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Ultrasound-assisted solvent extraction of porous membrane packed solid samples: a new approach for extraction of target analytes from solid samples

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Abstract

For the first time, a porous membrane-based method is proposed for the extraction of target analytes directly from the solid samples. This method involves the packing of solid sample inside a porous polypropylene membrane sheet whose edges are heat-sealed to fabricate a bag. This bag is immersed in a suitable solvent and the analytes are extracted by the application of ultrasound energy. The various factors that affect the extraction performance such as extraction solvent, ultrasonication time, and ultrasound power are suitably optimized. The scope of this extraction method is very general, it can be used for the extraction of different classes of analytes from a variety of solid samples using suitable extraction solvents. The beauty of this method lies in the fact that only the small molecules such as analytes can pass through the membrane while the interfering or complex matrix species cannot pass through the membrane bag to the extraction solvent. Previously, the solid samples were first digested/dissolved into liquid medium and then analytes were extracted by membrane-protected adsorbents involving adsorption and desorption steps. With the proposed procedure, the steps of digestion/dissolution and the adsorption of analytes onto a suitable adsorbent are eliminated. Likewise, the steps of filtration, and centrifugation are not required as the solid is effectively packed inside the membrane bag. Moreover, the extraction device is low cost, portable, easy to fabricate, and simple to use in extraction process. In this work, proof of the concept is demonstrated by the extraction of polyaromatic hydrocarbons from the soil samples using GC-MS. This method provided reasonably low LODs ranging from 0.19 to 0.93 ng/mg. The inter-day precision ranged from 87.5 to 109%, while recoveries varied from 75.1 ± 4.9 to 106.0 ± 4.5 %.

Keywords

Solvent extraction; membrane-packed solid samples; microextraction; environmental analysis; sample preparation

1. Introduction

Despite all the major developments in analytical instrumentation, sample preparation is of critical importance in quantification of the analytes in various matrices. The need of sample preparation arises due to the demand of trace level quantification, the new regulatory obligations, and the complex matrix compositions [1].One of the major objectives of sample preparation is to convert the sample into a form that can be introduced and analyzed by the analytical instrument. This can be accomplished by the removal of interferences, separation/preconcentration of the analytes, and (if required) conversion of the analytes into more suitable derivatives [2]. The selection of the suitable sample preparation method and related analytical instrument has great significance in analytical method development.

As far as the sample preparation is concerned, liquid-liquid extraction (LLE) and solid phase extraction (SPE) are two commonly used classical extraction techniques. They have advantages of better clean up and good extraction recoveries. However, both techniques consume large amounts of hazardous organic solvents and consist of multistep procedures. In addition, SPE also requires selective adsorbents for proper retention of the analytes. The synthesis of selective adsorbents involves the use of different chemicals in large quantities. In this way, both techniques are not environment friendly; also, they are time and labor extensive. As an alternative to classical LLE and SPE, the area of sample preparation is progressing toward the development of microextraction approaches that are characterized by miniaturization, simplification, and automation. Hence, the use of large amounts of organic solvents, synthetic sorbents, and the samples can be avoided. Solid phase microextraction [3], liquid phase microextraction [4], dispersive liquid-liquid microextraction [5], porous membrane protected micro-solid-phase extraction [6], and their modified versions are some examples of the widely accepted microextraction techniques.

Despite all the major advancements in the microextraction techniques, a kind of sample pretreatment or modification is generally required for the samples characterized by the complex matrix composition. Moreover, some of these methods cannot extract directly from the complex natured or solid samples. The cost, fragile nature of the extraction devices, and instability against certain solvents are among some major issues[7].

To deal with extraction of the analytes from the solid samples, the sorbent- and solvent-based microextractions generally require the digestion or dissolution of the solid samples in water or any other solvent. Further pretreatment or dilution may be needed based on the nature of the sample and selected microextraction technique. In sorbent-based techniques, two main steps are involved; first is the adsorption of the analytes from the sample onto the sorbent and second is the thermal or solvent desorption of the analytes from the sorbent.

Porous membrane protected micro-solid-phase extraction (µ-SPE) was first introduced by Basheer et al., in 2006 as an alternative to multistep SPE [6]. In µ-SPE, few milligrams of sorbent are packed inside a porous polymer membrane sheet which is heat sealed to fabricate a tea-bag like µ-SPE device. The µ-SPE device is then used for the adsorption of the analytes from the sample solution. The unique feature of µ-SPE is its direct use in complex samples as sorbent is effectively protected inside the membrane bag and interfering species cannot adsorb on it. That is why it has been used for a wide variety of matrices [8]. After the adsorption, analytes are back extracted into a suitable solvent. µ-SPE has been widely used for the extraction of analytes from environmental [6,9–29], food [30–37], and biological samples [38–48].

µ-SPE cannot extract directly from the solid samples they need to be digested [38,41] or dissolved into a liquid [33]. In this work, we propose for the first time, a new idea for the direct extraction from the solid samples into a suitable solvent. Instead of packing the sorbent inside the porous membrane bag, we suggest packing the solid sample inside the bag. The analytes are extracted by immersing the solid sample containing bag inside the suitable solvent through the aid of the ultra-sonication. This approach eliminates the step of adsorption as analytes are directly extracted into the suitable solvent. Moreover, no sample cleanup is needed, because the interfering species cannot come out of the porous membrane. This technique results in a clear extract that can be directly injected into the analytical instrument. The proposed methodology is fast and easy to perform. In addition, no specific instrumentation is required. Depending on the solvent used, it can be considered green due to such reasons: small volume of sample as well as solvent is required, small amount of waste is produced, no much energy is consumed, depending on characteristic of analytes – several group of compounds can be extracted in single extraction. In addition, this technique can be applied for samples with complex matrices because PP membrane effectively secures the sorbent from fats, proteins and other large biomolecules. In this work, PAHs were extracted from the soil samples to demonstrate the proof of the concept. However, this idea is also extendable for variety of analytes present in various solid samples.

