z*) | CID[a] voltage (V) | ESL[b] (*m*/*z*) | Product ion (*m*/*z*) | $t_R$[c] (min) |
|---|---|---|---|---|---|
| 1-Nitronaphthalene | 173 | 59 | 76.0 | 129 | 11.4 |
| 3-Nitrobiphenyl | 199 | 71 | 87.6 | 153 | 12.6 |
| 2-Nitrofluorene | 211 | 98 | 92.9 | 165 | 14.5 |
| 3-Nitrofluoranthene | 247 | 72 | 99.0 | 217 | 17.9 |
| 1-Nitropyrene | 247 | 73 | 99.0 | 217 | 18.9 |

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

as sorbent and eluent, respectively, for subsequent experiments.

*3.2.2.2. Retention flow-rate*

The flow-rate at which the sample passes through the minicolumn was optimised by aspirating the standard solution to the minicolumn at flow-rates between 1.0 and 3.5 ml/min. The results obtained showed a maximum retention of the analytes at 3.0 ml/min, so it was selected for further experiments.

*3.2.2.3. Elution flow-rate and volume of eluent*

Both variables were optimised jointly taking into account that the elution kinetics influences the volume of eluent necessary for total elution, which intended to be the minimum possible in order to obtain the highest preconcentration factor. The carrier into which the acetonitrile was injected was air. Its usage had the aim of dragging away from the system the aqueous phase before passage of the eluent, thus avoiding both dilution and presence of water in the eluate. Elution flow-rates between 0.25 and 1.5 ml/min and volumes of eluent from 0.25 to 2 ml were assayed. Finally, an elution volume of 0.5 ml at 1.0 ml/min were the optimum values found.

*3.2.2.4. Breakthrough*

In order to determine the breakthrough of the $C_{18}$-Hydra minicolumn, the volume of sample containing 5 $\mu g\ ml^{-1}$ of each analyte passed through the sorption material was studied in the range 10–100 ml. The signal provided by the eluate remained constant up to 70 ml and decreased for higher volumes, so in subsequent experiments, volumes higher than 70 ml were not used so as not to surpass the breakthrough volume of the minicolumn. Under these conditions, quantitative retention and elution of the analytes was achieved.

### *3.3. Optimisation of the pressurised liquid extraction modes*

*3.3.1. Static pressurised liquid extraction*

A full two-levels factorial design was selected as screening method of the main variables affecting the extraction efficiency, namely temperature, pressure and static extraction time. These three variables were optimised in order to obtain the best recoveries in a single extraction cycle. Table 2 (static extraction mode) shows the upper and lower values tested for each variable in this first screening design. These values were selected from the available data and experience gathered in the preliminary experiments.

The main factor affecting the extraction of these analytes was the temperature, which was significant and with a positive effect for all the analytes; thus showing that higher values should be tested. The extraction time was a statistical significant variable for most of the analytes but not for 3-nitrobiphenyl. As the effect of this variable was also positive, higher values should also be tested. The pressure was not a significant variable, so the highest value tested, 150 bar, was selected for further experiments as this pressure was enough to maintain the water in the liquid state under the temperatures used.

A second full two-levels factorial design was performed in order to test higher temperatures and longer extraction times (Table 2, static extraction mode). In this case, the time was not a significant variable for the analytes removal in the range under study, so the lowest value tested (10 min) was selected for further experiments in order to make the whole extraction process as short as possible. The temperature was significant with a positive effect on the response. Thus, higher values should be tested. However, higher temperatures cause deterioration of the system by increasing water corrosiveness. It would make mandatory the use of special materials (e.g., Hastelloyd) which would lead to the increase of total costs. For this reason, the highest value tested (300 °C) was selected for subsequent experiments.

Finally, several static extraction cycles were performed under the optimised extraction conditions in order to obtain quantitative recovery of the analytes. After two static extraction cycles, three of the analytes studied (3-NB, 2-NF and 3-NFa) were quantitatively extracted; while three static cycles (30 min) were necessary for quantitative removal of 1-NN and 1-NP.

