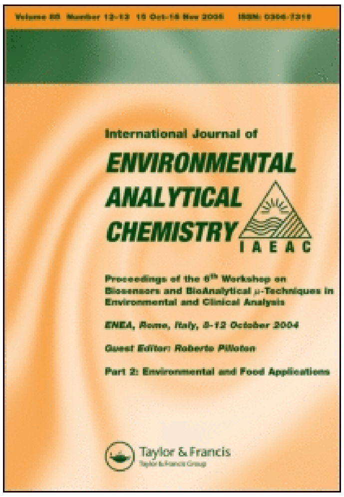

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DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN MANGROVE SEDIMENTS: COMPARISON OF TWO INTERNAL STANDARD SURROGATE METHODS AND QUALITY-CONTROL PROCEDURES

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Two internal standard surrogate (ISS) methods, ISS-1 (with *m*-terphenyl as a single ISS) and ISS-2 (using five deuterated PAHs as a multi-ISS), for the determination of polycyclic aromatic hydrocarbons (PAHs) in mangrove sediments were compared. The recovery percentages of 16 PAHs except naphthalene in HS-6 (a certified reference material) were high, ranging from 69.3 to 111.8% for ISS-1 and from 71.8 to 120.3% for the ISS-2 method. Similarly, the PAH recovery percentages in spiked mangrove sediments and clean sand were lower for ISS-1 than for ISS-2, but both methods met the accurate acceptance criteria for PAH recoveries. The reproducibility (i.e. the method precision) between the two ISS methods was also comparable and satisfactory, with relative standard deviation values in most cases within 20% of the data variability. These results indicate that both ISS methods were acceptable for the determination of PAHs in mangrove sediments, despite the fact that the matrix of mangrove sediments may interfere with the PAH recovery efficiency.

Keywords: Deuterated PAHs; *m*-Terphenyl; Matrix effects; Recovery efficiency

INTRODUCTION

Marine sediments are known as sinks for many contaminants including PAHs [1]. Elevated concentrations of polycyclic aromatic hydrocarbons (PAHs) have been recorded in coastal sediments near highly industrialized areas [2,3]. The methodology for the determination of PAHs in sediments has improved rapidly in the past 20 years, but the absence of any standardized protocol makes data comparisons difficult [4]. Risks of producing errors (including systemic and manual errors) have been reported in many steps of analysis. For instance, samples subjected to freeze-drying

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prior to extraction have been shown to undergo a partial loss of naphthalene [4], but the use of wet sediments for extraction has also been found to be unsatisfactory because of the low PAH recovery [5]. The non-polar solvents used for extraction have been found to cause insufficient moistening of hydrophilic regions in the organic matter, thus decreasing the extraction efficiency [6]. The low-molecular-weight PAHs were lost during the concentration steps [7], while the high-molecular-weight PAHs could be lost due to their incomplete vaporization during gas chromatography (GC) determination [5]. Therefore, strict quality control in PAH extraction and determination procedures is essential.

The quality-control procedures generally include the analysis of procedural blanks, certified reference materials (CRMs), and spiked samples [8]. Procedural blanks are used to estimate any contamination produced by other sources such as solvent and glassware used during the analytical steps. CRMs are used to evaluate the accuracy of the methodology, and spiked samples are often used to assess the efficiency of recovery (i.e. the procedure efficiency). Spiked samples can also indicate analytical accuracy when the analytical response for a determinant in an unspiked sample is due to the determinant alone (i.e. no matrix interference) and is not subject to any bias [9]. In addition, the precision of a method, often estimated by replicate analyses of a homogenous sample, is also important [10].

Satisfactory quality control depends on a reliable method for quantification which is often carried out by adding a known amount of internal standard surrogates (ISSs) to compensate for the losses involved in the analytical procedures including the extraction and clean-up steps [11]. A chemical is qualified as ISS if it behaves identically to the compounds of interest and is not found in nature; thus, its recovery is considered representative of that of target determinants, and the losses of the contaminants during analytical procedures can be compensated for [9]. Deuterated PAH compounds [10,12], *m*-terphenyl [13–15], 1,1′-binaphthyl [16], and benzo[b]chrysene have been used as PAH ISSs [17]. The use of deuterated PAHs as ISSs is the most accurate quantification method because they have the same structures as their target compounds, except the atom ‘H’ is replaced by its isotope ‘D’, which guarantees behavior identical to that of the compound of interest. Theoretically, each parent PAH compound can be presented most appropriately by its deuterated substitute. However, it is very costly to use too many deuterated PAHs as ISSs. The deuterated PAH ISSs would also introduce a risk of interference in separation and identification of their parent PAHs. Therefore, it is usual practice to select three to six deuterated PAH ISSs covering the low-, medium-, and high molecular weights to represent the 16 priority PAHs [10,11].

In addition to using several deuterated PAHs ISS, a single ISS such as *m*-terphenyl, a synthetic chemical with a similar structure to that of the four-ring PAHs, has been used for PAH quantification [13–15]. A single ISS is commonly used in complicated sediment samples because of its convenience and simplicity of manipulation. Nevertheless, how well a single ISS such as *m*-terphenyl could represent the 16 PAH compounds with wide-ranging molecular weights and structural differences still remains unclear. Structural differences may cause different physico-chemical behaviors throughout the analytical steps, thus affecting the method accuracy. The interference between a single ISS and the complex matrix in any sediment is also uncertain.

