

Validation of various extraction techniques for the quantitative analysis of polycyclic aromatic hydrocarbons in sewage sludges using gas chromatography-ion trap mass spectrometry

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Abstract

A new GC-ion trap MS method has been developed for the determination of polycyclic aromatic hydrocarbons (PAHs) in sewage sludge samples. The sludge samples were extracted with Soxhlet, Soxtec, and pressurized liquid extraction (PLE) using 1:1 (v/v) dichloromethane (DCM):*n*-hexane solvent mixture. A multi-layer clean-up (silica/Al₂O₃) column were used, followed by gel permeation chromatography (GPC) to eliminate the interfering organic compound as well as the lipids. The extracts were quantified with GC–EI–SIS and GC–EI–MS–MS. The method was successfully applied to determine the concentration of PAHs present in sewage sludge samples collected from four waste water treatment plants (WWTPs). The method recovery values varied from 61.5 to 90.5%, 65.0 to 91.8% and 60.0 to 93.4% for Soxtec extraction, Soxhlet extraction and PLE extraction, respectively. The total concentrations of the 16 PAHs in the sewage sludge samples were found to vary from 1.56 to 6.18 mg/kg. The concentration of PAHs in WWTPs did not significantly vary.

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1. Introduction

Sewage sludge are applied to agricultural land as fertilizer, which represent an economic way to use high amount of sludge produced by the wastewater treatment plants. It is estimated that sewage sludge are produced annually around 1.07×10^8 kg (51%) and used in agriculture [1]. It is well recognized that xenobiotic organic compounds present in sewage sludge may have a negative impact on soil organisms [2–4]. Sludge which are spread on land mostly contains polycyclic aromatic hydrocarbons (PAHs) that are persistent, exhibiting a high accumulate potential and producing a high carcinogenic and mutagenic toxicity [5,6]. PAHs are

hydrophobic in nature and can easily removed from sewage and adsorbed on solid particles during the activated treatment of wastewaters and remain un-graded due to their potential biological recalcitrance [7]. Maximum acceptable concentrations of some PAHs in sludge have been set in some countries. The European Union has imposed that the total concentration of PAHs in sewage sludge for agriculture use to be 6.0 mg/kg [8]. The source of organic pollutants was mainly from: (i) human excretion products and (ii) household disposal [9]. These organic pollutants containing over 300 organic compounds as have been identified [10]. PAHs in sewage sludge have certain characteristics such as: (i) high lipophilicity; (ii) harmful to biota and mutagenic toxicity and (iii) highly persistence in soil [11]. A survey of organic pollutants in the sewage sludge collected from four Kuwait's wastewater treatment plants (WWTPs) was carried out in order to obtain a contemporary data on the concentration of organic pollutants in sewage sludges. There is a requirement for basic information regarding the concentrations of PAHs in sewage sludge to assess the environmental impact of these compounds.

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The objective of the present work was to develop a sensitive method in order to measure the concentration of the 16 US Environmental Protection Agency (EPA) PAHs in sewage sludge collected from four different wastewater treatment plants WWTPs. A secondary objective was to optimize and validate various extraction techniques which are Soxtec, Soxhlet and pressurized liquid extraction (PLE) and to compare their performance. This methodology has been validated by analyzing PAHs in different sewage sludge samples. To our knowledge, similar work related to this work has not been done in Kuwait and the present paper provides a valuable information related to the levels of PAHs contents in the four WWTPs, differing in the origin of the wastewater and the treatment process. This paper will provide information about the PAHs content and to which extent variations in PAHs can be expected.

2. Experimental

2.1. Sample collection and pretreatment

Sewage sludges were sampled from four municipal WWTPs distributed in different cities of Kuwait: (a) Rikka; (b) Al-ardiya; (c) Jahra and (d) Umm Al-Hyman. Sludge samples were collected in the following months: January, February, March and April in the year of 2004. The sewage sludge samples were stored in glass bottles, pre-cleaned with acetone and *n*-hexane. They were kept frozen after collection to avoid any deterioration during storage. Before extraction, sludges were dried at 40 °C, grinded in a hammer mill, and sieved to get the pieces of wood and stones. Samples were stored in a glass bottles with aluminum foil protected covers until analysis.

2.2. Reagents and chemical

All reagents and chemicals were of HPLC-grade. Dichloromethane (DCM) was purchased from BDH (UK, AnalaR grade) and *n*-hexane was from Panreac Quimica (Spain). Anhydrous sodium sulfate was from Scharlau, Barcelona, Spain and was cleaned by baking at 400 °C for 12 h before the use. Aluminium oxide was from EM Science (Germany), activated at 400 °C for 12 h. Silica was from Aldrich-Germany and activated at 400 °C for 12 h prior to use. Copper bars were cut into a small piece and activated with 30% hydrochloric acid for 30 s. The bars were then cleaned with acetone and *n*-hexane and were added to the sewage sludges before extraction in order to remove the sulfur.