2. Experimental

2.1. Materials and chemicals

A multi-component certified standard solution (QTM PAH mix) containing 17 PAHs (Acenaphthene, Acenaphthylene, Anthracene, Benzo(a)anthracene, Benzo(b)fluoranthene, Benzo(ghi)perylene, Benzo(a)pyrene, 2-Bromonaphthalene, Chrysene, Dibenz(ah)anthracene, Fluoranthene, Fluorene, Indeno(1,2,3-cd)pyrene, Naphthalene, Phenanthrene, Pyrene; listed in Table 1) at a concentrations of 2000 µg/mL (in methylene chloride) was purchased from Sigma Aldrich (St. Louis, MO, USA). Benzo(a)anthracene-d12 was also obtained from Sigma Aldrich (St. Louis, MO, USA) and was used as internal standard (IS). HPLC-grade solvents (acetone, methanol and n-hexane) were delivered from Fisher (Loughborough, UK). Polypropylene (PP) flat membrane sheet roll (Type PP 1E (R/P), pore size: 0.1 µm, wall thickness: 100 µm) was obtained from Membrana (Germany).

2.2. Collection and preparation of soil samples

The real soil samples were collected from the side of the road from the two places: village placed 50 km from Gdańsk (1-6) and city center of Gdańsk (7-13; North of Poland), while soil for method optimization and validation was collected from the place at the seaside (Gdańsk). The real samples were collected from the surface of the sandy road, and 5 cm under this point, to present differences between concentration of selected PAHs in surface soil samples and in samples coming 5 cm under the surface.

All samples were transported to the laboratory in glss/plastic tubes. Then, they were dried and homogenized. For optimization procedure, 25 g of soil was spiked with 1.25 mL of PAHs standard solution (stock solution: 1 µg/cm3) dissolved in 20 mL of acetone. Such prepared soil was used for further optimization experiments.

2.3. Fabrication of extraction device and extraction procedure

The membrane bag was prepared by heat-sealing the edges of porous polypropylene (PP) membrane sheet. One end was kept open for filling of solid soil samples. 2.5 mg of soil sample (spiked with 50 ng/mL of PAHs mixture standard solution or real) was filled and remaining end was heat-sealed. The dimensions of membrane device were 0.8 cm × 0.8 cm. The membrane device was placed in a 4 mL glass vial, and extraction solvent was added. Then, the vial was subjected to ultrasound bath and the extraction was allowed to take place for 25 min. The membrane device was then removed from the vial, and the extract was dried in the stream of nitrogen at 40°C. Then n-hexane (100 µL) was added into the vial to reconstitute the analytes. The resulting extract was then transferred to 200 µL insert placed in autosampler vials and 2 µL aliquot was injected into GC–MS system for analysis.
Each optimization experiment was conducted in triplicate. The parameters that affect the efficiency of extraction including extraction solvent and its volume, extraction time, and ultrasound power were suitably optimized. Extraction efficiency was evaluated based on comparing of chromatographic peak areas.

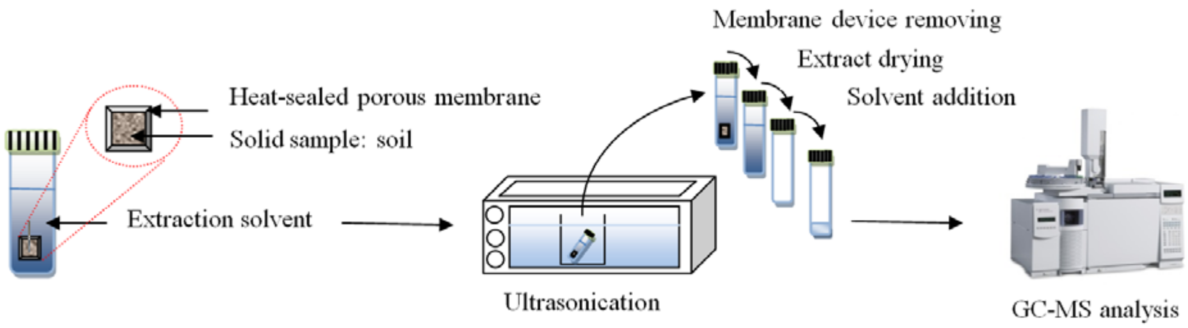

Figure 1. The workflow of the developed analytical procedure for PAHs determination in soil samples

2.4. Preparation of stock solutions, calibrators and quality control samples

Stock solution of analytes was prepared in methanol by diluting the certified standard solution to reach a concentration of 10 µg/mL. Stock solution of the IS was prepared also in methanol at a concentration of 10 µg/mL. All solutions used for calibration and validation were stored at -20°C prior to analysis.

The calibrators (n=3) were prepared in methanol by diluting the stock solution of analytes to concentrations of 12.5, 25, 50, 62.5, 100, 250, 500, 1000, 2500, 5000 ng/mL what correspond the concentrations of 0.5, 1, 2, 2.5, 4, 10, 20, 40, 100, 200 ng/mg of soil (these values were calculated as the mass of analytes added to the samples). The concentration of the IS in each calibrator was maintained at 500 ng/mL (20 ng/mg of soil).