Another very important factor affecting the PAH extraction efficiency in sediments is the complexity of the sample matrix, such as organic substances [6] and levels of contamination [18]. It is particularly difficult to extract PAHs efficiently from a matrix containing a high amount of organic matter because of its low extraction efficiency and the co-extraction of non-target substances [6]. The sediments in mangrove swamps have unique properties, being reduced, anaerobic and rich in salt, organic matter, iron and sulfide content, and have significant spatial variations. Even in a small city like Hong Kong SAR, with an area of around 1000 km^2, mangrove sediments vary from soft deep muddy substrates to very sandy, compact, hard and shallow sediments [19]. Mangrove sediments in Hong Kong also have different organic matter contents and degrees of PAH contamination [13]. However, no study has been conducted to evaluate the matrix effects of mangrove sediments on the PAH extraction efficiency. The present study therefore aims: (1) to compare two ISS methods, *m*-terphenyl (a single ISS) with several deuterated PAHs ISS (a multi-ISS), for PAH quantification; (2) to estimate the matrix effects of mangrove sediments on the PAH extraction efficiency; and (3) to develop an applicable method for the determination of PAHs in mangrove sediments.

EXPERIMENTAL

Preparation of Spiked Samples

Surface sediments (2–3 cm) were collected from two mangrove swamps, namely Sai Keng and Ma Wan. Sai Keng sediment was sandy with a low organic matter content (1.23–4.83%) and PAH contamination (total concentration of the 16 EPA priority PAHs ranging from 356 to 1811 ng/g dry wt) [13], while Ma Wan sediment was muddy and had a relatively high organic matter content (3.42–9.02%) and greater PAH contamination, with the total concentration of the 16 PAHs varying from 791 to 3358 ng/g dry wt (from our preliminary investigation). The sediments collected from Sai Keng and Ma Wan mangrove swamps had 1.59 ± 0.33% and 8.44 ± 0.68% organic matter, respectively, based on triplicate analyses on each sediment. A PAH-spiked sample was prepared by adding 10 μL of a stock standard solution to 10 g of freeze-dried sieved (180 μm) sediments or clean sands in a 250-mL clean conical flask. Clean sand was used as the control (without any matrix interaction). The stock standard solution contained a mixture of 16 EPA priority PAHs, each at a concentration of 1000 μg/mL. The conical flasks were then tightly sealed with parafilm to minimize volatilization of the low-molecular-weight PAHs. The spiked sediments or sands were subjected to the aging process for 24 h at room temperature (22 ± 2°C) in the dark. The intrinsic mangrove sediments, without any PAH addition, were regarded as the non-spiked sediments. Four replicates were prepared for each spiked and non-spiked sample.

Quality-control Experiment

The quality-control procedures for PAH determination included procedural blanks, spiked samples, and CRMs (CRM Sediment HS-6, NRC, Canada). PAH compounds were extracted from all the samples using a series of identical procedures summarized in Fig. 1.

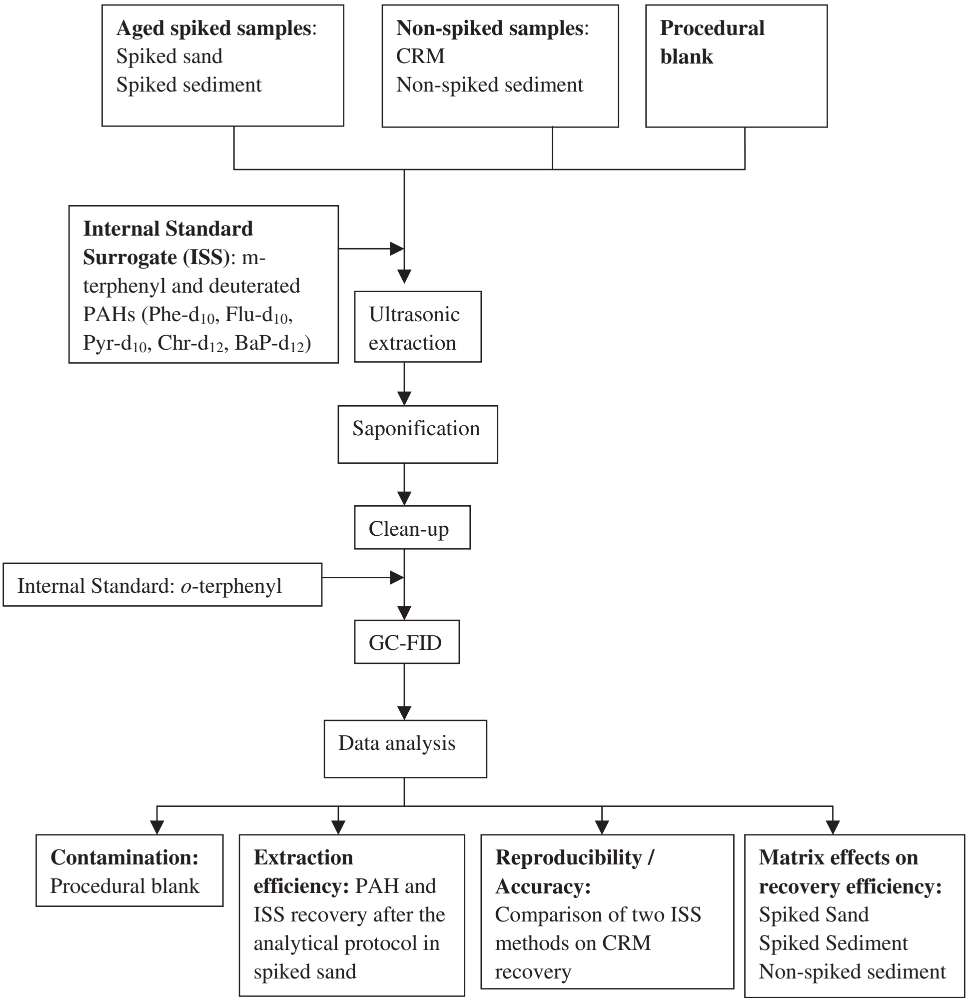

FIGURE 1 Schematic diagram summarizing the quality-control procedures for the determination of PAHs in mangrove sediments.

