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# Development of a sample preparation procedure of sewage sludge samples for the determination of polycyclic aromatic hydrocarbons based on selective pressurized liquid extraction

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ABSTRACT

An automated, simple and sensitive method based on selective pressurized liquid extraction (SPLE) was developed for the analysis of polycyclic aromatic hydrocarbons in sewage sludge samples. The new sample preparation procedure consists of on-line clean-up by inclusion of sorbents in the extraction cell, and combines elevated temperatures and pressures with liquid solvents to achieve fast and efficient removal of target analytes from complex sewage sludge matrices. The effects of various operational parameters (e.g. sample pretreatment, extraction solvent, temperature, pressure, static time, etc.) on the performance of SPLE procedure were carefully investigated, obtaining the best results when SPLE conditions were fixed at 140 °C, 1500 psi, static time of 5 min and n-hexane as extraction solvent. A new programmed temperature vaporization–gas chromatography–tandem mass spectrometry method based on large volume injection (PTV–LVI–GC–MS/MS) was also developed and analytical determinations were performed by high performance liquid chromatography coupled with fluorescence detection and GC–MS/MS. The extraction yields for the different compounds obtained by SPLE ranged from 84.8% to 106.6%. Quantification limits obtained for all of these studied compounds (between 0.0001 and 0.005 $\mu g\,g^{-1}$, dry mass) were well below the regulatory limits for all compounds considered. To test the accuracy of the SPLE technique, the optimized methodology was applied to the analysis of a certified reference material (sewage sludge (BCR088)) and a reference material (sewage sludge (RTC-CNS312-04)), with excellent results.



## 1. Introduction

Sewage sludge may be a useful material to be used in agriculture as fertilizer or soil conditioner because of its high organic matter content, as well as nitrogen and phosphorous concentrations. However, because of the physical–chemical processes involved in wastewater treatment, sludge tends to concentrate pollutants with low water solubility and adsorption capacity [1].

High concentrations of different contaminants have been found in sewage sludge samples [2–4]. Consequently, the application of sewage sludge on agricultural land can lead to the accumulation of toxic pollutants and pathogenic microorganism in soils, plants and grazing animal [5], as well as enter in the food chain or be transported toward rivers or groundwater [6].

Polycyclic aromatic hydrocarbons (PAHs) are well-known carcinogens and mutagens [7] which are present in sludges [8–9]. In wastewater treatment plants, PAHs are almost completely removed from wastewater (up to 90%), being concentrated in sludge because of their poor solubility in water and high adsorption capacity on solid particles [2]. As a consequence, the range of PAH concentrations in sewage sludge is varying widely from a few micrograms up to several hundred milligrams per kg of dry matter and depends on the origin of wastewater as well as the mineralization level of organic matter [9,10].

In order to improve the sewage sludge management and control the quality of the sludge that is going to be applied to soil, the third draft of a European Union (EU) directive has set maximum acceptable concentrations of some organic compounds. The total concentration of PAHs (sum of 11 compounds) in sewage sludge for agriculture use, have been regulated to 6 $mg\,kg^{-1}$ dm (dry mass) [11].

One of the main problems for the implementation of the future EU directive is the need of accurate, sensitive, rapid and low cost procedures for the routine determination of these and other pollutants in sewage sludge. Sample preparation and especially extraction is a critical step in PAH analyses because these hydrophobic compounds are strongly sorbed to the solid materials. Traditionally, Soxhlet extraction has been used for the extraction of PAHs in sewage sludges [12–13], but this procedure does not provide enough energy to release the analytes

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rapidly. In consequence, it requires very long extraction times and large amounts of organic solvents. New approaches have been used in order to overcome these disadvantages and improve automation. Thus, microwave assisted extraction (MAE) [14], supercritical fluid extraction [15], ultrasonic extraction (USE) [9], matrix solid-phase dispersion (MSPD) [16] and pressurized liquid extraction (PLE) [8,12,13,17] have been proposed as alternative techniques.

The clean-up of the extracts is also a very important step in determination of PAHs in sludge samples. Selective extraction of specific compound from sewage sludge is a very complicated task because it can contain a large variety of pollutants as well as organic matter, especially lipidic substances. Thus, in spite of a good optimization of extraction parameters to obtain a selective method, complex mixtures of organic compounds and matrix components are frequently present in the extracts and must be eliminated in order to achieve accurate analytical determinations. Solid-phase extraction [14,18], gel permeation chromatography [12] and selective inmunosorbents [19] are usually employed to clean sludge extracts before final separation and quantification.

PLE is a low solvent consuming, fast, effective and automated extraction technique, which was introduced in 1996 [20–21]. PLE is an attractive technique because 24 samples can be processed sequentially in unattended operation. Moreover different sample sizes can be accommodated and filtration is not required after extraction [21]. PLE offers also the possibility of controlling the selectivity of the extraction by loading a stationary phase in the extraction cell. The amount of co-extracted interferences can be reduced by adding different sorbents to the PLE cells. Additionally, the sample itself can also be mixed and dispersed with the same or with a different sorbent. Thus, a one-step PLE method avoids the exhaustive clean-up of extracts prior to analysis increasing the possibilities of automation.

PLE has been chosen for the extraction of a wide range of compounds from various matrices [22]. Some examples are the extraction of parabens and triclosan from indoor dust [23], organophosphate triesters from sediments [24] and pharmaceuticals [25] from sewage sludge samples. In particular, it has been used for extraction of PAHs from soils [26], sediments [27], atmospheric particulate matter [28], pine needles [29], food and biological samples [30] and sewage sludges [8,12,13,17].

Some comparative studies carried out between PLE and conventional techniques, such as Soxhlet extraction, showed that the performance of PLE was consistently equivalent or better than traditional methods [8]. Flotron et al. compared traditional techniques (Soxhlet and USE) with PLE and MAE for the effectiveness of extracting PAHs from sewage sludge and PLE appeared to be a promising technique, giving high recoveries with moderate extraction times and solvent volumes [13].

The aim of this study was to develop and validate a new selective pressurized liquid extraction (SPLE) method that integrates exhaustive extraction with in-cell clean-up. The optimized SPLE procedure allows the selective extraction of 19 PAHs (naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Anth), fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (B[a]A), chrysene (Chry), 5-methylchrysene (5-MC), benzo[*e*]pyrene (B[e]P), benzo[*b*]fluoranthene (B[b]F), benzo[*k*]fluoranthene (B[k]F), benzo[*a*]pyrene (B[a]P), dibenzo[*al*]pyrene (DB[al]P), dibenz[*ah*]anthracene (DB[ah]A), benzo[*ghi*]perylene (B[ghi]P) and indeno[1,2,3-*cd*]pyrene (I[1,2,3-cd]P)) from sewage sludge, obtaining extracts clean enough to be analyzed directly by two different chromatographic methods, and leading to substantial time and solvent savings as well as less sample manipulation compared to PLE with off-line clean-up.

The influence of the different experimental parameters (e.g. sample pretreatment, extraction solvent, temperature, pressure, static time, etc.) on the yield of the sample preparation step was investigated and operating conditions were optimized. Moreover, a new programmed temperature vaporization–gas chromatography–tandem mass spectrometry (PTV-GC–MS/MS) method based on large volume injection (LVI) was developed and subsequently employed to analyze sewage sludge extracts.

A certified reference material (certified sludge (BCR088)) and a reference material (sewage sludge (RTC-CNS312-04)) were used to validate the proposed method. Finally, the applicability of the developed procedure was tested by the determination of PAHs in some real sewage sludge samples.

## 2. Experimental

### 2.1. Reagents, standards and materials

Elemental analyses were carried out in shared research facilities at the University of Santiago de Compostela.

An ultrasonic water bath was purchased from Selecta Ultrasounds (Barcelona, Spain). A Turbo Vap II automated nitrogen evaporator (Zymark, Hopkinton, MA, USA) was used to evaporate the extracts obtained by SPLE.

