

OPTIMIZATION OF SOLID-PHASE EXTRACTION AND GC-MS/MS DETERMINATION OF PAHs IN BULGARIAN SEWAGE SLUDGE

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Abstract

In this study, a method for the solid-phase extraction (SPE) and GC-MS/MS determination of the priority polycyclic aromatic hydrocarbons (PAH) in sewage sludge was developed. Two types of SPE sorbents (C18 ec and HLB) were evaluated for the separation and pre-concentration of the analytes. The initial extraction from the sludge matrix, column conditioning, and final elution were optimized using organic solvents of varying polarities, and the recovery for each PAH was evaluated. Satisfactory recoveries (96–114%) for all PAHs were achieved using acetone for the initial extraction, HLB (500 mg/3 mL) SPE columns, conditioning with dichloromethane/acetonitrile, and elution with acetone. The optimized method was applied to the analysis of PAHs in sewage sludge from Bulgarian wastewater treatment plants (WWTPs). Total PAH concentrations in urban sludge ranged from 393 µg/kg to 1780 µg/kg, while mixed domestic industrial sludge exhibited a higher concentration of 5980 µg/kg.

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Introduction. Wastewater treatment processes generate millions of tons of sludge annually in wastewater treatment plants (WWTPs) [1–3]. Sewage sludge is highly heterogeneous, with high concentrations of essential elements as well as potentially toxic elements (PTEs) [3,4] and persistent organic pollutants (POPs) [5–7]. In the EU-27, 27% of sewage sludge is used in agriculture [8], with Bulgarian and European legislation setting strict limits for PTEs and POPs [9,10]. Polycyclic aromatic hydrocarbons (PAHs) are among the most widespread POPs; sixteen are classified as priority pollutants [11], necessitating their strict monitoring [12–15]. GC-MS is the most commonly used method for PAH analysis, preceded by appropriate sample preparation – most frequently solid-phase extraction (SPE) [13–15]. Literature reviews indicate that various solvents and SPE columns of different polarities are employed for PAH extraction and clean-up [13–16]. While the PAH content in sewage sludge has been extensively studied globally [13–17], data regarding their concentrations in Bulgarian sewage sludge remain scarce [18].

The aim of the present study was to optimize a method for the quantitative extraction, clean-up, and GC-MS/MS determination of PAHs. This was achieved by assessing: (1) the efficiency of solvents of different polarities for PAH extraction from the sewage sludge matrix; (2) the capacity and selectivity of C18 and HLB sorbents, as well as the influence of solvents used for clean-up; and (3) the application of the method for PAH analysis in sewage sludge from Bulgarian WWTPs.

Experimental. Reagents. HPLC-grade hexane (n-Hex), dichloromethane (DCM), ethyl acetate (EA), acetone (Ace), methanol (MeOH), and acetonitrile (ACN) were used throughout the experiments. For the clean-up of PAHs, two types of SPE columns from Macherey-Nagel (GmbH & Co. KG, Germany) were evaluated: CHROMABOND C18 ec (octadecyl-modified silica gel, endcapped, 100 mg/1 mL, 45 μ m) and CHROMABOND HLB (poly(divinylbenzene-co-*N*-vinylpyrrolidone)) in two dimensions (100 mg/1 mL, 30 μ m and 500 mg/3 mL, 60 μ m).

Sewage sludge samples and reference materials. Sewage sludge samples from eight urban wastewater treatment plants (WWTP 1 to WWTP 8) and one treating a mixed flow of domestic, municipal and industrial wastewater (WWTP 9) were analyzed. All samples were collected after the on-site dewatering process. Prior to extraction, the sludge samples were air-dried to a constant weight, ground, and homogenized. Sample preparation was optimized using the certified reference material (CRM) LGC6182 (Sewage Sludge – PAHs, UKAS Reference Materials, UK). PAH-Mix 18 in ACN (10 μ g/mL, LGC Standards, Dr. Ehrenstorfer, Germany) was used to prepare the calibration standards.