Extraction of PAHs

All samples including procedural blanks, CRMs (HS-6), spiked and non-spiked samples were extracted following the protocol modified from Lipiatou and Saliot [20]. Ten microliters of a stock solution mixture of five deuterated PAHs and *m*-terphenyl (1000 µg/mL for each compound) was spiked as an ISS prior to extraction. The sediment sample was then sonicated in an ultrasonic bath with a 100-mL mixture of trichloromethane and methanol (2 : 1 v/v) for 30 min, followed by a second extraction with a 100-mL mixture of trichloromethane and methanol (1 : 2 v/v). The two extracts were then combined, and 0.9 part of Milli-Q water was added to separate and discard the methanol phase after vigorous shaking on a horizontal shaker for 20 min. The trichloromethane phase was concentrated to around 1 mL by a rotary evaporator under a reduced pressure at 35°C. The extract was then saponified for 1 h in a water bath (75°C) by adding 15 mL of 2 M KOH (dissolved in a mixed solution of methanol and water at a ratio of 9 : 1). After cooling, 10 mL of Milli-Q water was added and extracted with 3–5 mL mixtures of hexane and diethylether in a sequence of 9 : 1, 8 : 2, 7 : 3, 6 : 4 and 5 : 5 (v/v). The extract was concentrated to near-dryness under a stream of pure nitrogen and redissolved in around 0.5 mL of hexane prior to cleanup.

Clean-up

The PAH fractions were eluted through a chromatographic column with 10 mL of distilled benzene after petroleum hydrocarbon fractions were eluted with 7 mL of distilled hexane. The column was 30 cm in length and 0.5 cm in internal diameter, and packed with 3 g of deactivated silica gel (addition of 3% w/w Milli-Q water after heating at 550°C for 8 h). The eluate was accurately concentrated to 500 µL using a gentle stream of pure nitrogen and packed into a dark brown GC vial. Ten microliters of *o*-terphenyl at a concentration of 1000 µg/mL were spiked to the concentrated sample prior to GC injection. This internal standard was used to calculate the absolute recovery of PAH and ISS compounds after extraction.

GC Analyses: Identification and Quantification

The concentrations and composition of PAH compounds were analyzed by a Hewlett Packard 5890 Gas Chromatograph with a flame ionization detector (GC-FID). A HP-5MS fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) was used for the analysis. The chromatographic conditions were as follows: helium was used as a carrier gas at a flow rate of 1.0 mL/min and a constant pressure of 13 psi. The injector port was used in the 'splitless' mode and held isothermally at 280°C during the duration of the run. The injection volume was 1 µL using a Hewlett Packard 7673 auto-injector. The purge time was 1 min at a flow rate of 50 mL/min. The detector temperature was 300°C. The oven temperature program for PAH analyses was 60°C (initial time, 2 min) to 120°C at a rate of 10°C/min, then 120°C to 300°C at a rate of 3°C/min, holding for 10 min. The GC conditions were tuned regularly for each batch of GC injection (every 10 samples) by the injection of a medium concentration of the standard PAH mixture (i.e. at a concentration of 20 µg/mL for each compound).

Identification and quantification of 16 PAH compounds, namely naphthalene (Nap), acenaphthylene (A), acenaphthene (Ace), fluorene (F), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[ah]anthracene (DA), benzo[ghi]perylene (BP), and indeno[1,2,3-cd]pyrene (IP), were based on matching their retention time with a mixture of PAH standards. The internal standard method was used for the quantification of PAHs [21].

Some samples were chosen and analyzed by a GC-MSD system consisting of a Hewlett Packard 6890 series II gas chromatogram equipped with a 5973 mass spectrometer and 6890 series auto-injector. An HP-5MS Ultra-2 fuse-silica capillary column (30 m long × 0.25 mm i.d. × 0.25 µm film thickness) coated with 5% biphenyl- and 95% dimethyl-polysiloxane (from HP Inc.) was employed. The chromatographic conditions were the same as that for GC-FID, except that the oven temperature program, which was 60°C (held for 2 min), from 60 to 120°C at 10°C/min, and from 120 to 300°C at 4°C/min (held for 7 min). The total run time of the program was 60 min. The GC–MS