Quality control (QC) samples were prepared in triplicate (n=3) at two concentration levels within the range of concentrations of calibration solutions: low - 500 (LQC; 20 ng/mg soil) and high 2500 (HQC; 100 ng/mg soil) ng/mL by adding appropriate volume of stock solution of analytes and the IS to the soils samples followed by extraction procedure and GC-MS analysis. QC samples were used for the evaluation of the repeatability.

2.5. GC-MS conditions

Analyses were performed using two equipments for procedure optimization and validation: a 7890A GC System (gas chromatography; Agilent Technologies, Santa Clara, CA, USA) equipped with an electron ionization (EI) ion source and a 5975C single quadrupole mass spectrometer (MS) (Agilent Technologies) and a 7890B GC System (gas chromatograph) with an EI ion source and a 5977B single quadrupole mass spectrometer (MS) (Agilent Technologies), respectively. Both GC systems were coupled with MPS (MultiPurpose Samper) robotic autosampler and a split/splitless CIS 4 injection system (Cooled Injection System) allowing for programming temperature of injection port (Gerstel GmbH & Co. KG). This temperature was initially set at 110°C and ramped up to 270°C at 10°C/s which was held to the end of analysis. The Pulsed Splitless mode for 1 min with initial injection pressure set at 50 psi for 0.5 min was used. Subsequently, split (20:1) mode was applied. The separation of analytes was carried out on a Phenomenex ZB-5 MS capillary column (30 m × 0.25 mm id, and 0.25 µm film thickness, Shim-pol, Izabelin, Poland) with helium at a purity of 99.999% as the carrier gas in a constant flow of 1 mL/min. The oven temperature was programmed at 70°C for 1 min, then increased to 200°C at 15°C/min, next increased to 270°C at 5°C/min and finally ramped up to 300°C at 10°C/min and held for 6 min. Post-run conditioning was carried out for 2 min at 300°C. The temperatures of the MS transfer line, ion source, and detector were set at 285, 230 and 150°C, respectively. The MS was operated in positive mode (electron energy 70 eV). Full-scan acquisition was performed with the mass detection range set at m/z 40-400 to determine retention times of analytes, optimize oven temperature gradient, and to observe characteristic mass fragments for each compound. For the identification and quantification of the analytes SIM mode was used with the ions listed in Table 1. All the ions were chosen due to their specificity and abundance. Data acquisition and analysis were accomplished by MassHunter GC/MS Acquisition software by Agilent Technologies (version B.07.05.2479) and Maestro 1 software by Gerstel GmbH & Co. KG (version 1.5.3.2/3.5). The optimization and validation was performed on two different instruments due to which difference in peak intensities was observed.

Table 1. Provides information on retention time and quantitative ion of analytes used for detection.

2.6. Method validation

The new developed membrane supported GC-MS-based method for PAHs' quantification was validated according to international guidelines in the field of our study [cyt.] in terms of: selectivity, linearity, sensitivity - limit of detection (LOD) and limit of quantification (LOQ), matrix effect, carry-over effect, recovery and repeatability.

The selectivity experiments were performed to verify the presence of endogenous or exogenous compounds in the retention times of the analytes and the IS. For this purpose, 6 various origin soil blank samples were analysed after the extraction step according to procedure described in section 2.3.

To compensate the variability of the detector signal during different analyses and losses of analyte in the extraction step (to increase repeatability), the internal standard calibration was performed. In order to increase the accuracy of the method, the weighted linear regression was applied to the calibration curves. The linearity of the weighted calibration curves were expressed as the correlation coefficient (r). The LOD and LOQ were assessed based on regression parameters of weighted calibration curves and calculated using the following formula: $LOD=3.3 \cdot S_b/a$, where S_b is the standard deviation of the intercept and a is the slope of the calibration curve. The values of limit of quantitation (LOQ) were calculated as three times LOD.

The matrix effects (ME) of the developed method was evaluated using procedure described by Matuszewski *et al*. [49]. ME were investigated at two concentration levels, similar to QC samples 500 and 2500 ng/mL and was calculated by comparing the responses (peak area of each analyte against peak area of the IS) for appropriate solution of analytes prepared in methanol (sets A, n=3) with those measured in blank soil extracts spiked after extraction procedure with the same analyte amount (sets B, n=3). The following formula was used ME[%]=B/A*100%.

The potential for carry-over of the analyte and the IS to the subsequent sample in the autosampler batch was evaluated by injecting 2 µL of methanol after calibration solution at the highest concentration level from the calibration curve (5000 ng/mL). The test was performed in six replicates.

The recoveries (in %) of the developed method were evaluated by comparing the analyte-to-IS peak area ratios of the spiked and extracted blank soil samples with the corresponding peak area ratios of the matrix extracts fortified with standards at concentrations of QC samples (n=3). In this test the IS was added after extraction as was suggested by Matuszewski *et al.* [49]. The repeatability of the method was determined as intra- and inter-assay accuracy and precision. Intra-day assay measurements were carried out by analysing QC samples (n=3). To determine the inter-day assay repeatability the tests were repeated over three different days. The accuracy (A%) of the method was calculated using following formula: $A=c_m/c_{nom}*100\%$ (c_m is the measured concentration of analytes in QC samples and c_{nom} is the appropriate nominal concentration). Precision was assessed as correlation coefficients (CVs) of above-mentioned measurements.

3. Results and discussion

3.1. Optimization of extraction procedure

Several parameters affect the extraction efficiency including extraction solvent and its volume, extraction time and ultrasound power. Thus, these parameters were examined during this experiment.