Instrumentation. Analyses were performed using a gas chromatograph coupled with a triple quadrupole mass spectrometer (GC-MS/MS, EVOQ GC-TQ,

Bruker, Germany). Separation was achieved on a low-polarity Rxi-5Sil Ms stationary phase (Crossbond, 1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane; 30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness, Restek, USA). Helium served as a carrier gas at a constant flow rate of 1 mL/min. A mechanical rotator (MX-RL-E, Citotest Scientific Co., Ltd., China), an ultrasonic bath (Elmasonic S10 H, Elma Schmidbauer GmbH, Germany), a vortex mixer (VELP Scientifica, Italy), and a centrifuge (ROTOFIX 32 A, Andreas Hettich GmbH, Germany) were utilized for PAH extraction. Purification and concentration were conducted using a CHROMABOND SPE vacuum manifold (Macherey-Nagel GmbH, Germany) and a sample concentrator (Stuart SBHCONC/1, Cole-Parmer UK).

Results and discussion. Optimization of GC-MS/MS method. Chromatographic conditions were optimized using PAH-Mix-18 in scan mode. The oven temperature programme started at 60 °C (1 min hold), increased to 180 °C at 7 °C/min (2 min hold), then to 270 °C at 6 °C/min (20 min hold), and finally to 290 °C at 10 °C/min (7 min hold). The injector and transfer line temperatures were maintained at 270 °C and 280 °C, respectively. These optimized conditions were subsequently applied to a selected ion monitoring (SIM) method. The resulting chromatogram for the studied PAHs and the monitored ions are presented in Fig. 1. The calibration curves were linear from 50 μ g/L to 1000 μ g/L ($R^2 \geq 0.99$) for all analytes. The detection limits ranged from 10 to 30 μ g/kg.

Solid-phase extraction procedure. While C18 and HLB sorbents typically require conditioning with the polar methanol-water mixtures [16, 17, 19] to retain PAHs from polar media, water was omitted in the present study. A 5 mm layer of anhydrous sodium sulphate was added to the top of the C18 (100 mg) and HLB (100 mg and 500 mg) columns, which were then initially conditioned with DCM (10 mL) followed by ACN (10 mL) to equilibrate the sorbent for sample loading. Subsequently, three separate 1 mL aliquots of a PAH standard solution (100 μ g/L) in ACN were loaded via gravity flow. The effluents were collected separately and analyzed by GC-MS/MS. The results indicated incomplete retention of PAHs on the C18 (100 mg) sorbent within the first 1 mL aliquot. For the HLB (100 mg) sorbent, breakthrough occurred in the second and third aliquots. These observations may be attributed to the high elution strength of ACN, though low recoveries have been reported for C18 (100 mg) applying methanol-water conditioning [19]. In contrast, the HLB (500 mg) achieved quantitative retention (87% to 100%) across all three aliquots, successfully counteracting elution strength through increased bed mass. Next, the retention capacity of HLB (500 mg) was tested on four separate SPE columns conditioned with solvents of increasing polarity (DCM, EA, Ace, and MeOH), each followed by ACN. While all conditioning sequences ensured quantitative retention, that involving DCM/Ace yielded the lowest baseline noise and was selected for further experiments. To optimize the elution step, five HLB columns (500 mg) were conditioned with DCM/ACN, loaded with 2 mL of a 200 ng/mL PAH standard solution via gravity flow, and then vacuum-dried.