Bio-Beads (Bio-Rad Labs., SX-3, 200–400 mesh) equilibrated overnight with 50 ml of DCM: *n*-hexane (1:1). The standard mixture of the 16 priority EPA PAHs (naphthalene—NAP, acenaphthylene—ACY, acenaphthene—ACE, fluorine—FLU, phenanthrene—PHE, anthracene—ANT, fluoranthene—FLT, pyrene—PYR, benzo[a]anthracene—BaA, chrysene—CHR, benzo[b]fluoranthene—BbF, benzo-

[k]fluoranthene—BkF, benzo[a]pyrene—BaP, indeno[1,2,3-cd]pyrene—IND, dibenzo[a,h]anthracene—DBA, benzo[g,h,i]perylene—BPY) at a concentration of 1 mg/ml in acetonitrile was obtained from Hewlett-Packard (HP; #No. 8500-6035), and deuterated internal standards (1000 µg/ml): [²H₁₀] acenaphthene (acenaphthene-d¹⁰), [²H₁₀] phenanthrene (phenanthrene-d¹⁰), [²H₁₂] chrysene (chrysene-d¹²) and [²H₁₂] perylene (perylene-d¹²) was supplied by Ultra Scientific (North Kingstown, USA; No. 60 76). Calibration graphs were Constructed and prepared by diluting the stock solutions with *n*-hexane. The final concentration of the deuterated internal standard in the calibration solutions was 1.0 µg/ml.

2.3. Soxhlet extractions

Dried sludge samples (2.5 g) were extracted with a mixture of 150 ml DCM–*n*-hexane (1:1) for 18 h. Activated copper bar (0.5 cm) were added to the sample before extraction. The extracts were concentrated in a rotary evaporator at 40 °C, the solvent was exchanged into *n*-hexane and the final volume were adjusted to 1 ml.

2.4. Soxtec extractions

Dried sludge samples (2.5 g) were extracted with 50 ml mixture of DCM: *n*-hexane (1:1). The total extraction process was fixed at 3 h (2 h for extraction and 1 h for rinsing). The extracts finally were concentrated to 1 ml.

2.5. Accelerated solvent extraction

2.5 g of dried sludge sample was mixed with 3 g of hydromatrix and extracted with 60 ml DCM:*n*-hexane (1:1) solvent for 20 min. PLE conditions were as follows: pressure = 100 atm; temperature = 100 °C; heating time = 5 min; static time = 3 min.; flush volume = 60% of extraction cell volume; purge = N₂, 50 s; number of cycle = 3, total volume of extract = 60 ml.

2.6. Spiking procedure

Immediately prior to extraction, sewage sludge sample was spiked with the mixture and deuterated standard. The sample were extracted and subjected to a multi-clean up process. Spiked and non-spiked samples were compared systematically.

2.7. Instrumentation and apparatus

An ASE 200 instrument (Dionex, Sunnyvale, CA, USA), was used to carry out the extraction. Stainless steel extraction cells and glass collecting vials were used. Sludge samples were mixed with 3 g of hydromatrix. Soxtec (2055 Soxtec Avanti, Hoganas, Sweden) was used.

All analyses was carried out using CP-3800 gas chromatograph coupled with a Saturn 2200 ion-trap spectrometer (Varian, Walnut Creek, CA, USA). Samples were separated in a DB-5MS (30 m, 0.32 mm I.D., 0.1 μm film thickness) capillary column (Varian ChromPakck). Varian 8200CX auto-sampler was used for all samples injected. Helium was used as a carrier gas at 1 ml/min constant flow rate.

Oven temperature was programmed as follow: 70 °C held for 3.5 min, then increased at 25 °C/min to 180 °C, held for 10 min, and finally increased at a rate of 10 °C/min to 300 °C.

The injection volume was 1 μl and injection was in split-splitless mode. Operational ion-trap conditions are listed in Table 1.

Automatic gain control (AGC) was used to optimize the sensitivity by filling the trap with the target ions. The value was optimized to 5000 for the electron impact ionization (EI) mode. The operational conditions for GC–EI–SIS are the same as mentioned in Table 1.

Collision induced dissociation (CID) waveform was selected for all compounds as summarized in Table 2. MS instrument parameters were optimized and the values were summarized in Table 1.

Perfluorotri-*n*-butylamine was used as a reference gas. MS–MS ion preparations and conditions are shown in Table 2.

2.8. Clean-up of the extracts

The extracts in all cases, were concentrated to 2–3 ml using a rotary evaporator and then to 1 ml under a stream of nitrogen. The extracts were passed through a mixed silica/ Al_2O_3 column to remove co-extractive substances. The column was packed at the bottom with cotton wool and then filled with 10 g of activated silica and 5 g of activated Al_2O_3 . 1 cm of sodium sulfate was added at the top of the column, the column was rinsed with 30 ml of DCM and 30 ml of *n*-hexane.

Table 1
GC–EI–MS–MS ion trap operational temperature for the analytical method of PAHs

I. Ion preparation parameters	
CID waveform	Resonant and non resonant
Mass isolation Window	1
Isolation time	5 ms
Excitation time	5 ms
Ejection amplitude	20 V
Broadband amplitude	30 V
Modulation rate	30 $\mu\text{s}/\text{step}$
II. Ionization parameters	
Ion trap temperature	250 °C
Transfer temperature	280 °C
Manifold temperature	35 °C
Axial modulating voltage	4.0 V
Emission current	80 μA
Electron multiplier voltage	1550 V
Scan rate	0.25 s/scan
Pre-scan ionization time	1500 μs
Target TIC	20,000
Maximum ionization time	25,000
RF pump value	650 m/z
Background mass	45 m/z
Count threshold	1 counts
Isolation window	3.0 m/z

The analytes of interest were eluted with 120 ml DCM:*n*-hexane (1:1), the collected extract was concentrated to 1 ml by rotary evaporator for manual gel permeation chromatography (GPC).