Acetonitrile (ACN) and methanol (gradient-grade, Lichrosolv), n-hexane, dichoromethane and acetone (Suprasolv) were purchased from Merck (Darmstadt, Germany). Ultrapure water was produced by means of a Milli-Q system supplied by Millipore (Bedford, MA, USA). Anhydrous sodium sulphate was supplied by Panreac (Barcelona, Spain). EPA-610 Polycyclic aromatic hydrocarbon mixture and B[e]P (solid, 98.5%) were supplied by Supelco. 5-MC (10 $\mu g\,mL^{-1}$) and DB[al]P (10 $\mu g\,mL^{-1}$) were from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Potassium hydroxide (Pellets, 85%+, AC), Florisil (60–100 mesh), aluminium oxide activated neutral (150 mesh) and sea sand (50–70 mesh) were purchased from Sigma–Aldrich (Madrid, Spain), and silica gel (230–400 mesh) was obtained from Merck. Silica gel and Florisil contain appreciable traces of PAHs and consequently required washing in columns with a hexane–acetone (50:50, v/v) mixture and were kept stored dry before use. BCR 088 was supplied by the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) and RTC-CNS312-04 reference material was obtained from LGC Standards (Teddington Middlesex, UK).

Cellulose filters for ASE 200 extraction cells (20 mm) was obtained from Restek and Durapore filters (Millex GV, 13 mm, 0.22 $\mu m$) were supplied by Millipore.

### 2.2. Samples

Optimization of SPLE parameters was carried out with a pool of different sewage sludge samples, with total carbon content of 32.6%. The pooled sample was obtained by mixing four different sewage sludge samples from urban sludge treatment plants located in the area of Galicia.

The reference materials, BCR 088 and RTC-CNS312-04, are dried sewage sludges. Additionally, real samples were used to test the applicability of proposed method. Six secondary sewage sludge samples were collected in different wastewater treatment plants in Galicia.

The moisture content of the materials was gravimetrically evaluated after drying sample portions of materials in an oven at 105 °C for 12 h. The lipid content of samples was evaluated by means of the Bligh and Dyer method [31]. Information about studied samples is compiled in Table 1. As can be seen, materials with different characteristics (lipid content and total carbon) were taken for this study.

**Table 1**
Properties of analyzed sewage sludge samples.

| | Sewage sludge samples | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pooled sample | S1 | S2 | S3 | S4 | S5 | S6 | BCR088 | RTC-CNS312-04 |
| Moisture content (%) | 8.9 | 6.6 | 5.4 | 3.8 | 10.3 | 34.1 | 17.0 | 12.1 | 8.4 |
| Lipid content (%) | 6.2 | 6.3 | 8.9 | 1.3 | 20.8 | 13.2 | 15.8 | 9.0 | 6.1 |
| Elemental analysis (%) | | | | | | | | | |
| Carbon | 32.6 | 38.0 | 35.9 | 21.7 | 42.3 | 33.0 | 50.0 | 27.1 | 30.4 |
| Nitrogen | 5.5 | 6.4 | 6.1 | 2.6 | 3.1 | 3.4 | 3.1 | 3.1 | 4.3 |
| Sulphur | 0.8 | 0.9 | 0.8 | 0.2 | 1.0 | 0.3 | 0.2 | 1.6 | 1.3 |

Spiked samples were prepared by adding a standard solution of analytes in acetonitrile. Pooled sewage sludge sample was spiked with PAHs at levels between 0.05 and 5.72 $\mu g\,g^{-1}$, taking into account the concentration values in blank sample. The spiking procedure consisted of mixing, forty grams of pool with 10 mL of a standard solution of studied PAHs in ACN. The mixture was mechanically stirred and allowed to air dry at room temperature for 24 h with occasional mixing. Then, the solvent was slowly evaporated under frequent homogenization. This procedure was carried out several months before sample analysis and all spiked and non-spiked samples once prepared were lyophilized and stored in amber glasses at 4 °C.

### 2.3. SPLE procedure

Extractions were performed on an ASE 200 system (Dionex, Co., Sunyvale, CA, USA) equipped with a 24-sample carousel, 11-mL stainless steel cells, and 40-mL collection vials. PLE cells were precleaned by sonication with a hexane–acetone (1:1, v/v) mixture for 15 min in order to obtain reproducible blanks and avoid the presence of interfering compounds that could difficult the trace level determination of PAHs.

Under the final conditions, 0.2 g (weighed accurately) of lyophilized sample was first soaked in 1 mL of saturated potassium hydroxide solution in methanol (KOH (MeOH) sat.) in a glass mortar, then 1 g of Florisil and 0.5 g of anhydrous sodium sulphate ($Na_2SO_4$) were added and the mixture was thoroughly blended with the pestle to obtain a homogeneous mixture.

Two cellulose filters were placed at the bottom of each 11 mL cell to avoid the collection of suspended powders in the extraction. A thin layer of sand followed by 1 g of Florisil and 2 g of silica were introduced in the cell in order to perform in situ clean-up. After loading the clean-up sorbents, the homogeneous sample mixture was transferred to cells. The remaining volume of the cell was filled up with sand in order to reduce the void volume in the cells and avoid solvent channelling as well as an undesirable increase in the total extract volume. Finally, a third cellulose filter was placed on the top and the cell was tightly closed and placed into the carousel of the ASE system.

All extractions were performed by preheating the cell for 6 min before filling with solvent (preheat method). PAHs were removed using n-hexane in a single static extraction cycle at 140 °C and 1500 psi for 5 min. The total flush volume and the cell purge time were 11 mL (100% of its capacity) and 60 s, respectively.

Three milliliters of the total SPLE extract (21 ± 1 mL) was concentrated to 0.5 mL under a stream of nitrogen in the Turbo Vap. Then, 2 mL of ACN was added and the mixture again concentrated to 0.5 mL. The final concentrated extract was then transferred to a 1 mL volumetric flask and the volume made up to the mark with water. The extract was filtered through a 0.22 μm Durapore filter and 20 μL were injected into the high performance liquid chromatography (HPLC) system.

Obviously, the solvent change stage was not necessary to GC–MS/MS analysis. Therefore, 1 mL of SPLE extract was directly filtered through a 0.22 μm Durapore filter and 25 μL was injected into the GC–MS/MS system.

### 2.4. Instrumental analysis

#### 2.4.1. HPLC-fluorescence detection analysis

HPLC chromatographic separations were developed in a system comprising a 600E pump with a gradient controller (Waters, Milford, MA, USA) and a fluorescence detector (Flu) (HP Series 1100-Agilent, Waldbrom, Germany). The injector (Rheodyne Model 7725i, Cotati, CA, USA) was fitted with a 20 μL loop. Analytical column temperatures were controlled with a MetaTherm 9540 oven (MetaChem, Torrance, CA, USA). The analytical column was a 250 mm × 4.6 mm I.D. Waters PAH $C_{18}$ column (particle size 5 μm). A Waters guard-pak, with Nova-Pak $C_{18}$ inserts, was used to protect the analytical column (both purchased from Waters). Agilent Chemstation Software (Rev. A. 06.03 [509]) was used for data acquisition.

A binary solvent system made of ACN and water was used for chromatographic separations at 1.5 mL $min^{-1}$. The gradient elution program was as follows: initial conditions, 50% ACN for 3 min, then a linear ramp to 100% ACN within 17 min and holding at 100% for 8 min. The column temperature was set at 35 °C. Detection was performed at selected fluorescence wavelength programming to obtain the better sensitivity and minimal interference. The excitation/emission wavelengths pairs (nm) were set as follows: 267/330 for Naph; 275/315 for Ace and Flu; 247/357 for Phe; 238/418 for Anth, Flt and Pyr; 286/410 for B[a]A, Chry and 5-MC; 294/425 for B[e]P, B[b]F, B[k]F, B[a]P, DB[al]P, DB[ah]A and B[ghi]P; and 245/500 for I[1,2,3-cd]P.

#### 2.4.2. PTV–LVI–GC–MS/MS analysis

In order to validate the developed analytical methodology and for comparative purposes, some sewage sludge samples were also analyzed by GC–MS/MS method, using LVI.