3.1.1. Extraction solvent

The extraction solvent should be carefully selected as it has significant importance in extraction process. Affinity between extraction solvent and analytes in terms ofpolarity is an important parameter to consider. One mixture of organic solvents (acetone: n-hexane, 1:1 v/v) and three organic solvents and with varying polarity index (n-hexane, dichloromethane and toluene) were employed as extraction solvent. N-hexane was found the most effective compared to other examined solvents and it wasselected as an optimum extraction solvent (Fig. 2). PAHs were effectively extracted into n-hexane due to non-polar nature of both the PAHs and solvent.

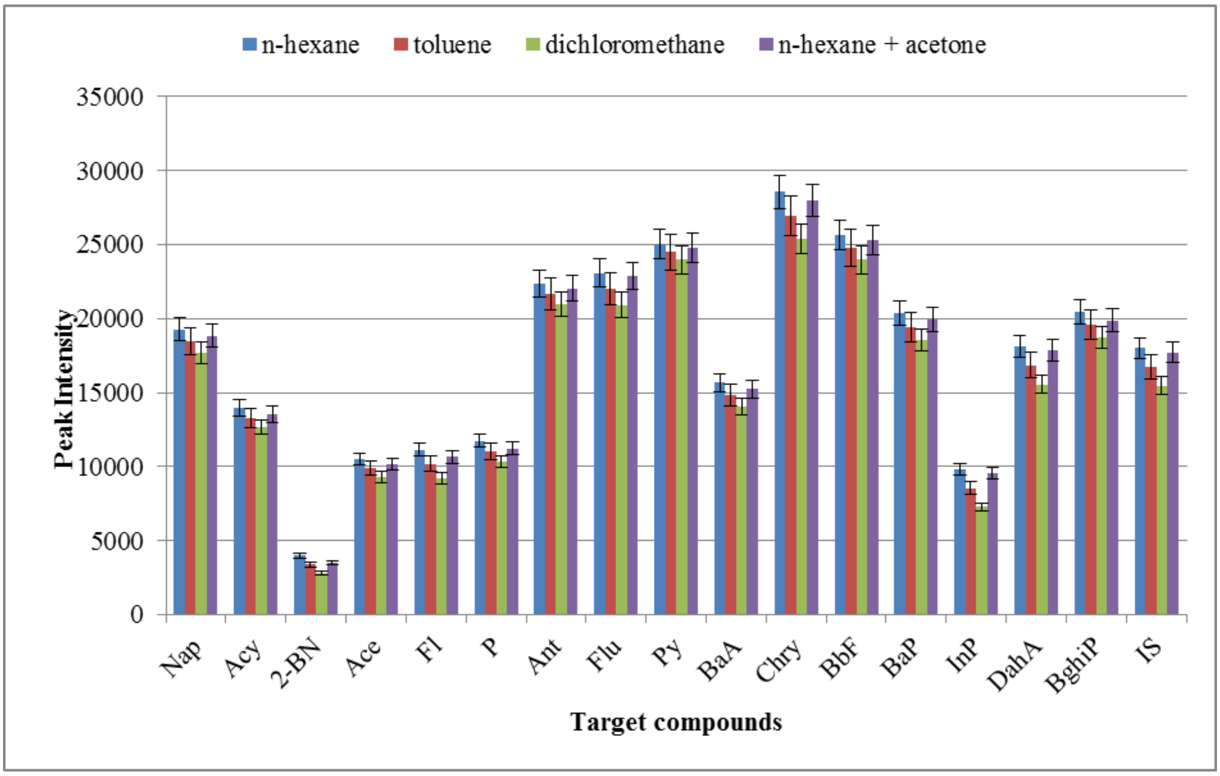

Figure 2. Selection of extraction solvent based on peak intensity for the determination of PAHs with ultrasound-assisted solvent extraction of porous membrane packed solid samples.

To elute the target compounds from porous membrane packed solid samples in a reproducible manner, the volume of extraction solvent should be sufficient enough to completely immerse the membrane device. In these experiments, we selected a constant volume of solvent as 1 mL, which was enough to completely immerse the solid sample containing membrane bag. After the completion of the extraction, the solvent was evaporated to dryness and reconstituted in 100µL of n-hexane.

3.1.2. Time of extraction (ultra-sonication)

Since proposed here procedure is time dependent, the mass transfer of analytes increases with extraction time until an equilibrium or steady state is attained. Thus, the time of extraction was examined in the range of 10–35 min. After 25 min, no further increase in peak areas of analytes was observed till 35 min. However, some decrease was observed. This attributed to rise of temperature by longer sonication times, which may evaporate analytes to the headspace and they can escape upon opening the vial. The longer times may also cause degradation of analytes.

Hence, extraction time of 25 min was selected as optimum extraction time (Figure 3).