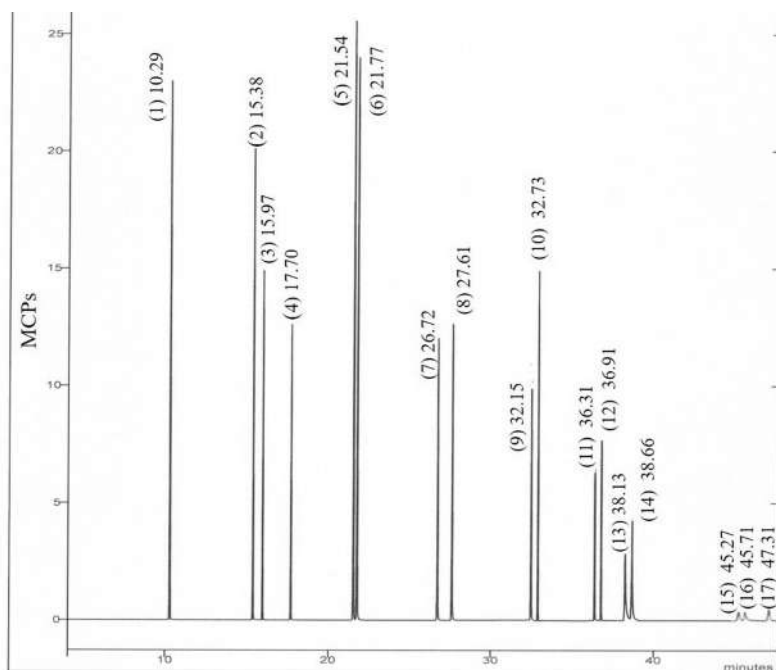


Fig. 1. GC-MS chromatogram of standard solution of PAHs. The Peaks: (1) naphthalene (m/z 128); (2) acenaphthylene (m/z 152); (3) acenaphthene (m/z 153); (4) fluorene (m/z 166); (5) phenanthrene (m/z 178); (6) anthracene (m/z 178); (7) fluoranthene (m/z 202); (8) pyrene (m/z 202); (9) benzo[a]anthracene (m/z 228); (10) chrysene (m/z 228); (11) perylene (m/z 252); (12) benzo[b]fluoranthene (m/z 252); (13) benzo[k]fluoranthene (m/z 252); (14) benzo[a]pyrene (m/z 252); (15) indeno[1,2,3-cd]pyrene (m/z 276); (16) dibenzo[a,h]anthracene (m/z 278); (17) benzo[ghi]perylene (m/z 276)

Each column was eluted with 10 mL of a solvent of varying polarity: n-Hex, DCM, EA, EA/Ace (1:1, v/v) and Ace. The eluates were concentrated to a few drops under a gentle stream of nitrogen. Solvent exchange was performed by adding two 1-mL portions of ACN; for n-Hex extracts, an intermediate step involving two 1-mL portions of Ace was included to ensure miscibility. Finally, the extracts were reconstituted in 0.5 mL of ACN and analyzed by GC-MS/MS. Recovery results are shown in Fig. 2. The results indicated poor recoveries (below 60%) for low-molecular-weight (2-ring) and high-molecular-weight (HMW, 4–6 ring) PAHs using n-Hex; for 2-ring and the more volatile 3-ring PAHs using DCM; and for 2-ring PAHs using EA and EA/Ace. The low recoveries of the 2–3 ring species may be attributed to volatilization losses during the nitrogen evaporation step. Conversely, the poor recovery of HMW compounds with n-Hex is likely due to its insufficient elution strength. Quantitative recovery across the entire PAH range, regardless of molecular weight, was achieved using Ace. These findings demon-