The PTV–LVI–GC–MS/MS analysis was performed using a Varian 450-GC gas chromatography (Varian Chromatography Systems, Walnut Creek, CA, USA) coupled to an ion trap mass spectrometer Varian 240-MS with a waveboard for multiple MS ($MS_n$) analysis operating in the external trap mode. The chromatograph was equipped with an automatic injector CP-8400 autosampler and a 1079 programmed temperature vaporization injector (both from Varian) with a split liner with frit (3.4 mm × 5.0 mm × 54 mm, Siltek deactivated) (Restek, Bellefonte, PA, USA). The system was operated by Varian MS Workstation v6.9.1 software. Separation was carried out on a J&W HP-5MS capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness) from Agilent Technologies (Palo Alto, CA, USA).

Helium (purity 99.999%; Carburos Metálicos, A Coruña, Spain) was employed as carrier gas at a constant column flow of 1.5 mL $min^{-1}$. The GC oven temperature was programmed from 60 °C (held 3 min) to 230 °C at 15 °C $min^{-1}$; then, ramped to 250 °C $min^{-1}$ at 10 °C $min^{-1}$, increased at a rate of 3 °C $min^{-1}$ up to 280 °C (held 3 min) and finally increased to

**Table 2**
Selected MS/MS experimental parameters and retention times of target compounds.

| Compound | Retention time (min) | Parent ion (*m*/*z*) | Excitation storage level (V) | Excitation amplitude (V) | Quantification ion (*m*/*z*) |
|---|---|---|---|---|---|
| Naph | 7.72 | 128 | 48.8 | 1.4 | 102 |
| Acy | 10.32 | 152 | 57.9 | 2.0 | 150 |
| Ace | 10.60 | 153 | 58.3 | 1.2 | 152 |
| Flu | 11.46 | 166 | 63.3 | 1.2 | 165 |
| Phe | 13.01 | 178 | 67.8 | 1.7 | 176 |
| Anth | 13.09 | 178 | 67.8 | 1.7 | 176 |
| Flt | 14.96 | 202 | 77.0 | 2.8 | 200 |
| Pyr | 15.36 | 202 | 77.0 | 2.8 | 200 |
| B[a]A | 17.92 | 228 | 86.9 | 2.5 | 226 |
| Chry | 18.01 | 228 | 86.9 | 2.5 | 226 |
| B[b]F | 21.29 | 252 | 96.0 | 3.7 | 250 |
| B[k]F | 21.38 | 252 | 96.0 | 3.7 | 250 |
| B[e]P | 22.29 | 252 | 105.2 | 3.4 | 250 |
| B[a]P | 22.48 | 252 | 105.2 | 3.4 | 250 |
| I[1,2,3-cd]P | 27.01 | 276 | 105.2 | 4.3 | 274 |
| DB[ah]A | 27.18 | 278 | 105.9 | 3.8 | 276 |
| B[ghi]P | 28.18 | 276 | 105.2 | 4.3 | 274 |

300 °C at 3 °C min$^{-1}$ (held 2 min). The total running time was 38 min.

Aliquots of 25 μL of sample extract were injected into the GC system operating at a syringe injection flow rate of 20 μL s$^{-1}$. The initial injector temperature of 60 °C was held for 0.3 min and then increased at 100 °C min$^{-1}$ to 300 °C (held 25 min). The injector split ratio was initially set at 20:1. The splitless mode was switched on from 0.3 to 3 min. At 3 min, the split ratio was set at 50:1 and reduced to 20:1 at 10 min. Cryogenic cooling with $CO_2$ was applied when the injector temperature was 280 °C in order to reach the initial injector temperature as fast as possible before continuing with the next injection.

The ion trap mass spectrometer was operated in the electron impact (EI) ionization positive mode (+70 eV) using an external ionization configuration. The trap, manifold and transfer line temperatures were maintained at 220, 40 and 300 °C, respectively. General parameters were as follows: filament/multiplier delay, 6.5 min; multiplier offset +200 V, filament emission current 90 μA, automatic gain control target value 5000 counts, and collision induced dissociation (CID) waveform, resonant. Specific conditions for each target compound are listed in Table 2. The target analytes were identified by retention times and EI-MS/MS libraries of standards.

## 3. Results and discussion

### 3.1. PTV–LVI–GC–MS/MS optimization

Optimization of the chromatographic conditions was accomplished using a standard mixture solution of all target compounds in n-hexane. Firstly, different oven programs were tested in order to obtain a suitable separation of the studied PAHs. The injector program was based on a previously reported PTV–LVI–GC–MS/MS methodology [32] for analysis of PAHs in water but parameters such as injector heating rate and injection volume were evaluated to obtain good sensitivity and improve the peak shapes.

In order to investigate the influence of the heating rate, a standard mixture of PAHs was repeatedly injected, using different injector programs. The initial temperature (60 °C) was increased to 300 °C at 70 °C min$^{-1}$, 100 °C min$^{-1}$ and 180 °C min$^{-1}$. All studied compounds showed better responses at higher heating rates, the influence of the heating rate being more pronounced for less volatile PAHs. The PAH responses decreased slightly at 100 °C min$^{-1}$ whereas an important decreasing of the signal was observed at 70 °C min$^{-1}$. The opposite effect was observed in the peak shapes, obtaining a deterioration of peak shape (mainly for more volatile compounds) by increasing the heating rate at 180 °C min$^{-1}$. Therefore, 100 °C min$^{-1}$ was chosen as the optimum injector heating rate in order to avoid peak distortion while obtaining high responses for the target compounds.

The effect of injection volume was also investigated. Different injection volumes (7, 14, 25, 50 and 70 μL) were tested to obtain the higher sensitivity without deterioration of peak shape. The experimental results showed that the sensitivity was increased with increasing the injection volume from 7 to 25 μL while keeping good shape of peaks. However, distorted and/or double peak were observed when the injection volumes were increased to 50 and 75 μL. Consequently, an injection volume of 25 μL was established.

The MS/MS detection mode was chosen and the conditions were carefully optimized to improve the selectivity and sensitivity of PAH determinations. A parent ion was chosen for each compound by taking into account their *m*/*z* and their relative abundance (both as high as possible) in order to increase sensitivity. The optimization of the excitation amplitude voltage for each PAH was carried out using the automated method development option included in the MS/MS software tool kit. The optimum valued for this parameter was reached when the secondary spectrum showed multiple and intense product ions while the parent ion intensity remained at around 10%. The effect the CID amplitude was studied in the resonant and non-resonant modes for every compound. Poor fragmentation was obtained in non-resonant mode and the resonant waveform type was required for obtaining a suitable dissociation of the PAHs. Optimized MS/MS conditions for each target compound are detailed in Table 2.

### 3.2. Optimization of conditions for SPLE

To achieve fast and efficient extraction of analytes from solid matrices using PLE, proper operational parameters (sample preparation, temperature, pressure, extraction time, number of cycles and flush volume) and an appropriate extraction solvent or mixture solvents, with polarities closely matching that of the target compounds, should be selected. Thus, the effect of the different extraction parameters on the extraction efficiency was evaluated to obtain optimal extraction conditions for PAHs from sewage sludge samples.

#### 3.2.1. Sample treatment optimization

Sample preparation is an essential part of every solvent-based extraction procedure. While many sample types can be efficiently extracted without any pretreatment, other samples require some manipulation for an efficient extraction to occur. The effect of sample matrix depends on sample composition. Solid environmental

**Table 3**
Amount of residue in sewage sludge extracts and values of recovery of analytes obtained by different SPLE conditions.