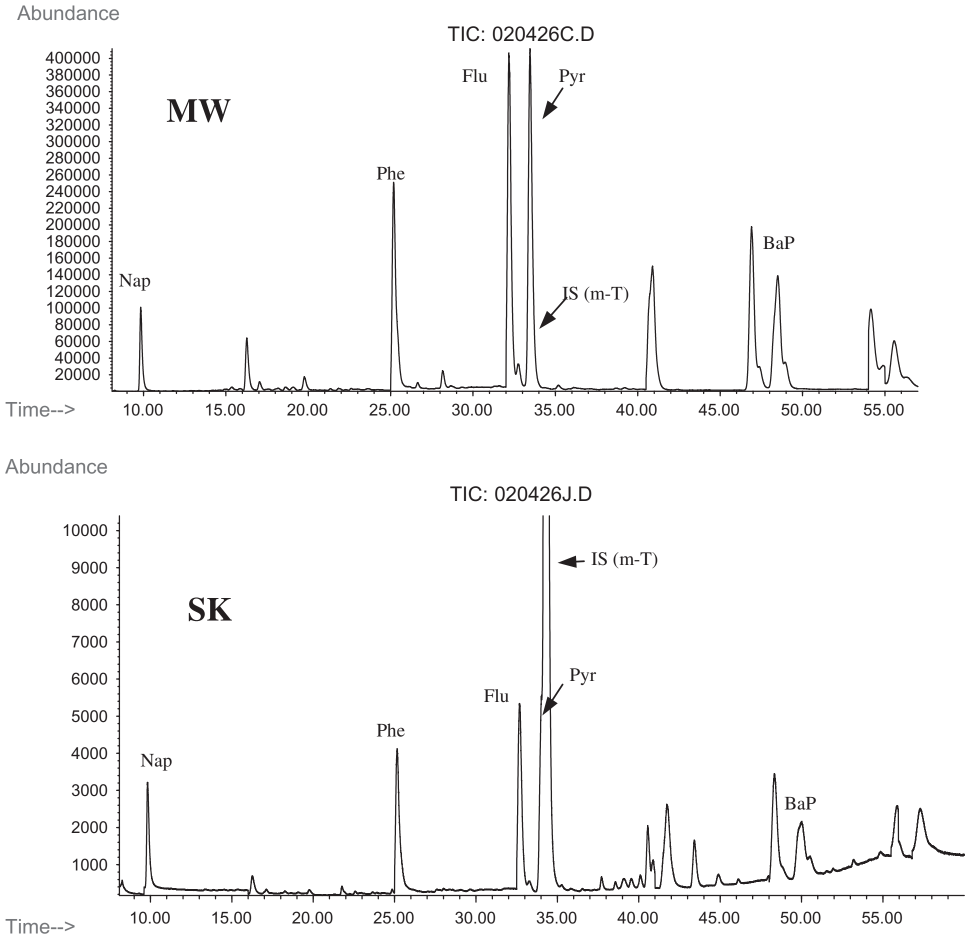

FIGURE 2 Two typical chromatograms showing the PAH profiles in MW (upper chromatogram) and SK sediments (lower chromatogram) obtained from GC-MSD using the TIS mode.

interface temperature was maintained at 280°C. The MS was operated in the total ion scan (TIS) mode with electron impact ionization at 70 eV, and the mass-to-charge ratio scan ranged from 50 to 350 amu. A selected ion-monitoring (SIM) mode was employed after a delay of 4 min. PAHs were monitored at an m/z of 128 (naphthalene), 152 (acenaphthylene), 153 (acenaphthene), 166 (fluorene), 178 (phenanthrene, anthracene), 202 (fluoranthene, pyrene), 228 (chrysene, benzo[a]anthracene), 252 (benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene), 276 (indeno[1,2,3-cd]pyrene, benzo[ghi]perylene), and 278 (dibenzo[ah]anthracene). The MS ion source and mass filter (quad) temperatures were held at 230 and 150°C, respectively. Typical chromatograms showing the PAH profiles for Ma Wan and Sai Keng sediments are shown in Fig. 2.

Comparison of Two ISS Methods

Two kinds of ISS, *m*-terphenyl and a mixture of five deuterated PAHs, were spiked to the CRM sample (Sediment HS-6) for comparison. The deuterated PAHs were Phe-d_{10},

TABLE I ISSs used for calibration

PAHs[a]	*ISS-1*[b]	*ISS-2*[c]
Nap	*m*-T	Phe-d_{10}
A	*m*-T	Phe-d_{10}
Ace	*m*-T	Phe-d_{10}
F	*m*-T	Phe-d_{10}
Phe	*m*-T	Phe-d_{10}
Ant	*m*-T	Phe-d_{10}
Flu	*m*-T	Flu-d_{10}
Pyr	*m*-T	Pyr-d_{10}
BaA	*m*-T	Chr-d_{12}
Chr	*m*-T	Chr-d_{12}
BbF	*m*-T	BaP-d_{12}
BkF	*m*-T	BaP-d_{12}
BaP	*m*-T	BaP-d_{12}
IP	*m*-T	BaP-d_{12}
DA	*m*-T	BaP-d_{12}
BP	*m*-T	BaP-d_{12}

[a] Nap: naphthalene, A: acenaphthylene, Ace: acenaphthene, F: fluorene, Phe: phenanthrene, Ant; anthracene, Flu: fluoranthene, Pyr: pyrene, BaA: benzo[a]anthracene, Chr: chrysene, BbF: benzo[b]fluoranthene, BkF: benzo[k]fluoranthene, BaP: benzo[a]pyrene, IP: indeno [1,2,3-cd]pyrene, DA: dibenzo[ah]anthracene, BP: benzo[ghi]perylene. [b] *m*-T: *m*-terphenyl. [c] Phe-d_{10}: deuterated phenanthrene, Flu-d_{10}: deuterated fluoranthene, Pyr-d_{10}: deuterated pyrene, Chr-d_{12}: deuterated chrysene, BaP-d_{12}, deuterated benzo[a]pyrene.

Flu-d_{10}, Pyr-d_{10}, Chr-d_{12}, and BaP-d_{12}. Their target compounds for calculation are shown in Table I.

Limits of Detection

The smallest quantity (1 μL) of an extract (500 μL) was injected into the GC-FID, and the limit of detection (LOD) for each PAH compound was equal to three times 'the signal-to-noise ratio', assuming a zero blank. The quantity was then normalized to the sample size, and the estimated LOD value for each individual compound of 16 PAHs in sediments ranged from 0.05 to 0.5 ng/g.

Statistical Analyses

A non-parametric one-way ANOVA test on ranks was used to test for any differences in recovery percentages among 16 PAH compounds of the same sample. A non-parametric two-way ANOVA test was conducted to determine any differences in PAH recovery percentages in the CRMs (CRM HS-6) using the two ISS methods for calculation, with PAH compounds and ISS methods as the two factors. A non-parametric three-way ANOVA test was performed to determine any differences in PAH recovery percentages of the spiked samples, and the three factors were PAH compounds, sample types, and ISS methods. If the ANOVA results showed significant differences in any factor, the Student–Newman–Keuls method, a pairwise multiple-comparison procedure, would be carried out to determine where the difference lay. The non-parametric one-way and two-way ANOVA tests were conducted using a commercial software package called Sigma Stat (Version 2.0, Jandel Scientific, USA), whereas the non-parametric three-way ANOVA was carried out using another software package, named SPSS (Version 10.0, SPSS Inc., USA).

RESULTS

Procedural Blanks

None of the 16 PAH compounds were detected in four replicates of the procedural blanks, indicating that no contamination from sources such as reagents, solvents, and glassware was generated during the analytical procedures.