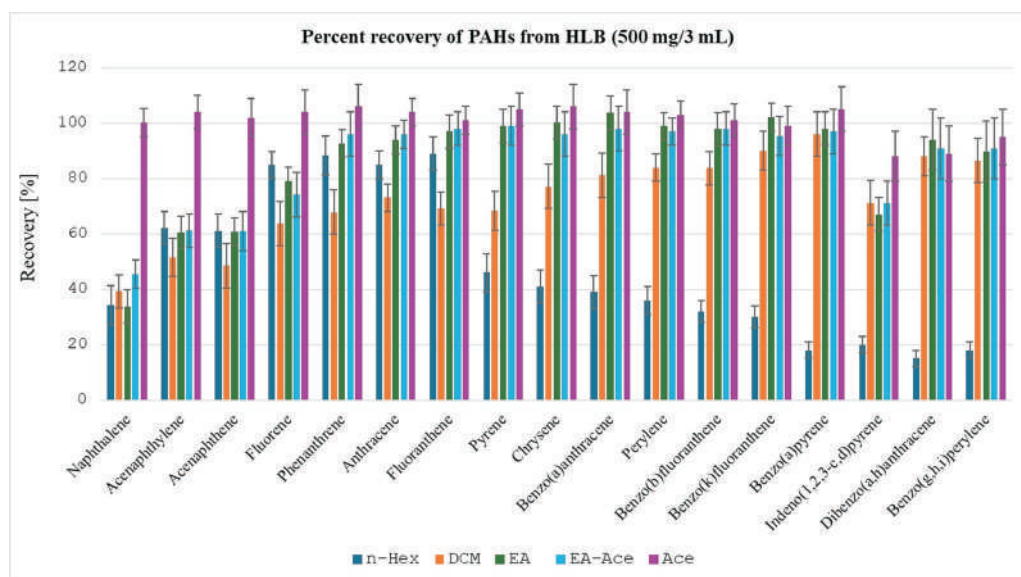


Fig. 2. Recoveries of PAHs obtained with different elution solvents (n-Hex, DCM, EA, EA-Ace and Ace)

strate that Ace is highly effective for the elution of PAHs from the HLB polymeric matrix and possesses high analyte affinity, which minimizes volatilization losses.

Optimization of the extraction of PAHs from sewage sludge. To evaluate PAH extraction efficiency from the sludge matrix, CRM LGC6182 (Sewage Sludge) was extracted using various solvents and mixtures: n-Hex, DCM, EA, Ace, and 1:1 (v/v) mixtures of n-Hex/EA, DCM/Ace, and EA/Ace. Samples were processed according to established methods [20] with slight modifications. Briefly, 10 mL of the respective solvent was added to 1 g of the sample. The mixture was shaken on a mechanical rotator (2 h), followed by ultrasonic extraction (1 h) and vortexing (30 min). The extracts were then centrifuged at 3600 rpm (15 min), decanted, and treated with activated copper for desulphurization. Concentration and solvent exchange into 3 mL of ACN were then performed. The concentrated liquids were cleaned up on HLB (500 mg) columns, eluted with 10 mL of Ace, evaporated under nitrogen, and reconstituted in 0.5 mL of ACN. Analyte concentrations and the total procedural standard deviations ($n = 6$) are summarized in Table 1.

n-Hexane proved ineffective for quantitative PAH extraction, yielding low recoveries for most compounds (58–88%). Similarly, extraction with n-Hex/EA, EA, and EA/Ace led to unacceptable results, with recoveries in the ranges 74–130%, 85–135%, and 90–130%, respectively. While DCM and DCM/Ace are commonly used extractants [14–16], they led to abnormally high recoveries (up to 160%) under the current experimental conditions and were consequently discarded. This

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Experimental concentrations (mean $\pm$ SD) of PAHs in the individual extracts of CRM LGC6182 [$\mu\text{g}/\text{kg}$]