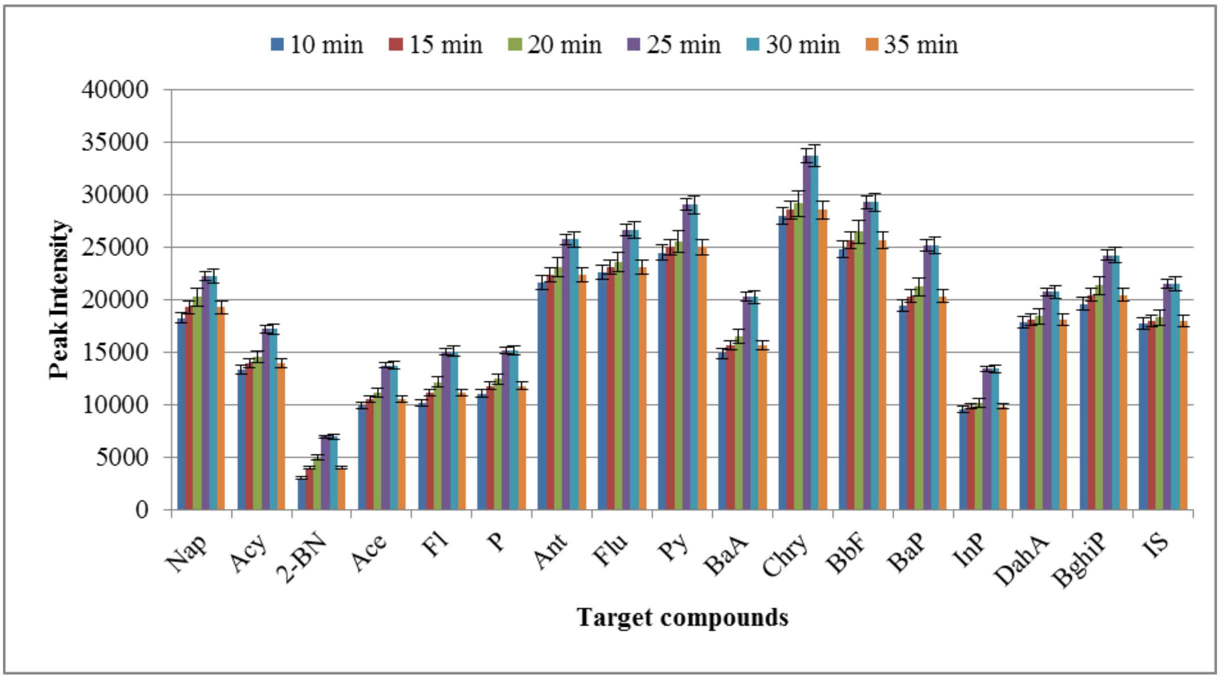

Figure 3. Selection of the extraction time based on peak intensity for the determination of PAHs with ultrasound-assisted solvent extraction of porous membrane packed solid samples.

3.1.3. Ultrasound power

Because the extraction process was supported by ultra-sonication, ultrasound power was evaluated in the range of 10 W – 100 W. Peak areas were increased up to 60 W and then became constant. However, after the application of 80W and higher powers, a significant decrease in the peak areas of the analytes were observed. It can be attributed to the fact that higher ultrasound power can increase temperature, which may result in evaporation of analytes in headspace over the vial. The second reason can be speculated as degradation of analytes under intensive sonication for longer times. Hence, 60 W was selected as an optimum ultrasound power.

3.2. Method validation

The developed GC-MS-based method includes three ions (1 quantifier and 2 qualifiers). An example chromatogram in SIM mode of all analytes at a concentrations of 1000 ng/mL and the IS (500 ng/mL) is presented in Fig. 4a. The increased sensitivity, better peak shape, and the better SNR was enabled by careful optimization of chromatographic conditions, such as the temperature of the injector, the initial and final column temperature, the temperature rate and carrier gas flow, as well as the injection mode (split, splitless by different period of the time, and pulsed splitless using various pressure conditions maintained by different time).

No interfering peaks of additional naturally occurring substances in soil in retention times of analytes and the IS which could have obstruct the quantification were reported in the soil blank samples investigated for selectivity (Fig. 4b). Therefore, the presented method can be considered as specific and selective for the determination of PAH in soil samples. No significance MEs were observed for most analytes, because there were determined in the range of 89.8-111. Such MEs varied between 80-120% can be perceived as soft and can be neglected [50]. Only for Chrysene there was observed high enhancement of the detector signal while was injected in matrix extract compared to the signal injected in the solvent (ME=160-188%). Therefore, to avoid necessity of preparation matrix-match calibration solutions for calibration, this compound was not used for further analysis. A carry-over effect was not observed. Seven- or six-point calibration curves were constructed using the peak area ratio (analytes vs IS) plotted against the concentration (number of replicates for each level n=3). The method was shown to be linear within the tested calibration ranges. The details on curves' range for each analyte and corresponding weighting factors are shown in Table 2. The data of correlation coefficients (r) of the weighted calibration curves, their regression parameters and LODs and LOQs for each analyte are presented also in Table 2. The accuracy, precision, recoveries data for intra- and inter-day measurements, and MEs values are summarized in Table 3. Importantly, for three compounds (Fluorene, Benzo(a)pyrene, and Indeno(1,2,3-cd)pyrene) the recoveries were below 80%, and therefore, there were took into account to calculate accuracy and precision.

Based on the obtained validation parameters which fulfil the established international criteria for analytical methods, it could be stated, that the presented method for the quantification of PAH in soil samples is characterized by high accuracy and precision and can be used for the analysis of real samples.