Bio-Beads (Bio-Rad Labs., SX-3, 200–400 mesh) were equilibrated overnight with DCM:*n*-hexane (1:1). The column was packed with approximately 6 g of Bio-Beads, the sample was eluted with 75 ml of DCM:*n*-hexane (1:1). The first 18 ml of the eluent was discarded and the next eluant containing analytes of interest were collected. The column was washed with 35 ml of the same solvent and prepared for the next sample.

Table 2
Ion selection and conditions for the GC–EI–MS and GC–EI–MS–MS analysis of PAHs

PAHs compounds	Retention time (min), RSD (%)	Parent ion (m/z) ^a	Wave-form type	ESL (m/z)	CID amplitude (V)	Ion (m/z) ^b
NAP	5.29 \pm 0.69	128	R	56	0.5	128
ACY	7.34 \pm 1.6	153	NR	67	82	152
ACE	4.41 \pm 0.16	154	R	68	0.7	152, 154
FLU	7.94 \pm 0.15	165	NR	73	80	165, 166
PHE	9.17 \pm 0.67	178	NR	79	96	178
ANT	9.23 \pm 0.17	178	NR	79	96	178
FLT	12.19 \pm 0.24	201	R	89	1.5	202
PYR	13.09 \pm 0.28	201	R	89	1.5	202
BaA	21.17 \pm 0.19	227	R	101	1.4	228
CHR	21.27 \pm 0.16	227	R	101	1.4	228
BbF	24.78 \pm 0.14	250	R	111	2.5	252
BkF	24.85 \pm 0.12	250	R	111	2.5	252
BaP	25.53 \pm 0.12	250	R	111	2.5	252
BPY	27.88 \pm 0.09	277	NR	122	60	276
DBA	28.11 \pm 0.65	277	NR	122	60	278
IND	28.34 \pm 0.22	277	NR	122	60	276

^a For GC–EI–MS–MS.

^b For GC–EI–MS.

The collected eluant was concentrated by rotary evaporator to 1 ml. The extracts were finally passed through a final column filled with 5 g silica + 3 g Al₂O₃ to remove any co-extractive compounds that may cause instrumental interferences during the analysis. The extract was eluted with 120 ml of DCM:*n*-hexane (1:1), the first 18 ml of eluent was discarded and the rest were collected, which contains the analytes of interest. The extract was exchanged into *n*-hexane, concentrated to 1 ml to which 1 µg/ml of internal standard was added.

2.9. Removal of sulfur and lipids

The sulfur present in sewage sludges has to be removed before the GC–EI–MS and GC–EI–MS–MS determination to avoid any interference.

Copper bars (0.5 cm) were activated with 30% HCl for 30 s and then cleaned with acetone, *n*-hexane and the dried bars was added to the extract. Bio-Beads were used to remove the co-extracted lipids and other higher molecular weight substances which may interfere with the determination. Furthermore, adsorption chromatography of large and small mixed silica and alumina columns allow the removal of lipids and other organic compounds from the sewage sludge extract.

3. Results and discussion

Concern has been expressed over the transfer of toxic organics from the soil and into human food chain. Sewage sludge is a sink for contaminants present in municipal wastewater. There are four WWTPs in operation in Kuwait. All of these treatment plants receive domestic sewage in major quantities, such as 27,000 m²/day for Reqqa, 70,000 m²/day for Jahra and 27,000 m²/day for Om Al-Haymann). Sewage sludge produces at Ardiya WWTP is disposed of, as liquid sludge without digestion to specific disposal sites. Riqqa and Om Al-Haymann are equipped with aerobic digesters and in Jahra thickened undigested sludge is only dried on sludge drying beds. So, the suitability for land application requires investigation since the likelihood for transfer of pollutants from wastewater to edible parts of the plants exists. Volatilization of organic matters, such as PAHs from sludge, due to the unique and extreme temperature in summer (~55 °C) may have adverse effect on the environment. At these temperatures, PAHs are likely to volatilize along with other semi-volatile organic compounds and as potential source of chemicals to the environment. It is questionable, however, whether these chemicals are likely to deposit at such temperatures, but may remain in the vapor phase and subjected to long range atmospheric transport. The eventual fate of chemicals in sludge requires a detailed assessment and probably other disposal options needed to be evaluated particularly for Kuwait and other countries in the region that experience such uniquely high temperatures.

Table 3

Recoveries and relative standard deviations (RSD, %) obtained from spiked sewage sludge samples (0.2 µg/ml) analysed by GC–EI–MS–MS, using three different extraction techniques