Comparison of ISS and PAH Recovery Percentages in Spiked Sand After the Analytical Protocol

The recovery percentages of ISSs and PAHs in spiked clean sands after the extraction procedure are shown in Fig. 3. The PAHs with three or more rings had much higher percentages of recovery (70–90%) than that of the two-ring PAH (10–50%), suggesting that low-molecular-weight PAHs were easily lost during extraction. Naphthalene, the most volatile PAH, had the lowest percentage of recovery. The stability of PAHs during the extraction procedure did not increase in proportion to the molecular weight of the PAH, according to the pairwise multiple comparisons (Student–Newman–Keuls Method). Several PAHs, including phenanthrene (Phe), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF) and dibenzo[ah]anthracene (DA), had higher recovery percentages than their homologs (Phe-d_{10} and BaP-d_{12}). It seems that some ISSs were not the proper representatives of their target PAH compounds (Fig. 3).

Method Accuracy: Determination of CRMs

The recovery percentages (i.e. method accuracy) of 16 PAHs in CRMs (HS-6) using two ISS methods were high, except for naphthalene, ranging from 69.3 to 111.8% for ISS-1 (*m*-terphenyl as a single ISS) and from 71.8 to 120.5% for ISS-2 (five deuterated PAHs

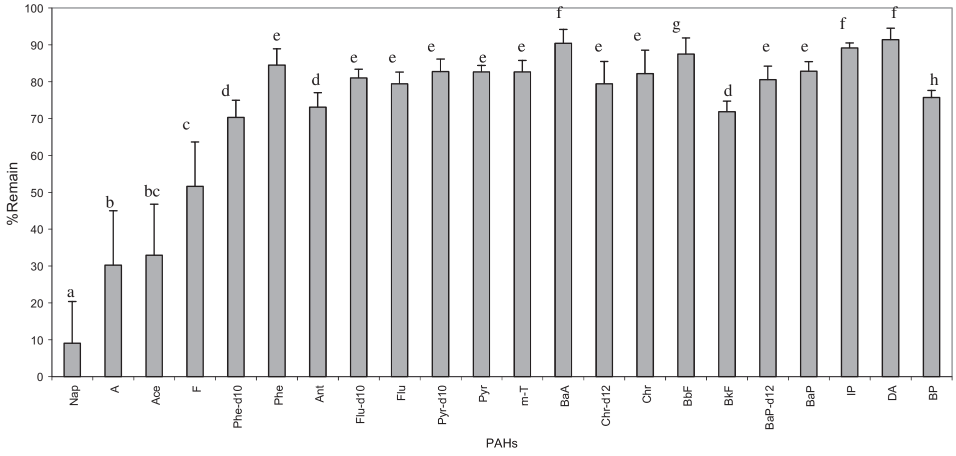

FIGURE 3 Percentage recovery (% remain) of PAH and ISS in spiked sand after the extraction. The legends are the same as those in Table I. The mean and standard deviation of four replicates are shown. Different lower-case letters indicate that significant differences in percentage recovery were found between PAH compounds at the probability level of 0.05.

TABLE II Comparison of the method accuracy calculated by two ISS methods using a standard marine sediment sample (CRM HS-6) (mean and standard deviation of four replicates are shown)

PAHs[a]	C_c[b]	C_m[c]		*RSD*[d]		*R%*[e]		
		ISS-1	*ISS-2*	*ISS-1*	*ISS-2*	*ISS-1*	*ISS-2*	*Mean of two ISS methods*[f]
Nap	4.1 ± 1.1	1.47 ± 1.20	1.51 ± 1.24	81.9	82.5	35.8 ± 29.4	36.7 ± 30.3	36.3^{A}
A	0.19 ± 0.05	0.14 ± 0.05	0.14 ± 0.05	33.4	33.1	74.1 ± 24.7	76.2 ± 25.2	75.3^{BC}
Ace	0.23 ± 0.07	0.16 ± 0.02	0.17 ± 0.02	11.9	10.4	70.0 ± 8.3	71.9 ± 7.5	71.0^{B}
F	0.47 ± 0.12	0.34 ± 0.05	0.35 ± 0.05	14.1	14.4	72.4 ± 10.2	74.6 ± 10.7	73.5^{BC}
Phe	3.0 ± 0.6	2.66 ± 0.09	2.73 ± 0.03	3.2	0.9	88.6 ± 2.9	91.1 ± 0.9	89.9^{BCD}
Ant	1.10 ± 0.4	1.23 ± 0.09	1.27 ± 0.10	7.7	8.0	111.8 ± 8.6	115.1 ± 9.2	111.7^{E}
Flu	3.54 ± 0.65	2.78 ± 0.10	2.69 ± 0.10	3.6	3.6	78.5 ± 2.8	76.0 ± 2.8	77.3^{BC}
Pyr	3 ± 0.6	2.18 ± 0.10	2.31 ± 0.06	4.6	2.6	72.6 ± 3.3	77.2 ± 2.0	74.9^{BC}
BaA	1.8 ± 0.3	1.25 ± 0.08	1.29 ± 0.07	6.7	5.5	69.3 ± 4.6	71.8 ± 3.9	70.6^{B}
Chr	2.0 ± 0.3	2.09 ± 0.12	2.16 ± 0.02	5.9	0.9	104.5 ± 6.2	107.8 ± 1.0	106.2^{DE}
BbF	2.2 ± 0.4	1.88 ± 0.14	2.27 ± 0.23	7.5	10.0	85.5 ± 6.4	103.0 ± 10.3	94.3^{CDE}
BkF	2.8 ± 0.6	2.53 ± 0.10	3.02 ± 0.05	4.1	1.7	90.3 ± 3.7	107.8 ± 1.8	99.1^{DE}
BaP	1.43 ± 0.15	1.23 ± 0.05	1.47 ± 0.06	3.9	4.0	85.8 ± 3.4	103.0 ± 4.1	94.4^{CDE}
IP	1.78 ± 0.72	1.79 ± 0.17	2.14 ± 0.14	9.6	6.5	97.3 ± 9.7	120.5 ± 7.9	108.9^{DE}
DA	0.49 ± 0.16	0.48 ± 0.09	0.57 ± 0.09	18.7	15.8	100.7 ± 18.2	116.1 ± 18.3	108.4^{DE}
BP	1.95 ± 0.58	1.37 ± 0.12	1.64 ± 0.09	8.7	5.6	70.3 ± 6.1	83.9 ± 4.7	77.1^{BC}