| Sample | Sample treatment conditions | | PLE conditions[b] | | Results | |
|---|---|---|---|---|---|---|
| | Dispersant | Type of additive[a] | Extraction solvent | $T$[a], flush volume | Residue (mg g$^{-1}$)[c] | Recoveries (%)[d] |
| Pooled sample | 1 g Flo + 0.5 g $Na_2SO_4$ | 1 mL KOH | DCM–MeOH | – | 22 ± 1 | 97 [16][e] |
| | | 0.5 mL KOH | Hex | 100 °C, 50% | 13 ± 3 | 78 |
| | | 1 mL KOH | Hex | 100 °C, 50% | 16 ± 3 | 95 |
| | | 1 mL KOH | Hex | 120 °C, 50% | 18 ± 1 | 98 |
| | | 1 mL MeOH | Hex | 120 °C, 50% | 29 ± 1 | 84 |
| | | – | Hex–Ace | 100 °C, 50% | 40 ± 2 | 78 |
| | | 0.5 mL KOH | Hex–Ace | 100 °C, 50% | 32 ± 3 | 88 |
| | | 1 mL KOH | Hex–Ace | 100 °C, 50% | 26 ± 1 | 96 |
| | | 1 mL KOH | Hex–Ace | 120 °C, 50% | 27 ± 1 | 99 |
| BCR088 | – | – | Hex–Ace | 120 °C, 60% [f] | 79 ± 5 | 82 [8] |
| | 1 g Flo + 0.5 g $Na_2SO_4$ | 1 mL KOH | DCM–MeOH | – | 20 ± 1 | 99 [16][e] |
| | 1 g Flo + 0.5 g $Na_2SO_4$ | 1 mL KOH | Hex | 120 °C, 70% | 21 ± 1 | 88 |
| | 1 g Flo + 1 g $Na_2SO_4$ | 1 mL KOH | Hex | 120 °C, 70% | 22 ± 1 | 83 |
| | 1 g Flo + 0.75 g $Na_2SO_4$ | 1.5 mL KOH | Hex | 120 °C, 70% | 20 ± 2 | 70 |
| | 1 g Flo + 1 g $Na_2SO_4$ | 1.5 mL KOH | Hex | 120 °C, 70% | 22 ± 1 | 73 |
| | 1 g Flo + 1.5 g $Na_2SO_4$ | 1.5 mL KOH | Hex | 120 °C, 70% | 24 ± 1 | 84 |
| | 1 g Flo + 2 g $Na_2SO_4$ | 1.5 mL KOH | Hex | 120 °C, 70% | 23 ± 1 | 79 |
| | 1 g Flo + 0.5 g $Na_2SO_4$ | 1 mL KOH | Hex | 140 °C, 100% | 24 ± 1 | 99 |

0.2 g of sample. Data for three replicates. Flo, Florisil; $Na_2SO_4$, anhydrous sodium sulphate; KOH, saturated methanolic potassium hydroxide solution; MeOH, methanol; DCM–MeOH, dichloromethane–methanol (90:10, v/v) mixture; Hex, n-hexane; Hex–Ace, hexane–acetone (50:50, v/v) mixture; (–) not employed.

[a] Amounts of solvent or saturated methanolic potassium hydroxide solution added in the blending step.
[b] Remaining PLE operational parameters were in all experiments 1500 psi, 1 static cycle of 5 min and 60 s of purge.
[c] Average (mg g$^{-1}$ sample) of three determinations ± standard deviation.
[d] Average of PAHs recoveries, calculated considering values obtained with MSPD procedure [16].
[e] Recoveries calculated considering values obtained with MAE procedure [14].
[f] Cell, 1 g alumina followed by 0.5 g of sample and 1.5 g of diatomaceous earth; PLE conditions, 1500 psi, 2 cycles of 5 min and 60 s of purge.

samples, such as sewage sludge, sediments or soils, can differ significantly in their physical–chemical properties, type of compounds present or particles size. These parameters affect the sorption and retention of analytes [21].

On the other hand, the complexity of an analytical procedure increases with the number of organic compounds present in the sample. Thus, sewage sludge is often regarded as one of the worst environmental matrices to extract as it can contain high concentrations of organic chemicals and their degradation products as well as high amounts of organic matter. In order to solubilize the analytes during the extraction, proper conditions should be used to overcome the interactions between the organic fraction and target compounds. This often results in dirty extracts that present large concentrations of co-extracted substances and require extensive clean-up steps before the final analysis. In-depth clean-up of extracts prior to chromatographic analysis can be avoided by performing an in situ clean-up step by adding certain sorbents to the PLE cells. In this way, lipids and other coextractable materials are prevented from coming out to the extract.

To the best of our knowledge, the application of PLE with in-cell clean-up to the determination of PAHs in sewage sludge has only been reported by Trably et al. [8]. This method involves the extraction of 14 PAHs at 120 °C using a hexane–acetone (50:50, v/v) mixture as extraction solvent and 1 g of alumina as clean-up sorbent inside cell.

The first extraction assays were carried out using the previously reported PLE with in-cell clean-up method [8] but dark-coloured extracts resulted with high residues after solvent evaporation (Table 3). Therefore, the sample extracts were not clean enough for direct injection in the chromatographic systems (HPLC-Flu and GC–MS/MS).

Unsatisfactory results obtained in preliminary assays suggested that very complex sewage sludge samples can require some treatment before extraction by PLE. In some applications, the sample is mixed and dispersed with solid sorbents before loading it in the PLE cell. De la Cal et al. [33] described a SPLE method for the analysis of polybrominated diphenyl ethers congeners in sediment samples. Spiked samples were ground with alumina and cooper (1:2:2) and the mixture was loaded into the extraction cell on top of alumina. Later Losada et al. [34] reported a SPLE method for polybrominated diphenyl ethers in fish. The sample was mixed with $Na_2SO_4$ at a fish $Na_2SO_4$ ratio of 1:20 in a mortar until a homogeneous mixture was obtained. The mixture was loaded into the cell on top of 20 g of Florisil. Consequently, a second series of experiments were performed including a sample dispersion stage. Although the dispersion and the clean-up sorbents were adapted from an optimized MSPD procedure for sewage sludge, developed in our laboratory [16], a series of experiments were carried out to assess the effect of additive (KOH (MeOH) sat.) and $Na_2SO_4$ on the yield and selectivity of the PLE process. Previous studies showed that these parameters were important in terms of minimizing extract residues and maximizing recoveries of PAHs [14,16]. Therefore, different volumes of additive (0, 0.5, 1, and 1.5 mL) as well as amounts of $Na_2SO_4$ (0.5, 1, 1.5 and 2 g) were tested to achieve the optimal conditions. The obtained results were in good agreement with those obtained in the previous studies. As expected, the alkali treatment has a strong positive effect. Recoveries increased and the residue amounts decreased as the amount of alkali increased (Table 3). The recoveries obtained with 0.5 mL of KOH (MeOH) sat. were on average 10–40% lower than with 1 mL of additive. With regard to the amount of $Na_2SO_4$, it is interesting to mention that it is closely related with the amount of additive. As can be seen in Table 3, 0.5 g of $Na_2SO_4$ was enough to provide good recoveries when 1 mL of alkali was added in the blending stage. However, an increasing of alkali to 1.5 mL required 1.5 g of $Na_2SO_4$ to achieve good results in terms of recovery.

In summary, the sample preparation was demonstrated be a critical step in the determination of PAHs from complex matrices. The sample treatment enhances the yield of extraction and provides the additional advantage of decreasing the amount of co-extracted substances, producing directly analysable extracts by HPLC-Flu and GC–MS/MS. Therefore, the sample was dispersed in a glass mortar by using 1 mL of KOH (MeOH) sat. as additive and 1 g of Florisil and 0.5 g of $Na_2SO_4$. Two filters and a layer of sand were placed at

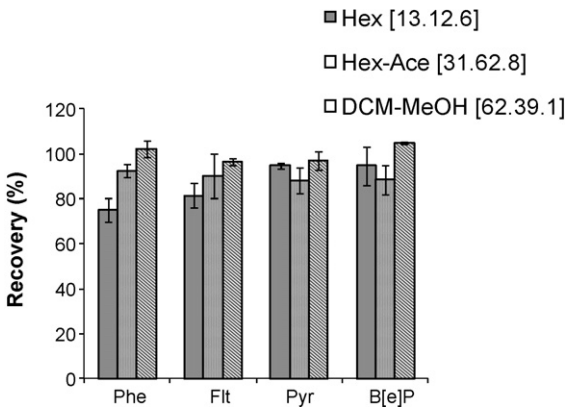


**Fig. 1.** Recoveries of some representative PAHs in pooled sewage sludge sample with different extraction solvents, during the optimization step. PLE conditions were 100 °C, one cycle of 5 min and 50% of flush volume. Hex, n-hexane; Hex–Ace, hexane–acetone (50:50, v/v) mixture, DCM–MeOH, dichloromethane–methanol (95:5, v/v) mixture. Values in brackets correspond to residues (average (mg $g^{-1}$ sample) ± standard deviation ($n$ = 3)) of pooled sewage sludge sample extracts. Data for triplicate extraction.

the end of the PLE cell. Then, the clean-up sorbents (1 g of Florisil followed by 2 g of silica) were introduced into the cell, followed by the dispersed sample. Finally, the remaining free space of the cell was filled up with sand to minimize cell void volume. These conditions were employed in all subsequent experiments.