*3.3.2. Dynamic pressurised liquid extraction*

The variables affecting this extraction mode (namely, temperature, extractant flow-rate and extraction time) were also studied by means of a full two-level factorial screening design (Table 2, dy-

Table 2
Optimisation of the extraction modes

| Extraction mode | Variable | Tested range | Analysis result | Effect on analyte removal | Optimum value |
|---|---|---|---|---|---|
| Static | *Screening study* | | | | |
| | Temperature (°C) | 100–200 | Significant | Positive | – |
| | Pressure (bar) | 50–150 | Non-significant | – | 150 |
| | Extraction time (min) | 5–10 | Significant | Positive | – |
| | Number of cycles | 1 | – | – | – |
| | *Full factorial design* | | | | |
| | Temperature (°C) | 200–300 | Significant | Positive | 300 |
| | Extraction time (min) | 10–20 | Non-significant | – | 10 |
| | Number of cycles | 1–4 | Significant | Positive | 3 |
| Dynamic | *Screening study* | | | | |
| | Temperature (°C) | 100–200 | Significant | Positive | – |
| | Flow-rate (ml/min) | 0.5–1.5 | Significant | Negative | 0.5 |
| | Extraction time (min) | 5–15 | Significant | Positive | – |
| | *Full factorial design* | | | | |
| | Temperature (°C) | 200–300 | Significant | Positive | 300 |
| | Extraction time (min) | 15–30 | Significant | Positive | – |
| | *Kinetic study* | | | | |
| | Extraction time (min) | 30–50 | Significant | Positive | 50 |

namic extraction mode). The pressure was not considered as not influence was observed in the optimisation of the static mode; thus, 150 bar was used to carried out the extractions.

The study showed that all the variables were significant in the range under study for most analytes. The temperature was the key variable again showing the highest significant and positive effect on the analytes removal. The extraction time also had a positive effect on the analytes recovery. The flow-rate showed a significant and negative effect for most analytes but not for 1-NP and 3-NFa for which it had a positive but non-significant effect. Thus, higher temperatures and longer extraction times should be tested as well as lower flow-rates. The lowest flow-rate already tested was 0.5 ml/min, it was selected as optimum as lower flow-rates were difficult to maintain in a reproducible manner.

Higher temperatures and longer extraction times were tested (Table 2, dynamic extraction mode) by means of a full two-level factorial design. The analysis of the design showed that temperature was a significant variable for all the analytes studied except for 3-NB. As it effect on the analytes removal was positive, higher temperatures should be tested. However, for the reason stated before, 300 °C was selected for further experiments. The extraction time was significant and positive for all the compounds, so a study of the extraction kinetics was performed in order to determined the time necessary for total removal of the analytes. The other extraction variables were fixed at their optimum values. After 40 min, 3-NB, 2-NF and 3-NFa were quantitatively extracted but 50 min was necessary for total removal of 1-NN and 1-NP. For this reason, 50 min was selected as optimum extraction time.

### *3.3.3. Static-dynamic pressurised liquid 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. A single static extraction, ranging between 10 and 15 min, was combined with a dynamic step (between 5 and 20 min). The temperature was fixed at 300 °C as it was the optimum variable for both modes studied separately, the pressure was fixed at 150 bar and the flow-rate for the dynamic step was also fixed at 0.5 ml/min, which was the optimum value found previously. Table 3 shows the results obtained for the

Table 3
Experiments and results (expressed as %recovery, $n=3$) for the optimisation of the static-dynamic mode

| Expt. | Static time[a] | Dynamic time[a] | Total time[a] | 1-NN (%) | 3-NB (%) | 2-NF (%) | 3-NFa (%) | 1-NP (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 5 | 15 | 47.5 | 68.8 | 62.0 | 64.4 | 50.1 |
| 2 | 15 | 5 | 20 | 61.3 | 74.1 | 69.8 | 74.5 | 66.6 |
| 3 | 10 | 10 | 20 | 74.5 | 86.9 | 83.2 | 89.2 | 79.2 |
| 4 | 15 | 10 | 25 | 86.3 | **99.3** | **97.2** | **100.2** | 84.1 |
| 5 | 10 | 15 | 25 | **96.8** | **98.5** | **103.2** | **98.1** | **99.0** |
| 6 | 15 | 15 | 30 | **102.9** | **99.1** | **102.6** | **99.9** | **104.3** |
| 7 | 10 | 20 | 30 | **103.4** | **100.4** | **97.7** | **101.6** | **100.5** |