	n-Hex	n-Hex + EA	EA	EA + Ace	Ace	Certified value
Naphtahlene	130 $\pm$ 9	148 $\pm$ 12	213 $\pm$ 17	207 $\pm$ 14	226 $\pm$ 15	200 $\pm$ 130
Acenaphthylene	31 $\pm$ 4	51 $\pm$ 5	53 $\pm$ 5	52 $\pm$ 4	45 $\pm$ 4	(40)
Acenaphthene	76 $\pm$ 8	83 $\pm$ 8	87 $\pm$ 9	89 $\pm$ 9	96 $\pm$ 11	(90)
Fluorene	137 $\pm$ 14	188 $\pm$ 17	204 $\pm$ 17	180 $\pm$ 18	163 $\pm$ 16	159 $\pm$ 68
Phenanthrene	644 $\pm$ 58	710 $\pm$ 57	801 $\pm$ 80	956 $\pm$ 86	810 $\pm$ 72	(800)
Anthracene	121 $\pm$ 11	168 $\pm$ 15	144 $\pm$ 13	163 $\pm$ 14	166 $\pm$ 15	162 $\pm$ 67
Fluoranthene	824 $\pm$ 74	1054 $\pm$ 89	1228 $\pm$ 120	1280 $\pm$ 115	1310 $\pm$ 118	1360 $\pm$ 260
Pyrene	810 $\pm$ 73	990 $\pm$ 79	1280 $\pm$ 114	1219 $\pm$ 109	1217 $\pm$ 108	1260 $\pm$ 430
Chrysene	602 $\pm$ 48	777 $\pm$ 78	810 $\pm$ 81	732 $\pm$ 56	783 $\pm$ 62	760 $\pm$ 180
Benzo[a]anthracene	460 $\pm$ 42	680 $\pm$ 61	702 $\pm$ 62	680 $\pm$ 61	601 $\pm$ 54	560 $\pm$ 210
Perylene	579 $\pm$ 46	812 $\pm$ 65	865 $\pm$ 77	911 $\pm$ 73	927 $\pm$ 74	–
Benzo[b]fluoranthene	505 $\pm$ 45	817 $\pm$ 74	875 $\pm$ 79	708 $\pm$ 64	697 $\pm$ 63	710 $\pm$ 140
Benzo[k]fluoranthene	263 $\pm$ 21	390 $\pm$ 35	407 $\pm$ 41	385 $\pm$ 31	331 $\pm$ 26	(300)
Benzo[a]pyrene	349 $\pm$ 39	380 $\pm$ 34	377 $\pm$ 33	386 $\pm$ 42	415 $\pm$ 42	406 $\pm$ 87
Indeno[1,2,3-c,d]pyrene	270 $\pm$ 32	301 $\pm$ 33	306 $\pm$ 34	341 $\pm$ 41	355 $\pm$ 43	360 $\pm$ 150
Dibenzo[a,h]anthracene	73 $\pm$ 9	114 $\pm$ 12	117 $\pm$ 14	112 $\pm$ 14	103 $\pm$ 12	(90)
Benzo(g,h,i)perylene	267 $\pm$ 32	361 $\pm$ 43	408 $\pm$ 53	415 $\pm$ 49	466 $\pm$ 54	460 $\pm$ 270

was likely due to matrix-induced interferences leading to the overestimation of peak areas. However, a column-washing step prior to elution was omitted to avoid potential PAH losses. The closest agreement between the experimental and certified values for all target PAHs was obtained using Ace and, therefore, it was selected for the extraction of PAHs from sewage sludge.

Application of the developed method for the analysis of PAHs in sewage sludge from WWTPs. The developed method was applied to determine PAH concentrations in sewage sludge collected from nine Bulgarian WWTPs. As not all information provided by the facilities is public, the WWTPs are presented only by numbers. The resulting concentrations are shown in Fig. 3.



Fig. 3. Results from the analysis of PAHs [$\mu\text{g/kg}$] in sewage sludge from Bulgarian WWTPs

The calculated relative standard deviation (RSD, %) for three replicates ($n = 3$) ranged from 8% to 15%. The results showed similar concentrations of PAHs in municipal WWTPs, with higher levels observed in the sludge receiving mixed domestic–industrial influent. In all samples, the total PAH concentrations remained below the maximum permitted limit of 6.5 mg/kg dw [9,10], confirming compliance with regulatory requirements for potential agricultural application.

Conclusions. A reliable method for sample preparation and GC-MS/MS determination of priority PAHs in sewage sludge was developed. Among the tested

SPE sorbents, HLB (500 mg/3 mL) columns demonstrated the highest retention capacity. The combination of DCM/ACN conditioning and Ace elution ensured quantitative recovery for all target analytes. Of the solvents evaluated, Ace proved to be the most effective extractant for PAHs from the sludge matrix. The method was successfully applied to sludge samples from nine Bulgarian WWTPs; results revealed higher PAH concentrations in sludge receiving mixed domestic–industrial influent compared to municipal WWTPs.

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