[a]Same legends as in Table I. [b]Cc: certified concentrations (ng/g dry weight). [c]C_m: measured concentrations (ng/g dry weight), ISS-1: *m*-terphenyl, ISS-2: five deuterated PAHs. [d]RSD (relative standard deviation) = $SD/C_m \times 100\%$, where SD is standard deviation of C_m. [e]R% (recovery percentage) = $C_m/C_c \times 100\%$. [f]Differences in recovery percentages between PAH compounds according to ANOVA test are shown by different superscript upper-case letters.

as a multi-ISS). The mean PAH recovery percentage calculated by ISS-2 (89.6%) was significantly higher than the mean obtained from ISS-1 (81.7%) according to the non-parametric two-way ANOVA test ($F = 9.99$, $p = 0.0024$), indicating that the number of ISSs used for calculation would affect the PAH recovery. The recovery percentages also varied among 16 PAH compounds of different molecular weights ($F = 6.29$, $p < 0.0001$). The reproducibility (i.e. method precision) was good for most of the PAH compounds using both ISS methods, and the relative standard deviations (RSDs) were within 20% except naphthalene and acenaphthylene (Table II). The recovery percentages of high-molecular-weight PAH compounds were generally higher than that of low-molecular-weight PAHs except anthracene and benzo[a]anthracene.

Recovery Efficiency: Determination of Spiked Samples

The background PAH concentrations of two mangrove sediments, based on a single ISS (*m*-terphenyl), are shown in Table III. It is obvious that PAH contamination in Ma Wan was more serious than that in Sai Keng mangrove sediments. The percentages of PAH recovery in spiked sediment or sand samples, after subtracting from their background PAH concentrations, were calculated using the two ISS methods (Table IV). The recovery percentages of the high-molecular-weight PAHs were significantly higher than that of the low-molecular-weight PAHs (in particular, the two-ring PAH). These findings indicate that the very volatile two-ring PAH (Nap) could not be effectively recovered by the ISSs used in the present study. Nevertheless, the recoveries of PAH compounds with molecular weights higher than that of Phe were around 100%.

The variability among four replicates of each PAH compound was within 20% except BkF. Significant differences in PAH recoveries were found among three spiked samples

TABLE III Background concentrations of PAHs (ng/g dry wt) in surface sediments of two mangrove swamps in Hong Kong SAR (mean and standard deviation of four replicates are shown)

PAH compounds[a]	*Sai Keng*	*Ma Wan*
Nap	28 ± 10	20 ± 14
A	11 ± 3	44 ± 16
Ace	20 ± 15	12 ± 5
F	6 ± 2	10 ± 2
Phe	21 ± 3	74 ± 7
Ant	13 ± 5	44 ± 17
Flu	26 ± 3	205 ± 15
Pyr	35 ± 6	225 ± 48
BaA	18 ± 5	153 ± 23
Chr	39 ± 8	203 ± 30
BbF	21 ± 3	223 ± 49
BkF	78 ± 28	316 ± 36
BaP	65 ± 16	195 ± 52
IP	136 ± 17	233 ± 51
DA	23 ± 10	230 ± 41
BP	79 ± 26	60 ± 25

[a] Same legends as in Table I.

TABLE IV Comparison of recovery percentages (%) of spiked sand (S) and two mangrove sediment samples (Sai Keng SW and Ma Wan MW) (mean and standard deviation of four replicates are shown)

PAHs[a]	*ISS-1*[b]			*ISS-2*[c]			*Mean % of two ISS methods*[d]
	S	*SK*	*MW*	*S*	*SK*	*MW*	
Nap	11.1 ± 11.5	7.0 ± 13.7	24.5 ± 11.4	12.6 ± 13.0	8.5 ± 15.1	26.9 ± 12.2	15.1^{A}
A	36.3 ± 14.2	49.9 ± 8.9	49.7 ± 9.5	42.2 ± 15.5	55.5 ± 10.5	55.0 ± 9.4	48.1^{B}
Ace	39.7 ± 13.2	47.4 ± 8.6	52.7 ± 10.6	46.1 ± 14.2	53.1 ± 10.7	52.7 ± 10.6	49.5^{B}
F	62.2 ± 11.3	73.3 ± 5.4	73.4 ± 4.4	72.9 ± 10.7	81.1 ± 5.8	80.8 ± 4.2	73.9^{C}
Phe	102.4 ± 6.4	83.7 ± 2.1	89.5 ± 4.8	120.8 ± 11.8	92.8 ± 3.2	98.9 ± 3.0	98.0^{DE}
Ant	88.3 ± 1.9	71.5 ± 3.4	85.1 ± 5.1	104.0 ± 1.9	79.0 ± 4.5	93.6 ± 3.5	86.9^{F}
Flu	96.2 ± 0.3	92.3 ± 12.8	95.2 ± 5.8	98.0 ± 0.8	102.0 ± 14.9	106.0 ± 4.4	98.3^{DE}
Pyr	100.1 ± 1.2	91.3 ± 3.4	97.2 ± 8.0	99.9 ± 1.7	101.0 ± 5.1	108.1 ± 5.8	99.6^{DE}
BaA	109.2 ± 1.3	98.2 ± 6.3	105.0 ± 8.7	114.1 ± 3.7	106.9 ± 3.6	114.9 ± 7.4	108.0^{GH}
Chr	99.4 ± 3.3	103.6 ± 8.3	103.0 ± 9.4	103.5 ± 1.2	113.2 ± 5.7	113.1 ± 7.9	106.0^{EG}
BbF	105.6 ± 1.2	92.9 ± 8.4	93.7 ± 8.1	108.6 ± 1.9	100.7 ± 9.5	102.5 ± 9.0	100.7^{DEG}
BkF	87.4 ± 0.6	97.5 ± 39.7	85.9 ± 7.5	89.2 ± 1.6	107.1 ± 44.2	94.8 ± 7.7	93.6^{D}
BaP	100.3 ± 0.6	86.6 ± 7.1	90.5 ± 6.3	102.9 ± 1.8	94.2 ± 6.1	98.3 ± 6.4	95.5^{D}
IP	108.2 ± 3.3	106.2 ± 6.4	97.9 ± 11.9	110.8 ± 3.6	114.4 ± 3.3	106.4 ± 12.9	107.3^{GH}
DA	110.9 ± 4.8	105.0 ± 16.7	115.7 ± 12.4	113.6 ± 4.6	115.4 ± 16.6	124.7 ± 13.2	114.2^{H}
BP	92.1 ± 1.7	96.6 ± 12.0	93.4 ± 10.2	92.1 ± 1.7	104.8 ± 9.7	100.6 ± 9.6	96.9^{D}