### 3.2.2. Solvent extraction choice

Optimization of the extraction process generally begins with an appropriate choice of the extraction solvent. The extraction of solid environmental samples, which consist of complex mixtures of different species at different concentration levels, is a complicated task. At elevated temperature and pressure, the extraction process proceeds faster, but the selectivity decreases, because the analytes are not the only compounds solubilized. Therefore, the extraction solvent must be able to solubilize the analytes of interest, minimizing the co-extraction of other matrix components. Consequently, the polarity of the extraction solvent should closely match that of the target compounds. It is also important to take into account the compatibility with the later treatment steps as well as the volatility of solvent if extract concentration is necessary.

In this study, extraction solvents were chosen on the basis of those employed in previously reported methodologies for the determination of PAHs from environmental matrices (sewage sludge and soil) [14,16,35]. Thus, a series of experiments were performed in order to evaluate the extraction capability of n-hexane, a hexane–acetone (50:50, v/v) mixture and a dichloromethane–methanol (95:5, v/v) mixture. As can be seen in Fig. 1, the dichloromethane–methanol (95:5, v/v) mixture provided better recovery values than n-hexane and hexane–acetone (50:50, v/v). However, this mixture provided extra interferences, as evidenced by yellow extracts with larger residue obtained after solvent evaporation. Experimental data did not show significant differences between n-hexane and the hexane–acetone (50:50, v/v) mixture in terms of recovery. However, lower sample matrix residues were found when n-hexane was used as extraction solvent; therefore, n-hexane was chosen and used in further experiments.

### 3.2.3. Optimization of SPLE parameters

Once the sample treatment conditions and the solvent extraction were established, initial experiments were carried out in order to evaluate the effect of the pressure on the extraction efficiency of SPLE. The application of high pressure allows maintaining the solvent in the liquid state while above their atmospheric boiling points. Furthermore, the use of high pressures facilitates extraction of analytes that have been trapped in matrix pores since the pressure forces the solvent into areas of the matrices that would not normally be contacted by solvent using atmospheric conditions [20,21]. Pooled sample of sewage sludge was extracted at 1500 and 1700 psi. The obtained results showed that an increase in the extraction pressure had no significant improvement in the extraction efficiencies, data not shown. In agreement with previously published results [20,28] for PLE, pressure was found to play no role other than to keep the extraction solvent liquid at the high temperatures used. Therefore, an extraction pressure of 1500 psi was chosen and used in further experiments.

The purge time controls the period during which nitrogen is passing through the stainless steel cell to sweep away all the solvent wetting the sample and the cell filling, at the end of the static extraction cycle. Purge time was set at default value (60 s) since it is not considered an important factor in the optimization of a PLE method.

Temperature is one of the most important parameters in PLE extraction. The use of solvents at elevated temperatures should give enhanced performance as compared to extraction to near room temperatures since the use of higher temperatures increases the capacity of solvents to solubilize analytes and increases the diffusion rate. In addition, increased temperatures also decrease the viscosity of liquid solvent (better penetration in matrix particles) and can disrupt the strong analyte–matrix interactions caused by van der Waals forces, hydrogen bonding, and dipole attractions of the analyte molecules and active sites of the matrix [20].

Initial experiments were performed at the extraction temperature recommended by Dionex [36] as starting point for all environmental applications (100 °C), but unsatisfactory results were obtained. Therefore, in order to achieve efficient extraction of target compounds, higher extraction temperatures (120, 140 and 160 °C) were tested. As expected, higher temperatures resulted in better extraction efficiency for the studied PAHs. The recovery values increased when the extraction temperature was increased from 120 to 140 °C. However, a higher temperature (160 °C) did not result in significantly higher extraction efficiency (data not shown). Therefore, 140 °C was chosen as the optimum extraction temperature.

Another experimental parameter investigated was the amount of solvent necessary to obtain complete extraction. The flush volume (referred as a percentage of the cell volume: 11 mL) divided by the number of cycles determines the amount of fresh solvent added between each extraction cycle. The flush volume may have a significant effect on recovery, especially during extraction where the PLE cells are packed with a stationary phase, as this may increase retention of the analytes.

In order to evaluate the influence of solvent volume on the yield of SPLE process, different percentages of flush volume were tested. The first experiments were carried out without adding fresh solvent in the PLE cell. Unsatisfactory results suggest that a very complex matrix such as sewage sludge requires higher percentages of flush volume to achieve an efficient extraction of PAHs. Then, a series of experiments were performed employing a 50% of flush volume (5.5 mL). The results showed that the flush volume was important in terms of maximizing recoveries of PAHs, since the recovery values were on average 20–30% lower when fresh solvent was not added; data not shown. Anyway, unsatisfactory results were also obtained when a percentage of 50% was used.

Consequently, higher percentages of flush volume (70% and 100%) were assayed. The results (Fig. 2) demonstrated that the recoveries of low molecular weight PAHs were unaffected by the increasing of flush volume. In contrast, increasing the flush volume from 70% to 100% significantly increases the recovery of high molec-