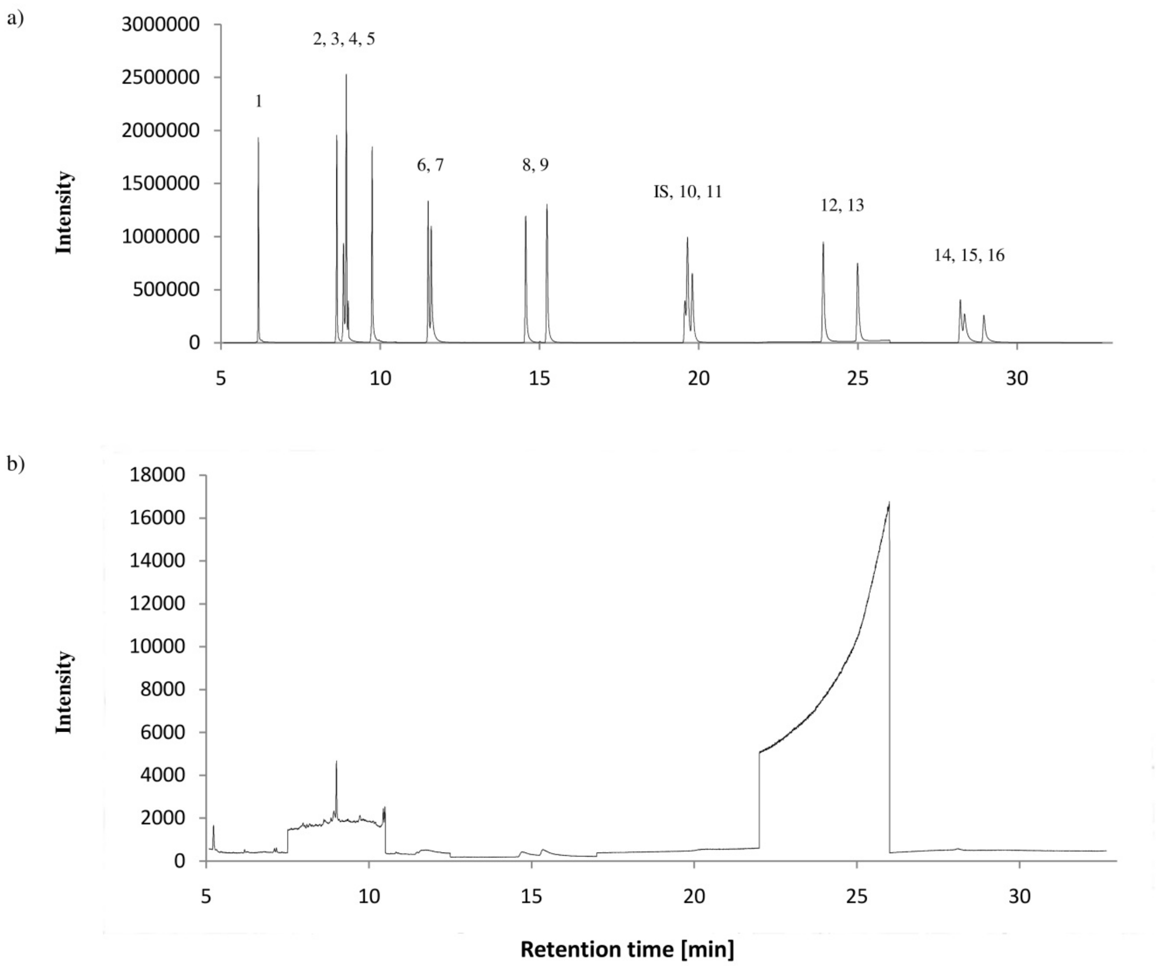

Fig. 4 GC-EI-MS chromatogram of a) mixture of PAH (1000 ng/mL) and the IS (500 ng/mL) in SIM mode, b) blank soil sample in SIM mode for selectivity test (numbers correspond compounds listed in Table 1).

3.3. Analysis of real samples

The proposed method was carried out to determine the PAHs levels in the real soil samples. Each measurement was performed four times. The information on concentration levels for PAHs determined in real samples are presented in Table 4. All of the compounds were determined in each sample. It was found that soil samples coming from village contain lower concentrations of PAHs than those coming from city center. In addition, in most cases, samples collected 5 cm under surface are characterized by lower concentration level of PAHs than those collected from the surface of the road. This was expected as in soils from large cities, along transport routes, in the vicinity of industrial plants, the level of these pollutants can be very high. It was expected that in village, some samples will be free of PAHs but this not happened. This can be because the village is placed close to Tricity (a metropolitan area in Poland consisting of three cities in Pomerania: Gdańsk, Gdynia and Sopot, as well as minot towns in their vicinity). And as it is well known, the transport of pollutants in the atmosphere poses a danger that in areas of limited anthropopressure - with minimal sources of pollution - their level can be significant.

4. Conclusion

For the first time, a simple and cost-effective method is proposed for the extraction of the target analytes from the solid samples. This method is based on packing of the solid sample inside a porous membrane bag which is subjected to solvent extraction under ultrasonication. This method eliminates many steps associated with conventional sorbent-based membrane extraction. The steps like sample pretreatment, digestion/dissolution, and adsorption of analytes on a selective adsorbent are omitted. In addition, it does not require special equipment for filtration and centrifugation. This method has shown excellent analytical figures of merit for the extraction of PAHs in soil samples. In comparison with conventional methods of PAHs determination presented in the literature [51, 52, 53], this method present lower LOD and LOQ, thus allow to determine ultra-trace concentration level of PAHs. In addition, it is faster and do not requires any additional instrumentation. The applications of this method can be further extended to other analytes present in variety of solid matrices. This work represents mainly a proof of concept, we expect some interesting applications of this method in the future.

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Authors' contribution

The idea of this work was proposed by Muhammad Sajid who also suggested the experimental design and contributed in manuscript preparation mainly in write up of the abstract, introduction, and conclusion and formatting of the rest of the manuscript. Mateusz K. Woźniak and Justyna Płotka-Wasylka performed collection of samples, experimentation, data analysis, and write up of experimental as well as results and discussion part.