Three-way ANOVA results:
PAHs: $F(15,383) = 166.690$; $p < 0.0001$
Samples (S *vs*. SK *vs*. MW): $F(2,383) = 3.089$; $p = 0.047$
ISS (ISS-1 *vs*. ISS-2): $F(1,383) = 45.839$; $p < 0.0001$
PAHs × Samples: $F(30,383) = 3.394$; $p < 0.0001$
PAHs × ISS: $F(15,383) = 0.295$; $p = 0.996$
Samples × ISS: $F(2,383) = 0.790$; $p = 0.455$
PAHs × Samples × ISS: $F(30,383) = 0.208$; $p = 1.000$

[a] Same legends as in Table I. [b] ISS-1: *m*-terphenyl; S: sand; SK: Sai Keng; MW: Ma Wan. [c] ISS-2: five deuterated PAHs. [d] Significant differences in recovery percentages between 16 PAH compounds are shown by different superscript upper-case letters.

($F = 3.089$, $p = 0.047$), among 16 different PAH compounds ($F = 166.690$, $p < 0.0001$), and between two ISS methods ($F = 45.839$, $p < 0.0001$). According to the non-parametric three-way ANOVA test, the differences in recovery percentages among 16 PAH compounds varied, depending on the types of samples ($F = 3.394$, $p < 0.0001$ for PAH × Samples) although these were not affected by the ISS methods (the interaction factors with ISS methods were not significant). Ma Wan spiked sediments had significantly higher PAH recovery percentages than Sai Keng sediments and clean sand (i.e. Ma Wan > Sai Keng = sand). These results suggest that the matrix effects of mangrove sediments would interfere with the PAH recovery percentages.

DISCUSSION

The differences in PAH recovery percentages between ISSs (deuterated PAH) and the target PAH compounds in the spiked sand after the analytical protocol show that ISSs behaved differently to their target compounds, even to their homologs such as Phe-d_{10} to Phe. Such differences, similar to those reported by previous workers [9], may arise during the extraction, reconcentration and saponification steps.

The number of ISSs used for calculation affected the accuracy and precision of the analysis. It was reported that the recovery percentages of a CRM (SRM 1941a, a marine sediment) calculated by six ISSs (97.4%) were slightly higher than that by a single ISS (96.1%), and the precision of the former calculation was higher with average standard deviations of 5.7% and 16.0%, respectively [10]. The present study also shows that the ISS-1 method (*m*-terphenyl) had slightly lower recovery percentages than the ISS-2 method (five deuterated PAHs). Nevertheless, both ISS methods had comparable and acceptable reproducibilities, indicating that the single-ISS approach was valid and showed more or less the same performance as the multi-ISS approach in quantifying PAH concentrations in sediments. Since increasing the number of ISS would increase the cost, the single ISS method was commonly used and has been applied to several recent environmental studies [13–16].

The PAH recoveries in sediments varied substantially among 16 PAH compounds, ranging from 20% to 130% (Table V). The recoveries of the low-molecular-weight PAHs, in particular, naphthalene, were low. Their high volatility and instability lead to losses during solvent evaporation procedures [7]. These suggest that it is necessary to use another ISS such as Nap-D8 to quantify naphthalene accurately, and its losses can be prevented by controlling the reconcentrations steps with a stopper such as nonane. Nonetheless, some researchers proposed excluding naphthalene, the most volatile compound, when determining PAHs in sediments [10]. Because of the complexity of marine sediments, some compromises on the criteria of accurate acceptance for a spiked sediment had been suggested by Burns *et al.* [22], including: (1) the PAH recoveries should be 40–120% for the 16 EPA priority PAHs; (2) one, and only one, PAH analyte is allowed to be below its minimum percentage recovery; and (3) the minimum percentage recoveries are at least 10% recovery for chrysene and benzo[a]pyrene and 20% for other PAH compounds. In the present study, the percentages of PAH recovery, irrespective of which ISS method was used, ranged between 40 and 120% (except naphthalene), indicating that both ISS methods fulfilled Burns' suggested criteria [22] and were acceptable for use in PAH quantification. The study also reveals that the recovery percentages of chrysene and dibenzo[ah]anthracene in spiked mangrove

TABLE V Comparison of different internal standard surrogate methods for calculation of PAH recovery from CRMs, standard reference materials (SRM) and spiked sediments