**Table 4**
Linearity, reproducibility, recoveries, LODs and LOQs of the proposed analytical procedure.

| Compound | PTV–LVI–GC–MS/MS | | | | | | HPLC-Flu | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Linearity | | Reproducibility[a] μg $g^{-1}$ ± RSD (%) | | LOD[a] S/N = 3 (μg $g^{-1}$) | LOQ[a] S/N = 10 (μg $g^{-1}$) | Linearity | | Reproducibility[a] μg $g^{-1}$ ± RSD (%) | | LOD[a] S/N = 3 (μg $g^{-1}$) | LOQ[a] S/N = 10 (μg $g^{-1}$) | Recovery ± RSD (%) [b] |
| | Calibration (μg $L^{-1}$) | $R^2$ | Pool[c] | Spiked pool[c] | | | Calibration (μg $L^{-1}$) | $R^2$ | Pool[c] | Spiked pool[d] | | | |
| Naph | 10–400 | 0.9995 | 0.2 ± 4.4 | 0.6 ± 6.5 | 0.03 | 0.09 | 1–200 | 0.9995 | 0.09 ± 3.1 | 0.5 ± 6.7 | 0.0004 | 0.001 | 85.2 ± 1.3 |
| Acy [e] | 20–800 | 0.9992 | 0.2 ± 5.5 | 4.0 ± 5.8 | 0.008 | 0.03 | | | | | | | 90.8 ± 1.1 [f] |
| Ace | 10–400 | 0.9998 | 0.1 ± 2.6 | 0.8 ± 1.6 | 0.02 | 0.08 | 1–200 | 0.9997 | 0.09 ± 5.5 | 0.7 ± 6.1 | 0.002 | 0.005 | 90.0 ± 1.1 |
| Flu | 2–80 | 0.9990 | 0.2 ± 2.9 | 0.8 ± 13.7 | 0.01 | 0.04 | 0.2–40 | 0.9997 | 0.2 ± 2.0 | 0.5 ± 3.4 | 0.0002 | 0.0005 | 84.8 ± 1.0 |
| Phe | 1–40 | 0.9979 | 1.8 ± 3.9 | 4.9 ± 1.2 | 0.01 | 0.04 | 0.1–20 | 0.9995 | 1.6 ± 2.9 | 3.4 ± 1.8 | 0.00008 | 0.0003 | 90.6 ± 1.4 |
| Anth | 1–40 | 0.9997 | 0.1 ± 4.6 | 0.3 ± 2.1 | 0.01 | 0.05 | 0.1–20 | 0.9994 | 0.08 ± 1.3 | 0.3 ± 9.1 | 0.0001 | 0.0004 | 97.5 ± 1.7 |
| Flt | 2–40 | 0.9992 | 0.5 ± 4.0 | 1.3 ± 4.4 | 0.004 | 0.01 | 0.2–40 | 0.9997 | 0.5 ± 0.1 | 1.2 ± 4.2 | 0.0006 | 0.002 | 100.2 ± 0.4 |
| Pyr | 1–40 | 0.9993 | 0.5 ± 1.7 | 1.2 ± 3.9 | 0.005 | 0.02 | 0.1–20 | 0.9996 | 0.5 ± 4.7 | 1.1 ± 2.1 | 0.0001 | 0.0003 | 98.6 ± 1.5 |
| B[a]A | 1–20 | 0.9989 | 0.09 ± 6.8 | 0.3 ± 4.1 | 0.01 | 0.05 | 0.1–20 | 0.9997 | 0.07 ± 2.9 | 0.3 ± 2.4 | 0.00004 | 0.0001 | 99.9 ± 1.6 |
| Chry | 1–20 | 0.9993 | n.d. | 2.2 ± 3.1 | 0.01 | 0.04 | 0.1–20 | 0.9998 | n.d. | 2.2 ± 2.1 | 0.0001 | 0.0004 | 105.5 ± 1.3 |
| 5-MC [g] | | | | | | | 0.1–20 | 0.9997 | n.d. | n.d. [h] | 0.0001 | 0.0003 | 100.0 ± 0.2 |
| B[e]P | 2–80 | 0.9971 | 0.2 ± 10.8 | 0.6 ± 4.5 | 0.01 | 0.04 | 0.2–40 | 0.9993 | 0.3 ± 1.2 | 0.8 ± 5.0 | 0.0006 | 0.002 | 95.1 ± 1.1 |
| B[b]F | 2–80 | 0.9976 | 0.09 ± 11.1 | 0.6 ± 3.6 | 0.02 | 0.06 | 0.2–40 | 0.9998 | 0.08 ± 2.5 | 0.5 ± 8.1 | 0.0002 | 0.001 | 102.7 ± 1.7 |
| B[k]F | 1–40 | 0.9994 | 0.06 ± 11.8 | 0.4 ± 5.1 | 0.02 | 0.06 | 0.1–20 | 0.9994 | 0.02 ± 5.5 | 0.3 ± 3.7 | 0.00003 | 0.0001 | 100.3 ± 1.5 |
| B[a]P | 1–40 | 0.9979 | 0.04 ± 7.9 | 0.3 ± 7.3 | 0.04 | 0.1 | 0.1–20 | 0.9992 | 0.04 ± 7.8 | 0.3 ± 2.6 | 0.00004 | 0.0001 | 99.3 ± 1.6 |
| DB[al]P [g] | | | | | | | 0.1–20 | 0.9991 | n.d. | 0.04 ± 5.9 | 0.0001 | 0.0004 | 97.0 ± 1.5 |
| DB[ah]A | 2–80 | 0.9985 | n.d. | 0.4 ± 4.6 | 0.005 | 0.02 | 0.2–40 | 0.9997 | 0.01 ± 11.2 | 0.4 ± 6.8 | 0.00007 | 0.0002 | 105.2 ± 0.7 |
| B[ghi]P | 2–80 | 0.9992 | 0.06 ± 4.2 | 0.2 ± 2.5 | 0.07 | 0.2 | 0.2–40 | 0.9995 | 0.06 ± 8.7 | 0.2 ± 4.1 | 0.00006 | 0.0002 | 106.6 ± 1.5 |
| I[1,2,3-cd]P | 2–40 | 0.9969 | 0.03 ± 3.1 | 0.1 ± 3.7 | 0.03 | 0.09 | 0.1–20 | 0.9998 | 0.03 ± 3.9 | 0.1 ± 8.0 | 0.0004 | 0.001 | 97.4 ± 1.5 |

Calibration, calibration range; $R^2$, determination coefficient; LODs and LOQs corresponding to overall analytical procedure; n.d., not detected.

[a] Concentration on dry weight basis.
[b] Spiked certified reference material (BCR088).
[c] $n = 3$ replicates.
[d] $n = 7$ replicates.
[e] Acy was not analyzed by HPLC-Flu.
[f] Recovery determined by GC–MS/MS.
[g] Compounds not investigated by GC–MS/MS.
[h] Pooled sewage sludge sample was not spiked with 5-MC.

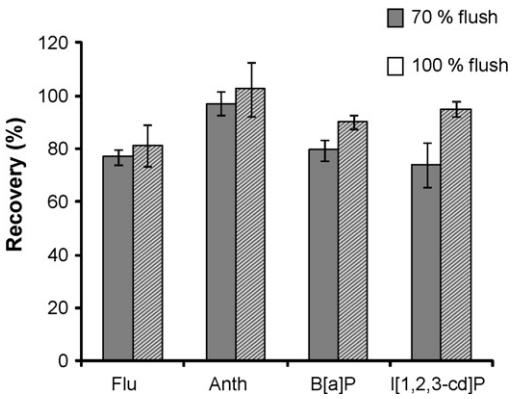


**Fig. 2.** Effect of the flush volume on the efficiency of the PLE extraction for some representative PAHs in BCR088. PLE conditions were 140 °C, one cycle of 5 min and n-hexane as extraction solvent. Data for triplicate extraction.

ular weight PAHs. Therefore, a flush volume of 100% was chosen for the method and used in further experiments.

The extraction process in PLE can be conducted in a static or dynamic mode. Although dynamic mode improves mass transfer, this type of extraction is rarely used, mainly because of higher solvent consumption compared with the static process. The static process begins with heating the cell with the sample to an appropriate temperature during the equilibration time and is followed by a so-called static extraction process. During this process, the analytes are isolated from the sample under static conditions. The static process can be repeated several times by using static cycles if low recoveries are obtained in a single stage. Thus, the use of static cycles was developed to introduce fresh solvent during the extraction process, which helps to maintain favourable extraction equilibrium [21]. Obviously, parameters such as number of static cycles and static time can affect to efficiency of PLE process and therefore these must be carefully evaluated.

The effect of static time was explored in conjunction with static cycles, in order to produce a complete extraction in the most efficient way possible. A series of experiments were performed with different static times and extraction cycles (1 cycle of 5 min, 2 cycles of 5 min, 2 cycles of 3 min and 3 cycles of 3 min). No significant differences were observed between one cycle of 5 min and two cycles of 5 min. However, recoveries slightly lower were obtained with two cycles of 3 min. In accordance with the results, a single static cycle of 5 min was considered able to obtain quantitative recoveries, which is advantageous in terms of extraction duration.

### 3.3. Performance of the analytical procedure

Calibration curves were prepared at six levels and each calibration level was injected in triplicate. The range of concentrations and other calibration figures of merit, as well as the detection (LOD, S/N = 3) and quantification (LOQ, S/N = 10) limits for the overall proposed method are summarized in Table 4. HPLC-Flu was demonstrated to be more sensitive than the optimized GC–MS/MS technique for the determination of PAHs but it should be stressed that all the analytes were quantified far below the limit imposed by