PAHs compounds	% Recovery ± RSD		
	Soxtec	Soxhlet	PLE
NAP	68.3 ± 19.7	65.6 ± 1.4	60.0 ± 8.2
ACY	61.5 ± 5.6	79.6 ± 0.77	66.4 ± 5.2
ACE	65.3 ± 14.4	73.9 ± 2.2	74.8 ± 7.9
FLU	71.9 ± 22.7	82.5 ± 0.59	75.3 ± 17.5
PHE	84.7 ± 16.3	83.9 ± 1.8	88.2 ± 2.9
ANT	88.2 ± 11.1	91.8 ± 1.1	87.9 ± 7.6
FLT	63.2 ± 11.7	86.3 ± 2.7	86.4 ± 9.2
PYR	64.7 ± 17.6	90.2 ± 3.7	86.0 ± 12.3
BaA	90.5 ± 5.1	76.8 ± 5.5	88.6 ± 10.8
CHR	87.7 ± 8.9	71.7 ± 5.6	91.9 ± 8.4
BbF	88.0 ± 2.7	90.4 ± 2.9	93.4 ± 7.1
BkF	86.4 ± 2.8	89.7 ± 1.5	91.4 ± 8.8
BaP	86.3 ± 2.3	90.8 ± 1.5	84.8 ± 9.6
BPY	87.3 ± 4.9	91.2 ± 3.1	91.0 ± 4.5
DBA	84.6 ± 1.7	89.7 ± 7.0	82.4 ± 9.3
IND	84.6 ± 4.7	90.3 ± 2.2	85 ± 7.9

3.1. Soxhlet extraction

Many agencies proposed that Soxhlet extraction is a method of choice for the extraction of nonpolar organic contaminants [12]. Extraction recoveries and relative standard deviations are summarized in Table 3.

Recoveries are calculated from the increase in peak areas between the non-spiked and spiked chromatograms using the deuterated internal standard method. The extraction recoveries were satisfactory, since they ranged from 65.0 to 91.8%. The relative standard deviations (RSDs, %) varied from 0.59 to 7.0% for the PAHs, the high value in RSD, could be explained due to the multi-step process such as spiking, extraction and evaporation.

3.2. Soxtec extraction

Recoveries varied from 61.5 to 90.5% with a relative standard deviations varied from 1.7 to 22.7%. The results obtained are presented in Table 3. The Soxtec method appears to be more simple and efficient when compared to Soxhlet extraction.

3.3. Pressurized liquid extraction

PLE recoveries of sludge samples varied from 60.0 to 93.4% with a RSDs varying from 2.9 to 17.5%.

Several papers have been successfully reported for the application of accelerated solvents extraction, such as for PAHs, OCPs, in environmental samples [13–17]. However, few papers have been published using ASE for determining PAHs in sewage sludge [13,15,17].

The use of higher extraction temperature and the role of high pressure for PLE reported to give comparable or higher extraction efficiencies compared with other extrac-

tion techniques [17]. Higher temperature $>140^{\circ}\text{C}$ was not recommended, which leads to the co-extraction of other organic substances or formation of degradation products of the parent compounds [14].

However, the use of high pressure keeps the solvent in the liquid phase and at high temperature facilitates a better penetration of the solvent into the sludge matrix. Therefore, PLE provide higher extraction efficiencies compared with other techniques.

3.4. Calibration and linearity of the method

Calibration curves were constructed with the internal standard multipoint calibration for the 16 PAHs. Calibration curves were constructed using the optimized conditions described for MS–MS. Quantification of the analyzed compounds were performed in the linear range of the calibration curve. The range of the concentration was appropriate to the levels found in environmental samples.

The separation of the 16 EPA PAHs was carried out in 40 min. The linearity of the curves were in the range 0.1–1.0 $\mu\text{g/ml}$ for GC–EI–SIS and in the range 0.05–0.5 $\mu\text{g/ml}$ for GC–EI–MS–MS. The correlation coefficient were ≥ 0.999 for most of the PAHs analyzed. The analytical data, linearity, correlation coefficient and regression equations of the calibration curves for both GC–EI–SIS and GC–EI–MS–MS are listed in Table 4.

3.5. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection is the minimum amount of target analyte that produce a chromatographic peak with a signal-to-noise ratio of three.

The LOD was obtained from the mean SD multiplied by a factor of 3 and the limit of quantification by the mean SD multiplied by a factor of 10. The results obtained are reported in Table 4.

LOD and LOQ values were ranged from 0.13 to 4.29 $\mu\text{g/ml}$ and 0.43 to 14.29 $\mu\text{g/ml}$, respectively for PAHs analysed by GC–EI–SIS and from 0.06 to 1.43 $\mu\text{g/ml}$ and 0.23 to 4.76 $\mu\text{g/ml}$, respectively for PAHs analysed by GC–EI–MS–MS.

3.6. Comparison of the extraction techniques

The extraction efficiency shows that higher values was obtained for PLE and Soxtec, this could be explained due to the temperature (100°C for PLE and 155°C for Soxtec) which enable a better extraction efficiency.

Soxhlet extraction efficiencies (high recoveries) could be due to the extended time used. PLE is a faster technique (30 min) compared with Soxhlet and Soxtec. Concentrated samples and multi step clean-up process were common for all of the extract techniques. Extraction time for PLE and Soxtec were short when compared to Soxhlet extraction. Less amount of organic solvents were consumed in PLE and Soxtec extraction. PLE appears to be a promising technique, consuming a small volume of solvent and moderate extraction time. Lower solvent consumption implies cost reduction, waste disposal and less exposure to solvent vapor. A general comparison parameter for the different extraction techniques are presented in Table 5.