[a] Expressed as min.

seven possible combinations of both modes, considering that the total extraction time never surpassed the optimum extraction time obtained in the static extraction mode previously optimised (30 min), which was much shorter than the dynamic one. As can be seen, quantitative recovery of all analytes was obtained for the experiments 5, 6 and 7 (see Table 3). With the experiment number 4, 3-NB, 2-NF and 3-NFa were quantitatively recovered but the efficiency of the extraction was lower than 90.0% for 1-NN and 1-NP. In order to make the extraction as short as possible, the experiment 5 was selected as optimum with a total extraction time of 25 min (10 min static extraction +15 min dynamic extraction), which was lower than those provided for both the static (30 min) and the dynamic mode (50 min), thus showing the suitability of a combination of both operational modes to shorten the time needed for quantitative recovery of nitro-PAHs from soil.

### 3.4. Features of the proposed methods

To confirm that the methods are suitable for its intended use, basic analytical requirements such as precision, linear dynamic range and both instrumental and method detection limits were evaluated.

#### 3.4.1. Precision

In order to evaluate not only the extraction efficiencies of the proposed methods but also the precision expressed as within laboratory reproducibility and repeatability were estimated in a single experimental set-up with duplicates [19] for every operational mode. The experiments were carried out using 4 g of soil in all instances. In all the experiments the optimal values obtained for the variables were used. Two extractions and measurements of the target compounds per day were carried out on 7 days. The repeatability, expressed as percent relative standard deviation, was between 3.5 and 5.2% for the static operational mode and between 4.7 and 6.2% for the dynamic one; meanwhile values ranging from 2.1 to 5.2% were obtained for the static-dynamic mode. The within-laboratory reproducibility study also showed similar results between the static (5.1–8.4%) and the static-dynamic (5.3–8.0%) modes and slightly higher values (7.1–10.0%) for the dynamic one (Table 4).

#### 3.4.2. Linear dynamic ranges and instrumental limits of detection (LODs)

These parameters were common for the three operational modes developed as the identification-quantification step was exactly the same in all instances. The calibration curves were run using standard solutions of the analytes in chromatographic-grade acetonitrile. The concentrations of the standards fitted within the linear portion of the calibration curve were between 30 and 2200 ng $ml^{-1}$ for

Table 4
Evaluation of the precision of the proposed extraction modes

| Analyte | Static mode | | Dynamic mode | | Static-dynamic mode | |
|---|---|---|---|---|---|---|
| | Sr | $S_{WR}$ | Sr | $S_{WR}$ | Sr | $S_{WR}$ |
| 1-NN | 4.0 | 8.4 | 6.2 | 9.3 | 5.2 | 8.0 |
| 3-NB | 3.5 | 5.9 | 4.7 | 7.3 | 2.1 | 5.3 |
| 2-NF | 5.2 | 6.1 | 4.9 | 7.8 | 4.4 | 6.5 |
| 3-NFa | 3.8 | 5.1 | 6.0 | 7.1 | 2.8 | 6.1 |
| 1-NP | 4.4 | 8.1 | 5.3 | 10.0 | 5.1 | 6.9 |

Sr, repeatability relative standard deviation; $S_{WR}$, within-laboratory reproducibility relative standard deviation.

Table 5
Linear dynamic ranges, regression coefficient, limits of detection and method detection limits for the analytes studied

| Analyte | Linear range[a] | Calibration curve | $r^2$ | LOD[b] | MDL[c] |
|---|---|---|---|---|---|
| 1-NN | 30–2200 | $y = 4.8 + 0.3x$ | 0.9987 | 30.0 | 1.9 |
| 3-NB | 10–2200 | $y = 143.8 + 3.7x$ | 0.9897 | 6.1 | 0.4 |
| 2-NF | 10–2200 | $y = 40.7 + 1.1x$ | 0.9963 | 9.2 | 0.6 |
| 3-NFa | 30–2200 | $y = 240.1 + 3.5x$ | 0.9965 | 7.3 | 0.5 |
| 1-NP | 10–2200 | $y = 86.3 + 1.2x$ | 0.9961 | 17.6 | 1.1 |

[a] Linear dynamic range (expressed as ng/ml).
[b] Limit of detection (expressed as pg).
[c] Method detection limit (expressed as ng/g).