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Table 1. List of target analytes, their retention times, and selected ions for SIM mode

L.p.	Detection widndow (time range [min])	Compound	Rt [min]	Quantitative ion	Qualitative ions
1	1 (5-7,5)	Naphthalene	6.13	128	127, 129
2	2 (7,5-10,5)	Acenaphthylene	8.65	152	151, 153
3		2-Bromonaphthalene	8.85	206	127, 208
4		Acenaphthene	8.95	153	154, 152
5		Fluorene	9.76	166	165, 167
6	3 (10,5-12,5)	Phenanthrene	11.52	178	176, 179
7		Anthracene	11.61	178	176, 179
8	4 (12,5-17)	Fluoranthene	14.59	202	200, 203
9		Pyrene	15.26	202	200, 203
10	5 (17-22)	Benzo(a)anthracene	19.67	228	226, 229
11		Chrysene	19.83	228	226, 229
12	6 (22-26)	Benzo(b)fluoranthene	23.94	252	250, 253
13		Benzo(a)pyrene	25.01	252	250, 253
14	7 (26-)	Indeno(1,2,3-cd)pyrene	28.24	276	277, 274
15		Dibenz(ah)anthracene	28.36	278	276, 279
16		Benzo(ghi)perylene	28.99	276	277, 274
18	5	IS	19.6	240	236, 120

Table 2 Quantification and calibration data for PAH analysed in this study

Analyte	Calibration range [ng/mg]	r	LOD [ng/mg]	LOQ [ng/mg]
Naphthalene	1 - 200	0.9995	0.32	0.97
Acenaphthylene	0.5 - 200	0.9993	0.19	0.57
2-Bromonaphthalene	1 - 200	0.9994	0.38	1.1
Acenaphthene	1- 200	0.9995	0.27	0.8
Fluorene	1.5 - 200	0.9992	0.53	1.6
Phenanthrene	1 - 200	0.9991	0.31	0.94
Anthracene	1.5 - 200	0.9994	0.51	1.5
Fluoranthene	2 - 200	0.9990	0.60	1.8
Pyrene	0.5 - 200	0.9992	0.25	0.77
Benzo(a)anthracene	1 - 200	0.9996	0.34	1.0
Benzo(b)fluoranthene	2 - 200	0.9995	0.63	1.9
Benzo(a)pyrene	1.5 - 200	0.9996	0.46	1.4
Indeno(1,2,3-cd)pyrene	2.5 - 200	0.9987	0.83	2.5
Dibenzo(ah)anthracene	2.5 - 200	0.9993	0.93	2.8
Benzo(ghi)perylene	2.5 - 200	0.9995	0.89	2.7

r - Correlation coefficient, *LOD* - limit of detection, *LOQ* - limit of quantification

Table 3 Summary of the validation study: accuracy (precision), recoveries±SD [%], and ME [%] (*n*=3)

Analytes	C	Intra-day Day1	Day 2	Day3	Inter-day	Recovery	ME [%]
Naphthalene	20	100 (5.2)	94.7 (3.0)	93.9 (3.6)	96.2 (3.4)	97.2 ± 1.6	97.5
	100	103 (1.7)	101 (1.4)	96.8 (1.6)	100 (3.2)	91 ± 2.2	96.5
Acenaphthylene	20	95.4 (7.5)	101 (3.9)	100 (4.8)	98.8 (3.0)	90.9 ± 1.7	99.8
	100	102 (1.3)	104 (1.8)	97.5 (4.1)	101 (3.3)	97.8 ± 23	102
2-Bromonaphthalene	20	96.4 (5.5)	92.1 (3.8)	98.2 (4.2)	95.6 (3.3)	100 ± 3.6	104
	100	104 (1.7)	101 (0.4)	99.1 (3.8)	101 (2.4)	98.6 ± 2.9	96.1
Acenaphthene	20	97.8 (7.3)	94.2 (3.5)	101 (6.1)	97.7 (3.5)	101 ± 3.8	101
	100	104 (1.5)	101 (1.4)	95.2 (4.5)	101 (4.5)	93.7 ± 4.2	98.8
Fluorene	20	96.4 (6.5)	93.1 (3.5)	102 (5.1)	97.2 (4.6)	77.5 ± 3.9	107
	100	107 (1.4)	100 (2.8)	97.8 (3.7)	102 (4.7)	78.6 ± 2.6	100
Phenanthrene	20	93.5 (4.9)	98.1 (6.4)	99.5 (5.1)	97.2 (3.2)	89.9 ± 5.7	109
	100	101 (1.8)	105 (2.6)	95.2 (3.5)	103 (2.7)	99.1 ± 1.2	108
Anthracene	20	94.6 (9.5)	94.2 (4.2)	98.1 (7.1)	95.6 (2.2)	98.9 ± 6.9	108
	100	109 (1.7)	102 (2.5)	99.6 (3.2)	104 (4.7)	98.7 ± 6.1	103
Fluoranthene	20	92.0 (6.0)	89.4 (5.3)	95.7 (4.1)	92.4 (3.4)	102 ± 9.2	107
	100	103 (1.8)	108 (3.0)	98.1 (4.1)	103 (4.8)	96.0 ± 2.9	109
Pyrene	20	90.3 (5.6)	87.2 (7.4)	85.1 (4.5)	87.5 (3.0)	102 ± 4.8	110
	100	102 (1.8)	105 (2.5)	101 (2.9)	103 (2.0)	94.2 ± 9.2	106
Benzo(a)anthracene	20	89.3 (7.6)	93,2 (5.0)	94.5 (6.1)	92.3 (2.9)	80.6 ± 4.9	111
	100	98.5 (2.6)	100 (2.7)	99.5 (3.3)	99.3 (0.8)	106 ± 4.5	108
Benzo(b)fluoranthene	20	90.0 (7.5)	87.2 (4.3)	91.2 (5.2)	89.5 (2.3)	94.1 ± 2.6	103
	100	102 (1.4)	99.6 (3.3)	96.7 (4.1)	99.4 (2.7)	98.4 ± 3.6	104
Benzo(a)pyrene	20	92.6 (8.3)	102 (5.7)	95.1 (7.2)	96.6 (5.0)	75.1 ± 4.9	103
	100	101 (1.5)	99.8 (3.8)	97.5 (2.4)	99.4 (1.8)	103 ± 3.5	105
Indeno(1,2,3-cd)pyrene	20	88.1 (3.5)	84.1 (4.5)	91.1 (4.9)	87.8 (4.0)	79.8 ± 2.6	89.8
	100	101 (2.3)	103 (4.0)	105 (3.9)	103 (1.9)	92.2 ± 2.8	91.5
Dibenzo(ah)anthracene	20	108 (6.5)	110 (2.9)	101 (5.2)	109 (1.1)	98.9 ± 4.2	109
	100	107 (4.2)	109 (3.5)	110 (4.5)	109 (1.4)	99.4 ± 5.6	107
Benzo(ghi)perylene	20	102 (4.6)	108 (3.4)	106 (4.1)	105 (2.9)	97.5 ± 3.6	108
	100	111 (0.9)	106 (4.1)	110 (1.1)	109 (2.4)	99.0 ± 3.4	106