3.7. Concentrations of PAHs in the real sewage sludge samples

The concentration of PAHs in the four WWTPs samples are given in Table 6. The results obtained from the four

Table 4
Linear range, correlation coefficient, regression equations, limits of detection and limit of quantification for the PAHs

PAHs	GC–EI–SIS					GC–EI–MS–MS				
	Linear range ($\mu\text{g/ml}$)	Corr. coeff. (r^2)	Reg. eq.	LOD ($\mu\text{g/l}$) S/N=3	LOQ ($\mu\text{g/l}$)	Linear range ($\mu\text{g/ml}$)	Corr. coeff. (r^2)	Reg. eq.	LOD ($\mu\text{g/l}$) S/N=3	LOQ ($\mu\text{g/l}$)
NAP	0.1–1.0	0.998	$Y=0.457x+0.0013$	0.13	0.43	0.05–0.5	0.997	$Y=4.057x+0.0703$	1.43	4.76
ACY	0.1–1.0	0.998	$Y=0.418x+0.0037$	0.21	0.71	0.05–0.5	0.996	$Y=0.84x-1.11 \times 10^{-4}$	0.61	2.03
ACE	0.1–1.0	0.994	$Y=0.2928x+0.0024$	0.25	0.83	0.05–0.5	0.999	$Y=0.8361x+0.0$	0.40	1.33
FLU	0.1–1.0	0.995	$Y=0.297x+0.0036$	0.23	0.78	0.05–0.5	0.999	$Y=3.4x-4.44 \times 10^{-16}$	0.38	1.25
PHE	0.1–1.0	0.998	$Y=0.423x-0.0019$	0.14	0.46	0.05–0.5	0.999	$Y=0.478x-0.0127$	0.28	0.93
ANT	0.1–1.0	0.999	$Y=0.38x+5.13 \times 10^{-4}$	0.19	0.65	0.05–0.5	0.997	$Y=0.4785x-0.0127$	0.31	1.04
FLT	0.1–1.0	0.999	$Y=0.4538x-0.0035$	0.20	0.67	0.05–0.5	0.999	$Y=1.0486x-0.0082$	0.17	0.57
PYR	0.1–1.0	0.999	$Y=0.416x-0.0051$	0.22	0.73	0.05–0.5	0.999	$Y=1.1x-1.11 \times 10^{-16}$	0.21	0.70
BaA	0.1–1.0	1.000	$Y=0.0114x+0.0354$	0.75	2.50	0.05–0.5	0.994	$Y=1.4278x-0.0824$	0.068	0.23
CHR	0.1–1.0	0.999	$Y=0.392x+0.00117$	1.0	3.33	0.05–0.5	0.994	$Y=0.3081x-0.4154$	0.1	0.34
BbF	0.1–1.0	0.998	$Y=0.0625x+0.002$	0.91	3.03	0.05–0.5	0.994	$Y=0.9520x-0.0695$	0.38	1.27
BkF	0.1–1.0	0.992	$Y=0.4489x+4.300$	1.43	4.76	0.05–0.5	0.999	$Y=1.7x-1.11 \times 10^{-16}$	0.52	1.72
BaP	0.1–1.0	0.998	$Y=0.492x-0.0021$	3.0	10.0	0.05–0.5	1.000	$Y=0.58x-5.5 \times 10^{-17}$	0.66	3.57
BPY	0.1–1.0	0.999	$Y=0.259x+0.00305$	4.29	14.29	0.05–0.5	0.999	$Y=0.49x-5.5 \times 10^{-17}$	0.49	4.0
DBA	0.1–1.0	0.999	$Y=0.004x+1.3 \times 10^{-18}$	3.33	11.11	0.05–0.5	0.997	$Y=0.08x-6.9 \times 10^{-18}$	1.07	3.57
IND	0.1–1.0	0.999	$Y=0.02x-2.3 \times 10^{-4}$	2.31	7.69	0.05–0.5	0.999	$Y=0.35x-2.7 \times 10^{-17}$	0.42	2.56

Table 5
Comparison of general parameters for all the extraction techniques used

Parameters	Soxhlet	Soxtec	PLE
Extraction time (h)	18	3	0.3
Solvent consumption (ml)	60	60	60
Re-concentration step	Yes	Yes	Yes
Clean-up	Yes	Yes	Yes
Cost	Low	Medium	Medium
Method development time	Low	Medium	High

WWTPs (Table 6) showed that the total sum concentrations of PAH (\sum PAHs) in the case of Umm (February 2004) using different extraction techniques (PLE, Soxhlet and Soxtec) varied in the range between 2.69 and 6.19 mg/kg. Ardiya (February 2004) sewage sludge sample with Soxhlet extraction, shows that the concentrations (\sum PAHs) is 5.860 mg/kg. Rikka WWTPs samples were collected and extracted with the three extraction process, the total concentrations of PAHs (\sum PAHs) varied in the range 1.56–3.48 mg/kg for March, 2004. However, Jahra sludge samples were collected in February and March and extracted with the three extraction process. The results shows that the \sum PAHs were varied between 3.28 and 3.77 mg/kg. The sum concentration of \sum PAHs collected in different months and extracted with different extraction techniques were not significantly varied for the four WWTPs.

However, Ardiya-WWTPs exhibit higher PAHs concentration.

Volatile PAHs compounds such as NAP, ACY, ACE exhibit higher relative standard deviations due to losses in evaporation process and in the case of Soxtec extraction, these compound were not detected due to higher temperature applied.

Phenanthrene, anthracene and pyrene in some cases are the most abundant compounds, as in the case of the Soxhlet extraction.

Fig. 1 illustrates the GC–EI–MS–MS of the target PAHs compounds extracted from sewage sludge sample (Umm Al-Hyman, February 2004) using PLE. Fig. 2 shows the GC–EI–MS–MS chromatogram of the sludge sample (Umm, February, 2004) extracted with Soxhlet extraction and spiked with 1.0 μ g/ml.