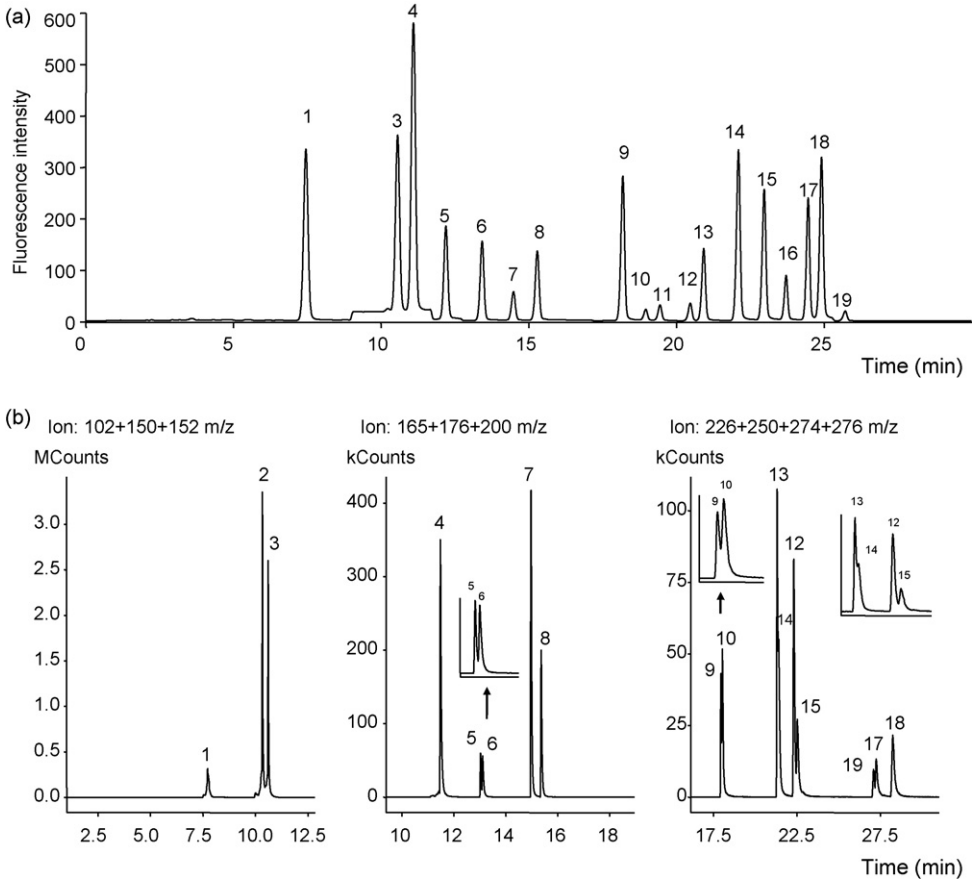


**Fig. 3.** HPLC-Flu (a) and PTV–LVI–GC–MS/MS extracted ion (b) chromatograms for a standard solution of studied PAHs at concentrations between 20 and 400 μg L$^{-1}$. Peak assignment: (1) Naph, (2) Acy, (3) Ace, (4) Flu, (5) Phe, (6) Anth, (7) Flt, (8) Pyr, (9) B[a]A, (10) Chry, (11) 5-MC, (12) B[e]P, (13) B[b]F, (14) B[k]F, (15) B[a]P, (16) DB[al]P, (17) DB[ah]A, (18) B[ghi]P and (19) I[1,2,3-cd]P.

**Table 5**
Measured concentrations and confidence intervals vs. certified and reference values in BCR088 and RTC-CNS312-04.

| Compound | BCR 088 | | | RTC-CNS312-04 | | |
|---|---|---|---|---|---|---|
| | Certified values ($\mu g\,g^{-1}$) | Measured ($\mu g\,g^{-1}$) $\bar{x} \pm 2SD$ ($n=7$) HPLC-Flu | Measured ($\mu g\,g^{-1}$) $\bar{x} \pm 2SD$ ($n=4$) PTV–LVI–GC–MS/MS | Reference values ($\mu g\,g^{-1}$) | Measured ($\mu g\,g^{-1}$) $\bar{x} \pm 2SD$ ($n=7$) HPLC-Flu | Measured ($\mu g\,g^{-1}$) $\bar{x} \pm 2SD$ ($n=4$) PTV–LVI–GC–MS/MS |
| Naph | | | | 2.58 ± 0.53 | 3.10 ± 0.39 | 2.94 ± 0.44 |
| Acy[a] | | | | 2.42 ± 0.53 | | 2.50 ± 0.63 |
| Ace | | | | 2.99 ± 0.46 | 3.42 ± 0.36 | 3.29 ± 0.67 |
| Flu | | | | 2.01 ± 0.32 | 2.31 ± 0.06 | 2.27 ± 0.25 |
| Phe | | | | 0.46 ± 0.11 | 0.56 ± 0.07 | 0.56 ± 0.03 |
| Anth | | | | 1.67 ± 0.27 | 1.89 ± 0.09 | 1.57 ± 0.36 |
| Flt | | | | 4.19 ± 0.57 | 4.66 ± 0.15 | 3.94 ± 0.33 |
| Pyr | 2.16 ± 0.09 | 2.09 ± 0.14 | 2.12 ± 0.10 | 4.17 ± 0.51 | 4.43 ± 0.73 | 4.56 ± 0.14 |
| B[a]A | 0.93 ± 0.09 | 0.86 ± 0.06 | 0.91 ± 0.14 | 1.45 ± 0.18 | 1.45 ± 0.20 | 1.53 ± 0.26 |
| Chry | | | | 1.12 ± 0.15 | 1.22 ± 0.11 | 1.17 ± 0.12 |
| B[e]P | 1.02 ± 0.07 | 1.11 ± 0.09 | 1.04 ± 0.11 | | | |
| B[b]F | 1.17 ± 0.08 | 1.12 ± 0.11 | 1.21 ± 0.09 | 0.24 ± 0.05 | 0.28 ± 0.03 | 0.25 ± 0.06 |
| B[k]F | 0.57 ± 0.05 | 0.53 ± 0.04 | 0.55 ± 0.01 | 0.68 ± 0.09 | 0.68 ± 0.07 | 0.60 ± 0.01 |
| B[a]P | 0.91 ± 0.09 | 0.86 ± 0.07 | 0.84 ± 0.08 | 0.87 ± 0.12 | 0.90 ± 0.09 | 0.77 ± 0.03 |
| DB[ah]A | | | | 0.41 ± 0.07 | 0.44 ± 0.02 | 0.43 ± 0.05 |
| B[ghi]P | | | | 0.84 ± 0.17 | 0.79 ± 0.09 | 0.69 ± 0.07 |
| I[1,2,3-cd]P | 0.81 ± 0.06 | 0.81 ± 0.05 | 0.85 ± 0.11 | 0.54 ± 0.12 | 0.55 ± 0.04 | 0.43 ± 0.01 |

Concentrations in dry weight basis.
[a] Acy was not analyzed by HPLC-Flu.

current regulations [11] with both proposed chromatographic techniques. Fig. 3 displays the HPLC-Flu and GC–MS/MS ion extracted chromatograms for a standard mixture solution.

Intermediate precision was assessed by series of independent experiments carried out on different days with spiked and non-spiked pooled sewage sludge samples. Average concentrations (on a dry mass basis) and precision between days are reported in Table 4.

Recoveries were evaluated by processing sewage sludge samples with standard additions at concentrations ca. 0.5, 1, 1.5 and 2 times the actual concentrations in original samples. SPLE extracts can be directly analyzed by GC–MS/MS whereas solvent evaporation stages are required in order to achieve extracts that can be injected in the HPLC system. Significant losses of some PAHs are produced during the solvent evaporation stages associated with sample preparation procedure. In the proposed SPLE method, the solvent evaporation step provides overall losses values ranged from 1% to 24%. Recoveries for the overall analytical process were calculated from the slope of the addition graph and the values of losses in the solvent evaporation process were taken into consideration for recovery calculation. The extraction process was highly efficient, with recoveries higher than 85% and relative standard deviations lower than 2% (Table 4). The combination of elevated temperatures and pressures allowed to obtain high recoveries of target compounds using a low polar solvent (n-hexane), providing clean extracts not only to analyze by HPLC-Flu but also by GC–MS/MS. Thus, the use of n-hexane as extraction solvent presented an improved selectivity versus the use of more polar solvents. Moreover, the developed SPLE procedure offers important advantages since it avoids the use of highly toxic chlorinated solvents, requires a low consumption of solvent and allows automated extraction process.