C - nominal concentration in ng/mg, *n* - number of measurements, ME - matrix effect

Table 4 Information on concentration levels for PAHs determined in real samples

	Concentration [ng/mg], n=4											
	Sample ID											
	SU	**5 cm/SU**	**SU**	**5 cm/SU**	**SU**	**5 cm/SU**	**SU**	**5 cm/SU**	**SU**	**5 cm/SU**	**SU**	**5 cm/SU**
Analytes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Naphthalene	90.0±2.2	79.8±1.5	94.8±2.2	71.8±1.6	34.3±1.0	31.4±1.1	192±3.7	152±2.9	257±3.8	205±3.7	116±1.8	110±1.9
Acenaphthylene	100±2.3	89.5±1.6	105±2.3	79.8±1.3	37.6±1.3	33.1±1.0	214±3.9	169±2.8	283±4.2	225±3.6	129±2.6	122±2.0
2-Bromonaphthalene	97.6±2.4	86.9±1.4	106±1.9	77.7±1.4	35.9±1.0	32.4±1.4	232±4.1	174±2.1	332±4.3	238±3.8	128±2.9	123±2.1
Acenaphthene	91.6±2.0	82.2±1.5	97.8±2.2	74.2±1.6	35.5±1.2	32.2±1.2	203±3.9	159±2.2	280±4.0	215±3.9	119±2.2	112±2.0
Fluorene	70.6±1.6	62.4±1.3	76.1±1.5	57.5±1.2	25.7±1.1	23.6±1.1	154±2.6	123±1.7	212±4.1	163±3.6	91.2±1.6	86.3±1.3
Phenanthrene	105±2.4	91.9±2.4	108±1.8	81.6±1.5	35.4±1.0	33.6±1.0	228±4.1	183±3.7	307±4.3	242±4.2	133±2.3	129±2.9
Anthracene	101±2.3	88.9±2.2	107±2.0	81.4±1.3	35.1±1.4	31.5±1.4	215±3.6	177±2.6	287±3.8	231±3.7	129±2.5	124±2.5
Fluoranthene	103±1.9	90.1±1.4	107±1.9	82.3±1.4	35.8±1.1	33.7±1.2	229±4.1	179±2.2	312±4.2	241±4.3	131±2.2	127±2.2
Pyrene	98.4±2.0	85.7±1.3	103±1.7	78.3±1.3	34.5±1.0	33.0±1.1	216±3.7	170±2.8	297±4.3	229±4.2	125±2.1	1202.7
Benzo(a)anthracene	108±2.3	93.8±1.6	114±1.8	86.9±1.5	35.4±1.2	23.90±0.93	247±3.8	192±3.9	341±4.5	260±3.8	138±2.9	135±2.5
Benzo(b)fluoranthene	99.2±2.4	86.6±1.5	105±2.3	80.8±1.6	33.4±1.1	30.9±1.1	229±3.9	178±2.8	317±4.2	241±4.1	127±2.6	125±2.1
Benzo(a)pyrene	106±1.9	93.3±2.4	113±2.0	84.8±1.9	33.7±1.0	31.0±1.2	248±4.1	192±3.6	342±4.5	260±3.8	138±2.9	135±2.2
Indeno(1,2,3-cd)pyrene	87.0±1.5	80.8±2.2	95.4±2.2	79.2±1.3	31.8±1.2	28.10±0.97	120±2.7	116±1.8	148±2.2	133±2.6	99.7±1.5	86.8±1.6
Dibenzo(ah)anthracene	143±2.9	134±2.9	128±2.9	133±2.9	59.2±1.2	54.2±1.2	209±3.9	199±3.7	260±4.2	236±3.9	170±2.8	146±2.9
Benzo(ghi)perylene	114±1.6	108±1.6	124±2.5	109±2.3	49.7±1.3	47.9±1.0	145±2.6	149±2.9	179±3.3	160±2.6	127±2.5	112±1.9
SU, surface of road; 5cm/SU, 5 cm under surface of road												