The GC–EI–MS–MS chromatogram of Rikka sludge sample (February, 2004), spiked with 1.0 μ g/ml PAH, using Soxtec extraction is presented in Fig. 3.

In comparison of the PAH data found in the literature, the PAHs values commonly varied between 0.1 and 100 mg/kg. A German study shows that the sum concentration of PAHs determined in sewage sludges varied between 2 and 100 mg/kg [18]. In another study they varied between 2 and 80 mg/kg [19]. In Spain sewage sludge sample extracted in 1995, shows that the total \sum PAHs values were found to vary in the range from 1.7 to 15 mg/kg [20] and in 2001, the total sum of \sum PAHs values were found in the range 1.13–5.52 mg/kg [21]. In the present study, the values varied in the range between 1.56 and 6.18 mg/kg for the

Table 6
Concentration of PAHs determined in sewage sludges from four WWTPs, using different extraction process

PAHs	Soxtec extraction				PLE extraction				Soxhlet extraction				
	Jahra (03/04)	Umm (02/04)	Rikka (01/04)	Rikka (03/04)	Rikka (04/04)	Jahra (02/04)	Jahra (02/04)	Rikka (02/04)	Umm (02/04)	Jahra (02/04)	Rikka (02/04)	Umm (02/04)	Ardiya (02/04)
NAP	n.d.	n.d.	n.d.	n.d.	n.d.	0.08 \pm 6.7	0.08 \pm 6.7	0.06 \pm 4.0	0.14 \pm 5.4	0.12 \pm 4.6	0.15 \pm 16.5	0.13 \pm 11.5	0.73 \pm 6.2
ACY	n.d.	n.d.	n.d.	n.d.	n.d.	1.8 \pm 0.66	1.8 \pm 0.66	n.d.	0.37 \pm 3.1	n.d.	0.05 \pm 5.9	0.05 \pm 6.1	n.d.
ACE	n.d.	n.d.	n.d.	n.d.	n.d.	0.36 \pm 1.4	0.36 \pm 1.4	n.d.	0.37 \pm 1.4	n.d.	0.05 \pm 9.4	0.06 \pm 13.1	n.d.
FLU	0.05 \pm 8.1	0.14 \pm 14.6	0.08 \pm 17.6	0.03 \pm 3.2	0.06 \pm 10.2	0.62 \pm 3.5	0.62 \pm 3.5	0.02 \pm 0.85	2.1 \pm 41.5	0.2 \pm 7.9	0.08 \pm 12.5	0.26 \pm 1.1	0.08 \pm 1.2
PHE	0.39 \pm 2.9	0.47 \pm 7.5	0.24 \pm 6.5	0.15 \pm 1.5	0.24 \pm 6.5	1.55 \pm 2.5	1.55 \pm 2.5	n.d.	0.27 \pm 2.7	0.31 \pm 3.2	n.d.	0.13 \pm 4.4	2.1 \pm 1.66
ANT	0.43 \pm 2.3	0.5 \pm 6.2	0.28 \pm 4.5	0.17 \pm 4.4	0.26 \pm 12.5	n.d.	n.d.	n.d.	0.28 \pm 2.8	0.15 \pm 16.5	n.d.	0.15 \pm 6.7	2.2 \pm 0.63
FLT	0.58 \pm 1.7	0.27 \pm 3.7	0.09 \pm 6.2	0.09 \pm 8.6	0.42 \pm 1.4	0.19 \pm 3.1	0.19 \pm 3.1	0.09 \pm 6.7	1.47 \pm 2.1	0.48 \pm 1.2	0.08 \pm 13.5	0.18 \pm 1.5	0.63 \pm 3.4
PYR	0.19 \pm 10.5	0.76 \pm 0.67	0.28 \pm 1.2	0.75 \pm 2.0	0.43 \pm 3.6	n.d.	n.d.	0.08 \pm 1.5	0.37 \pm 2.7	0.37 \pm 2.7	n.d.	0.13 \pm 5.8	0.7 \pm 5.0
BaA	0.14 \pm 4.3	0.39 \pm 1.28	0.68 \pm 1.5	0.65 \pm 2.6	n.d.	0.22 \pm 2.6	0.22 \pm 2.6	0.22 \pm 3.2	0.37 \pm 1.6	0.07 \pm 12.5	0.13 \pm 6.1	n.d.	0.02 \pm 3.3
CHR	0.28 \pm 5.4	n.d.	n.d.	0.15 \pm 10.4	n.d.	0.35 \pm 2.9	0.35 \pm 2.9	0.22 \pm 4.5	0.38 \pm 1.2	0.55 \pm 1.8	0.14 \pm 7.5	n.d.	n.d.
BbF	0.29 \pm 2.5	n.d.	n.d.	0.17 \pm 5.9	n.d.	n.d.	n.d.	0.14 \pm 3.7	0.26 \pm 3.4	0.38 \pm 0.76	0.18 \pm 1.4	0.36 \pm 4.8	n.d.
BkF	0.17 \pm 7.9	n.d.	n.d.	0.17 \pm 9.2	0.15 \pm 9.9	n.d.	n.d.	0.25 \pm 2.5	0.11 \pm 9.1	0.19 \pm 2.7	0.09 \pm 14.1	0.25 \pm 10.6	n.d.
BaP	n.d.	n.d.	n.d.	n.d.	n.d.	0.22 \pm 8.5	0.22 \pm 8.5	0.23 \pm 4.3	n.d.	0.23 \pm 2.4	0.31 \pm 4.0	0.29 \pm 3.4	n.d.
BGP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.47 \pm 2.7	n.d.	n.d.
DBA	n.d.	n.d.	n.d.	0.57 \pm 3.7	n.d.	n.d.	n.d.	0.7 \pm 3.8	0.66 \pm 4.9	0.49 \pm 5.2	0.74 \pm 5.4	0.7 \pm 4.4	n.d.
IND	n.d.	n.d.	n.d.	0.3 \pm 3.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
\sum PAHs	3.284	2.760	2.660	3.481	1.560	3.770	2.010	6.180	3.690	2.470	5.860	2.690	5.860