1-NN and 3-NFa and between 10 and 2200 ng $ml^{-1}$ for 3-NB, 2-NF and 1-NP. The formula of the calibration curve for each analyte as well as the correlation coefficients are shown in Table 5. The instrumental (LODs), expressed in picograms entering on-column and reaching the detector, which gives a signal that is $3\sigma$ above the mean blank signal (where $\sigma$ is the standard deviation of the blank signal), ranged between 6 pg for 3-NB to 30 pg for 1-NN (Table 5).

### 3.4.3. Method detection limit

As the amount of sample used in the three operational modes was the same, this parameter was also common to all them. The method detection limits (MDL) were estimated from the statistical information derived from the standard calibration curves used to determine the instrumental LOD [20]. The MDLs was estimated from the LOD multiplied by the final volume and divided by the sample mass and injected volume [MDL=(LOD×final volume)/(sample mass×injected volume)] [21]. Table 5 shows the MDL values obtained for each compound. They ranged between 1.9 ng $g^{-1}$ for 1-NN and 0.4 ng $g^{-1}$ for 3-NB.

## 3.5. Application of the proposed approaches to natural contaminated soils. Comparison with the reference EPA method 3540

Due to the lack of certified reference materials containing nitro-PAHs, the proposed methods were validated, in terms of extraction efficiency, by comparison with the reference EPA method 3540. The methods were applied to the extraction of the analytes in two soil samples naturally contaminated, provided by EMGRISA. The concentration of PAHs in these samples was known, but not that of nitro-PAHs. Table 6 shows the average estimated soil concentration obtained by the proposed methods and by the EPA method for each analyte as well as the $f$ value (amount of analyte extracted by PLE/amount of analyte extracted by EPA method 3540). As can be seen, the $f$ factor ranged between 0.9 and 1.2 for the static mode (Table 6, static mode), between 0.7 and 0.8 for the dynamic mode (Table 6, dynamic mode) and between 0.9 and 1.1 in the case of the static-dynamic mode (Table 6, static-dynamic mode). As can be seen, similar estimated soil concentrations were provided by both, the static and the static-dynamic modes showing the suitability of both approaches for the extraction of these compounds from natural contaminated soil. However, for the dynamic mode, 50 min of extraction was enough for quantitatively removal of the analytes from a spiked sample but not from the natural contaminated soils where the analytes are more strongly retained. A longer dynamic extraction time should be performed to obtain results similar to those provided by the EPA method. Longer dynamic extraction times were tested using both soils. Results similar to those provided by the EPA method (considering $f>0.9$) were obtained after 70 min of dynamic extraction time for soil A, while 65 min was necessary for soil B (Table 6, static-dynamic mode).

## 3.6. Comparison of the static, dynamic and static-dynamic operational modes

The three operational modes were compared in

Table 6
Application of the proposed approaches to natural contaminated soils; comparison with the reference EPA method 3540 (estimated soil concentration expressed as ng/g; $n=3$)