To check the performance of the analytical procedure, seven replicates of BCR088 (certified sludge) and RTC-CNS312-04 (reference sludge) were processed by the described procedure obtaining good agreement between the results and the certified and/or reference values (see Table 5 for mean concentrations and confidence intervals). Fig. 4 depicts typical chromatograms obtained by HPLC-

**Table 6**
Concentration of PAHs found in different sewage sludge samples by PLE-HPLC-Flu.

| Compound | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|
| Naph | 0.15 ± 0.8 | – | 0.3 ± 2.7 | 4.2 ± 2.9 | 7.6 ± 4.1 | 90.7 ± 3.1 |
| **Ace** | n.d. | 0.2 ± 0.5 | n.d. | 0.5 ± 5.5 | 0.8 ± 2.4 | 17.6 ± 4.6 |
| **Flu** | 0.05 ± 8.7 | 0.4 ± 6.8 | 0.09 ± 2.6 | 0.4 ± 0.9 | 2.2 ± 6.1 | 43.1 ± 4.0 |
| **Phe** | 0.25 ± 6.1 | 4.1 ± 0.9 | 0.5 ± 3.6 | 1.8 ± 6.2 | 14.9 ± 3.4 | 207.0 ± 6.3 |
| Anth | 0.01 ± 2.9 | 0.2 ± 4.6 | 0.02 ± 3.0 | 0.1 ± 3.7 | 0.5 ± 5.1 | 3.9 ± 7.8 |
| **Flt** | 0.20 ± 7.4 | n.d. | 0.3 ± 0.7 | 0.7 ± 1.9 | 7.2 ± 3.9 | 87.2 ± 4.8 |
| **Pyr** | 0.20 ± 8.4 | 1.1 ± 1.3 | 0.2 ± 1.6 | 0.4 ± 0.6 | 4.3 ± 4.0 | 29.8 ± 4.9 |
| B[a]A | 0.02 ± 2.5 | 0.1 ± 1.1 | 0.03 ± 2.1 | 0.04 ± 3.0 | 0.1 ± 1.8 | 0.3 ± 5.2 |
| Chry | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 5-MC | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| B[e]P | 0.08 ± 4.0 | 0.6 ± 1.1 | 0.1 ± 11.5 | n.d. | n.d. | n.d. |
| **B[b]F** | 0.04 ± 2.5 | 0.1 ± 1.7 | 0.05 ± 2.2 | 0.1 ± 4.0 | n.d. | n.d. |
| **B[k]F** | 0.01 ± 6.7 | 0.03 ± 11.0 | 0.02 ± 7.1 | 0.03 ± 6.8 | n.d. | n.d. |
| **B[a]P** | 0.03 ± 4.0 | 0.1 ± 9.6 | 0.03 ± 5.4 | 0.03 ± 8.6 | n.d. | n.d. |
| DB[al]P | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| DB[ah]A | 0.003 ± 2.5 | n.d. | n.d. | n.d. | n.d. | n.d. |
| **B[ghi]P** | 0.05 ± 4.1 | 0.1 ± 4.2 | 0.04 ± 2.8 | 0.04 ± 4.2 | n.d. | n.d. |
| **I[1,2,3-cd]P** | 0.02 ± 1.9 | 0.05 ± 3.5 | 0.03 ± 1.9 | 0.03 ± 5.6 | n.d. | n.d. |
| **ΣPAHs leg** | 0.9 | 6.0 | 1.3 | 4.0 | 29.3 | 384.6 |
| ΣPAHs | 1.1 | 7.1 | 1.8 | 8.4 | 37.5 | 479.6 |

Average ($\mu g\,g^{-1}$) ± relative standard deviation (RSD, %); concentration on dry weight basis; $n=3$ replicates per sample; n.d., not detected; (–), not available; **ΣPAHs leg**, sum of PAHs considered in EU legislation; ΣPAHs, sum of PAHs considered in this study.

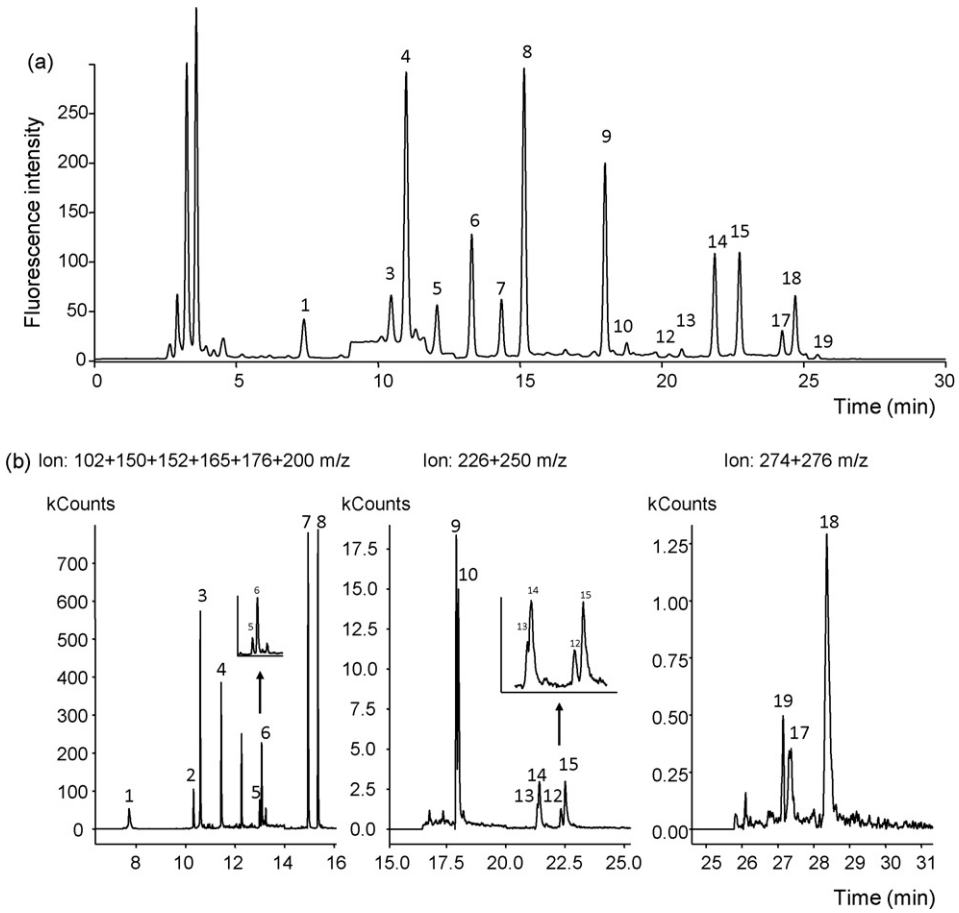


**Fig. 4.** Chromatograms obtained by (a) HPLC-Flu and (b) PTV–LVI–GC–MS/MS, corresponding to reference material (RTC-CNS312-04) extracts. Peak assignments as in Fig. 3.

Flu and PTV–LVI–GC–MS/MS for reference sludge (RTC-CNS312-04) working in the optimal conditions described.

The applicability of the proposed SPLE method was tested by determination of PAHs in several sewage sludge samples. All samples were also processed by two alternative techniques using MAE [14] and MSPD [16], with good agreement between the sample preparation techniques. Total concentrations between 1.1 and 479.6 $\mu g\,g^{-1}$ were found in sewage sludge samples. As can be seen in Table 6, two samples (S5 and S6, coming from highly industrialized areas) exhibited high contents of PAHs, exceeding the EU legal limit for the sum of selected PAHs. The PAH levels in the remaining samples were below the maximum allowed concentrations.

The optimized SPLE procedure appears to be an advantageous alternative, being fast (one single static cycle of 5 min is employed) and simple, avoiding use of chlorinated solvents, using low volumes of organic solvents and requiring small amounts of sample.

## 4. Conclusions

A new SPLE process has been developed and validated for the first time for the determination of 19 PAHs in complex sewage sludge matrices. Extraction conditions were carefully selected to achieve maximal recovery of PAHs contained in sewage sludge while eliminating most of the interfering matrix components. Sample treatment combined with the use of solid sorbents in the extraction cell allowed obtaining a directly analyzable extract. Another advantage of the proposed methodology is that most of the steps involved in the sample preparation procedure are performed automatically and up to 24 samples can be processed sequentially and unattended. Combining the efficiency with the automation of PLE significant improvements in the PAHs analysis throughput can be attained.

A PTV injection method was optimized for the GC–MS/MS analysis of PAHs. PTV injections offer the possibility of introducing large volumes of sample into the GC system, which improves the sensitivity, detection and quantification limits with respect to those obtained by conventional split/splitless injection. Thus, sewage sludge extracts obtained using the developed SPLE procedure were successfully analyzed by the optimized PTV–LVI–GC–MS/MS method and HPLC-Flu.

In summary, the developed methodology shows good performance, providing good recoveries for all studied compounds and allows the determination of PAHs in the low "microgram per kilogram" range in a reproducible and simple way. The proposed methodology appears to be a sensitive, selective and reliable analytical method, suitable to high throughput monitoring the PAH concentrations in sewage sludge as established by the EU Regulations.

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