Concentrations are given in (mg/kg). Relative standard deviations (RSD, %) are given for triplicate determination.

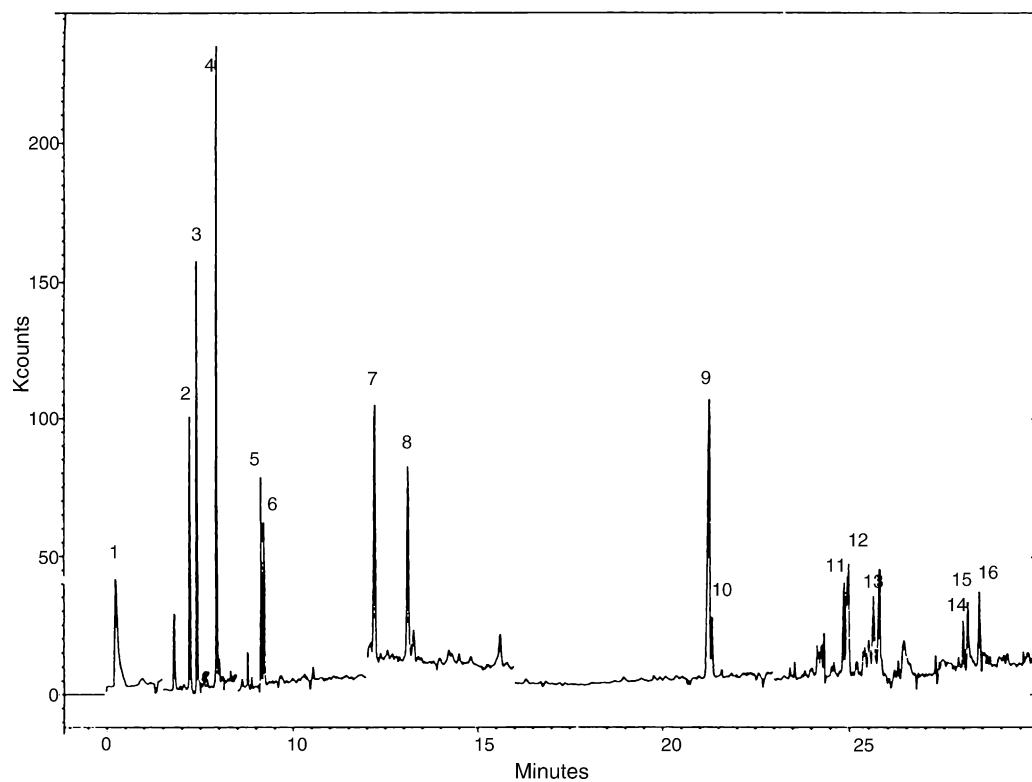


Fig. 1. GC–EI–MS–MS chromatogram of Umm Al-Hyman WWTPs sampled in February 2004, using accelerated solvent extraction. GC–EI–MS–MS conditions were the same as in Tables 1 and 2. Peak identifications: 1 = NPT, 2 = ACY, 3 = ACE, 4 = FLU, 5 = PHE, 6 = ANT, 7 = FLT, 8 = PYR, 9 = BaA, 10 = CHR, 11 = BbF, 12 = BkF, 13 = BaP, 14 = BPY, 15 = DBA, 16 = IND.