Sample sources	*Number of replicates*	*Internal standard surrogates*	*Recovery percentages of PAHs*	*Relative standard deviation*	*References*
CRM HS-6	4	Ace-d_{10}, Phe-d_{10}, Chr-d_{12}	Not calculated	24 ± 19%	[27]
CRM HS-6	2	*m*-Terphenyl	> 85%	5.8–24.9%	[15]
SRM 1941a	Not mentioned	Not mentioned	86% on average	Not calculated	[28]
CRM	Not mentioned	Not mentioned	70 (only for naphthalene)-102%	Not calculated	[29]
SRM 1941a	2	Nap-d_8, Phe-d_{10}, Pyr-d_{10}	85–90% for tetracyclic and more cyclic PAHs, 50–55% for tricyclic PAHs	< 10%	[3]
SRM 1941a	Not mentioned	Not mentioned	> 70%	< 6%	[12]
CRM HS-3	Not mentioned	Nap-d_8, Ace-d_{10}, Phe-d_{10}, Chr-d_{12}	87–111%	Not calculated	[30]
Spiked sediment	3	Not mentioned	Ranged between 30 and 70%, with the lower values for the lower-molecular-weight PAH compounds	±10–20%	[31]
Spiked sediment	Not mentioned	Not mentioned	65% on average, with the recovery of individual PAH ranging from 20% (Nap) to 95% (BaP, Pyr)	Not calculated	[32]
Spiked sediment	3	Ace-d_{10}, Phe-d_{10}, Chr-d_{12}	60–96%	5–15%	[11]

Spiked sediment	Not mentioned	Not mentioned	40% to > 90%	Not calculated	[33]
Spiked sediment	Not mentioned	Nap-d$_8$, Flu-d$_{10}$, Chr-d$_{12}$	40–120%	Not calculated	[22]
Spiked sediment	2	Not mentioned	50–85%	< 20%	[2]
Spiked sediment	2	Not mentioned	Not calculated	3–5% for tetra- and pentacyclic PAHs, 4–7% for tricyclic PAHs, 10–15% for Nap	[34]
Spiked Sediment	3	Not mentioned	62–98%	24–36% at highest concentrations, 24–63% at lower concentrations	[18]
Spiked sediment	Not mentioned	1,1′-Binaphthyl	> 70%	Not calculated	[16]
Spiked sediment	2	Ace-d$_8$, Flu-d$_{10}$, BbF-d$_{12}$	70–130%	± 20%	[35]
Spiked sediment	Not mentioned	Not mentioned	> 65% for all PAHs except Nap	Not calculated	[1]
CRM HS-6	3	Phe-d$_{10}$, Flu-d$_{12}$, Pyr-d$_{12}$, BaP-d$_{12}$	71.8–116.1% for all PAHs except Nap (36.7%)	< 20% except Nap and Ace	The present study
CRM HS-6	3	*m*-Terphenyl	69.3–111.8% for all PAHs except Nap (35.8%)	< 20% except Nap and Ace	The present study
Spiked sediment	4	Phe-d$_{10}$, Flu-d$_{12}$, Pyr-d$_{12}$, BaP-d$_{12}$	42.2–124.7% for all PAHs except Nap (8.5–26.9%)	< 20% except BkF	The present study
Spiked sediment	4	*m*-Terphenyl	36.3–115.7% for all PAHs except Nap (7.0–24.5%)	< 20% except BkF	The present study

sediment samples and certified marine sediment were greater than 100%. It is important to note that the recovery percentages may be affected by the co-elution of the homologs of some target PAHs in sediment samples such as triphenylene (chrysene homolog), dibenzo[ac]anthracene (dibenzo[ah]anthracene homolog), and benzo[j] fluoranthene (benzo[b]- and benzo[k]fluoranthene homolog).

One of the challenging tasks for the determination of PAHs in sediments currently confronting researchers is the development of an efficient method for extracting PAH compounds from complex matrices [23]. PAHs have been found to be preferably bound to organic substances of sediments via adsorption and diffusion processes [24,25], which decrease the accessibility of solvents to extract PAHs [4]. Another major problem for the determination of PAHs in soils high in organic matter is co-extraction of non-target substances, which may alter the PAH extraction efficiency [6]. The release of PAHs from the organic substances in sediments, thus enhancing the PAH extraction efficiency, could be promoted by two ways: (1) hydrolyzing organic matter like humic acids to facilitate a limited breakdown of the polymeric structures by cleavage of ester bonds; (2) introducing alkaline conditions to induce an extension of the humification processes due to mutual repulsion between negatively charged carboxyl, phenolic and hydroxyl functional groups, which then improves the solvent accessibility within the polymeric structures of humic acids, promotes the release of recalcitrant PAH fractions, and reduces the hydrogen-bonding ability [6,23].

The saponification method is generally considered an efficient digestion method to help the release of more PAHs from organic substances [6], although this method was also criticized for the formation of artefact compounds such as methyl esters of fatty acids and organosulfur compounds [5]. It has been reported that higher quantities of PAHs in soils were released only when an additional saponification step was employed, thus indicating that a large part of the PAHs strongly adsorbed to soil organic substances could not be released unless the soil was subjected to saponification [26]. In the present study, the inclusion of a saponification step during extraction significantly increased the extraction recoveries of PAHs in both spiked and non-spiked mangrove sediments, and the enhancement was more obvious in the Ma Wan mangrove sediments, the one with higher organic matter content, than that in the Sai Keng sediments.

CONCLUSIONS

A series of quality-control procedures was carried out to evaluate the method validity for the determination of PAHs in mangrove sediments. The present study demonstrates that both ISS methods, ISS-1 (*m*-terphenyl, a single ISS) and ISS-2 (five deuterated PAHs, a multi-ISS), were acceptable for PAH determination because of their comparable reproducibility and performance. The recovery percentages for most PAH compounds calculated using both methods satisfied the acceptance criteria suggested by Burns *et al.* [22]. The matrix effects of mangrove sediments would affect the PAH recovery efficiency. The reasonably high organic matter content in the Ma Wan mangrove sediments would reduce the losses of PAHs by evaporation and/or degradation during the extraction and concentration procedures, and the inclusion of a saponification step improved the PAH recovery efficiency. In spite of the sediment matrix effect, the analytical method developed in the present study for the determination of PAHs, in particular the simple and cheap ISS-1 method (*m*-terphenyl), was acceptable and applicable to mangrove sediments.

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