| Mode | Analyte | Soil A | | | Soil B | | |
|---|---|---|---|---|---|---|---|
| | | PLE method | EPA method | $f$ | PLE method | EPA method | $f$ |
| Static | 1-NN | 9.0±0.9 | 8.5±0.7 | 1.1[a] | 5.2±0.8 | 5.7±0.9 | 0.9[a] |
| | 3-NB | 9.7±1.0 | 9.5±0.5 | 1.0 | 10.2±1.3 | 9.3±0.4 | 1.1 |
| | 2-NF | 87.3±2.9 | 90.2±3.0 | 1.0 | 100.2±4.6 | 104. 2±2.4 | 1.0 |
| | 3-NFa | 199.3±10.1 | 199.6±9.4 | 1.0 | 229.6±10.5 | 246.7±8.3 | 0.9 |
| | 1-NP | 6.1±1.4 | 5.0±1.0 | 1.2 | 16.2±2.1 | 15.2±0.9 | 1.1 |
| Dynamic | 1-NN | 6.7±0.6[c] | 8.5±0.7 | 0.8[b] | 4.6±0.9 | 5.7±0.9 | 0.8[b] |
| | | (7.9±0.4)[d] | | (0.9) | (6.4±0.8) | | (1.1) |
| | 3-NB | 7.9±0.9 | 9.5±0.5 | 0.8 | 7.4±0.5 | 9.3±0.4 | 0.8 |
| | | (9.7±1.2) | | (1.0) | (9.1±0.4) | | (0.1) |
| | 2-NF | 72.2±4.1 | 90.2±3.0 | 0.8 | 86.5±3.2 | 104. 2±2.4 | 0.8 |
| | | (86.6±4.5) | | (1.0) | (110.5±4.2) | | (1.0) |
| | 3-NFa | 151.1±9.8 | 199.6±9.4 | 0.7 | 202.2±10.6 | 246.7±8.3 | 0.8 |
| | | (197.6±8.7) | | (1.0) | (254.1±9.1) | | (1.0) |
| | 1-NP | 4.1±0.8 | 5.0±1.0 | 0.8 | 11.9±1.7 | 15.2±0.9 | 0.8 |
| | | (4.6±0.5) | | (0.9) | (14.2±0.8) | | (0.9) |
| Static-dynamic | 1-NN | 7.8±0.6 | 8.5±0.7 | 0.9[e] | 6.1±0.5 | 5.7±0.9 | 1.1[e] |
| | 3-NB | 10.6±0.6 | 9.5±0.5 | 1.1 | 10.6±0.7 | 9.3±0.4 | 1.1 |
| | 2-NF | 88.4±2.2 | 90.2±3.0 | 1.0 | 95.9±2.7 | 104. 2±2.4 | 0.9 |
| | 3-NFa | 227.5±7.4 | 199.6±9.4 | 1.1 | 271.3±6.4 | 246.7±8.3 | 1.1 |
| | 1-NP | 4.7±0.9 | 5.0±1.0 | 0.9 | 14.5±1.1 | 15.2±0.9 | 1.0 |

[a] Amount of analyte extracted by three cycles static-PLE/amount of analyte extracted by EPA method 3540.
[b] Amount of analyte extracted by dynamic-PLE/amount of analyte extracted by EPA method 3540.
[c] Values obtained using 50 min of dynamic extraction.
[d] Values obtained using 70 and 65 min of dynamic extraction for soil A and B, respectively.
[e] Amount of analyte extracted by static (10 min)-dynamic (15 min)-PLE/amount of analyte extracted by EPA method 3540.

terms of experimental set-up, precision and extraction time.

The experimental set-up 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 the static-dynamic modes, 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.

Concerning the extraction time, the static-dynamic mode was the shortest one as it took only 25 min for total removal of the analytes; meanwhile the static and the dynamic mode needed 30 and 50 min (65–70 for the natural contaminated soil), respectively. It is clear that the worst operational mode in this case was the dynamic one. Regarding the time needed to carry out the filtration-preconcentration step, it directly depended on the total volume of extract obtained. The highest volume of extract was provided by the dynamic mode (between 25 and 35 ml). The static-dynamic mode provided the smaller volume (around 10 ml) compared with the around 15 ml (≈5 ml for each cycle) obtained when the static mode was performed.

## 4. Conclusions

A comparison of the three pressurised liquid extraction modes (namely, static, dynamic and static-dynamic) for the extraction of nitro-PAHs from both spiked and natural contaminated soils has been

performed. The use of a flow injection interface through which every pressurised extractor used was connected on-line with a filtration-preconcentration system has allowed the development of partially automated methods.

The results of the comparison of the three operational modes clearly shown that the static-dynamic mode constitutes the best option, providing efficiencies similar to those provided by the reference EPA method 3540 in a time shorter (25 min vs. 24 h). The performance of three 10-min static extraction cycles provided similar efficiencies and precision but in a longer time. The worst mode in terms of precision and extraction time needed was the dynamic mode.

All the extraction modes were performed using water as extractant, thus providing environmentally friendly methods that avoided the use of organic solvents.

Additional advantages derived from the use of GC–MS–MS for individual separation-identification/quantification are the high selectivity achieved, thus avoiding interference problems and the high sensitivity, allowing detection limits of the analytes at low pg levels.

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