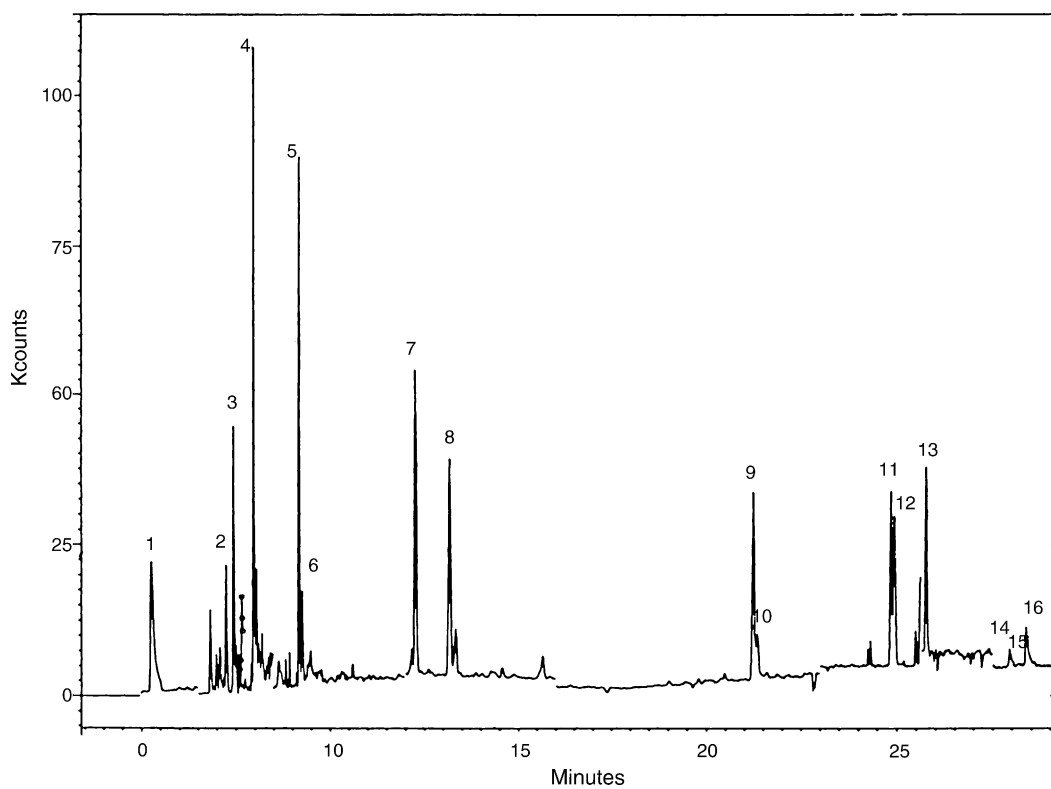


Fig. 2. GC–EI–MS–MS chromatogram of Umm Al-Hyman WWTPs samples in February 2004, spiked with 1.0 µg/ml PAHs standard, using Soxhlet extraction. Peak identification and MS–MS condition were the same as in Fig. 1.

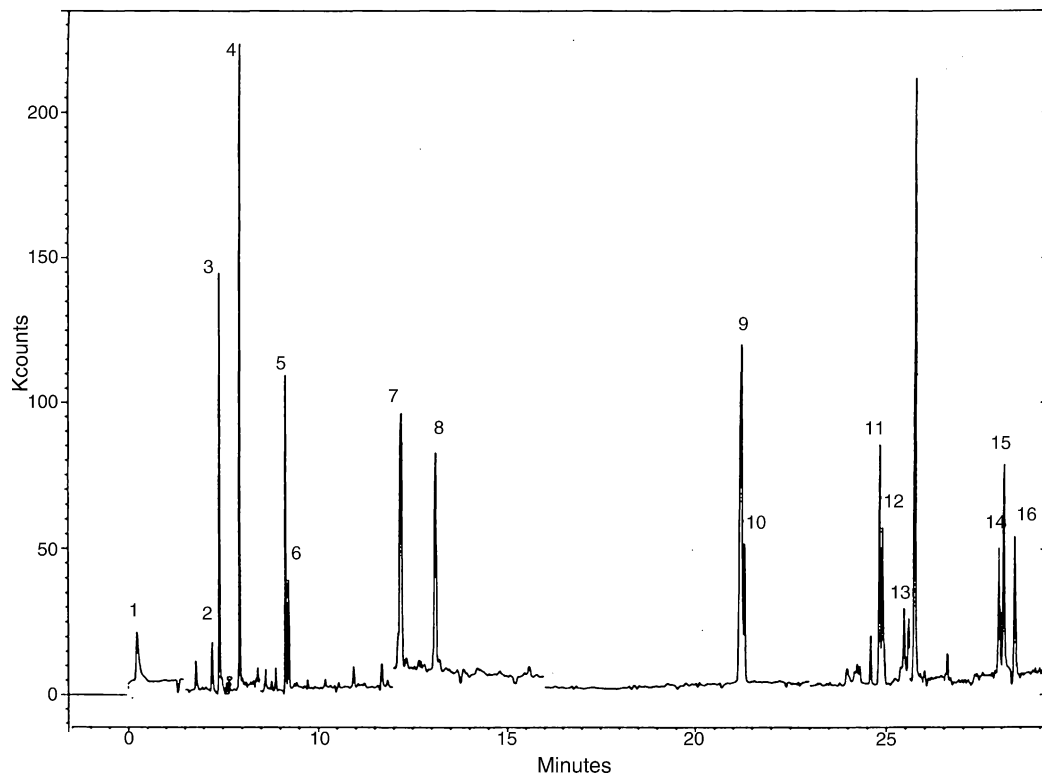


Fig. 3. GC–EI–MS–MS chromatogram of Rikka-WWTPs samples in February 2004, spiked with 1.0 µg/ml standard PAHs, using Soxtec extraction. Conditions were the same as in Fig. 1.

four WWTPs. However, the European community permits (6 mg/kg) for the sludge for farm land. The concentrations obtained in this study fall within the range of the European community and consistence with the previously reported values [20,21].

4. Conclusions

PLE, Soxhlet and Soxtec have proven to be efficient extraction techniques for PAH. PAH extracts were measured by GC–EI–SIS and GC–EI–MS–MS after multi-step clean-up process. The analytical methodology for PAHs determination was validated by analyzing sludges material and accurately analyzing PAHs in sewage sludges. The total concentrations of PAHs was in the low mg/kg and the amounts in this study fall within the range of the reported data. Some significantly differences between the sludge samples were observed, which contributed to the origin of the sewage sludge and type of the treatment method.

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