

Article

An Efficient and Greener Alternative for the Extraction of Polycyclic Aromatic Compounds from Sediments

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Abstract

This study details the validation of a novel microbead beating extraction (MBE) technique for the analysis of polycyclic aromatic compounds (PACs) in sediments. The method's performance was evaluated against international analytical validation criteria, including trueness, precision, measurement uncertainty and robustness. Limits of detection and quantitation were consistently low (≤ 6.5 and 21 ng g^{-1} , respectively), trueness for the majority of analytes fell within accepted performance criteria, and repeatability values for most analytes were generally $<10\%$. Analytical data confirm the method's reliability, with more than 80% of certified analytes in two certified reference materials (CRMs) meeting the satisfactory z-score ($|z| \leq 2.0$). Furthermore, the method's inter-laboratory repeatability, as measured by HorRat values, fell within the range recommended by the Association for Official Analytical Chemist for most analytes, and combined measurement uncertainties showed no statistical difference from the certified uncertainties of the CRMs. Incorporating an in situ cleanup step enabled the MBE method to substantially reduce extraction times ($<15 \text{ min}$) and reduces solvent consumption by $\sim 60\%$ compared with conventional pressurize fluid extraction while maintaining good quality data objectives. By meeting or exceeding well-established metrics for good laboratory performance, the MBE method demonstrates reliability, efficiency, and a greener approach for the routine analysis of PACs in sediments.

Keywords: polycyclic aromatic compounds; microbead beating extraction; method validation; extraction; sediment; environmental analysis



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1. Introduction

Polycyclic aromatic compounds (PACs) are a well-known diverse and environmentally ubiquitous group of chemicals that are composed of multiple fused aromatic rings [1]. The PAC family includes polycyclic aromatic hydrocarbons (PAHs), alkylated polycyclic aromatic hydrocarbons (APAHs), halogenated polycyclic aromatic hydrocarbons (HPAHs), heterocyclic polycyclic aromatic compounds (HPACs), which can contain either S-, N- and O- incorporated into the cyclic ring, and halogenated heterocyclic polycyclic aromatic compounds (HHPACs). They are primarily generated from incomplete combustion of organic matter and are widespread in the environment due to both natural processes

and anthropogenic activities such as fossil fuel combustion, industrial discharge, and urban runoff [2–4]. Owing to their hydrophobicity and chemical persistence, PACs tend to accumulate in sediments, where they pose significant ecological and human health risks due to their mutagenic and carcinogenic properties [5–10].

It is widely acknowledged that sample preparation remains the bottleneck in routine analysis of persistent organic compounds in environmental samples. Traditional extraction methods, such as Soxhlet extraction [11–16], ultrasonic-assisted extraction (UAE) [17–19], the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method [20–23], and pressurized fluid extraction (PFE) [24–28], have been widely employed for PACs in environmental matrices. While its exhaustive nature is well-established, Soxhlet extraction suffers from significant drawbacks, including a long extraction time (up to 24 h), high solvent consumption (often >150 mL per sample), and poor selectivity [29,30]. The co-extraction of bulk organic matter can produce complex gas chromatograms with artifact peaks, often necessitating extensive and laborious post-extraction cleanup steps to prevent contamination of the analytical instrument. Although originally developed as a rapid and inexpensive method for the analysis of organic compounds in food matrices, QuEChERS remains limited by its restricted solvent compatibility for a broad suites of analytes, low sample-to-extract ratios, susceptibility to matrix effects, and potential PAH contamination from the dispersants used [31–34].

Another major advancement in the analysis of PACs is solid-phase microextraction (SPME), a solvent-free method that integrates extraction and pre-concentration onto a single coated fiber. Further innovation has led to the development of magnetic solid-phase extraction (MSPE), which employs functionalized magnetic nanoparticles to selectively adsorb target analytes, followed by rapid separation using an external magnetic field [35,36].

Recent advances in sample preparation have led to the exploration of mechanical disruption techniques for environmental analysis. Microbead-beating extraction (MBE), a mechanical lysis method widely used in microbial DNA extraction, uses high-energy agitation of samples with microbeads to disrupt biological or solid matrices [37–39]. This principle has potential utility in the extraction of hydrophobic organic compounds from sediments by promoting enhanced physical breakdown of aggregates and facilitating solvent penetration [40,41]. Despite its proven success in biomolecular applications, bead-beating remains underutilized in environmental organic contaminant analysis, particularly for sediment-bound PACs.

To address the challenges of labor-intensive sample preparation, we investigated the application of MBE as a solvent-efficient, time-saving, and robust method for the extraction of PACs from sediments. We hypothesize that the bead-mediated mechanical agitation would enhance the mass-transfer of PACs from sediments into an appropriate solvent system. In addition, incorporation of a selective adsorbent within the extraction vessel is anticipated to retain co-extracted matrix components. Following moisture removal, we expect an extract that would be sufficiently cleaned for direct injection into a gas chromatographic system. The present research aims to optimize the extraction of a wide suite of PACs with varying chemical properties from sediments by evaluating its performance against the conventional PFE technique and validating its applicability for routine environmental monitoring.

2. Materials and Methods

2.1. Chemicals

Organic solvents were obtained from Fisher Scientific (Ottawa, ON, Canada). The suite of isotopically labelled internal standards used as recovery internal standard (RIS) comprised d_8 -naphthalene, d_8 -acenaphthylene, d_{10} -acenaphthene, d_{10} -fluorene, d_{10} -phenanthrene, d_{10} -pyrene, d_{12} -benzo(*a*)anthracene, d_{12} -chrysene, d_{12} -benzo(*b*)fluoranthene, d_{12} -benzo(*k*)fluoranthene, d_{12} -benzo(*a*)pyrene, d_{12} -indeno(1,2,3-*c,d*)pyrene, d_{14} -dibenz(*a,h*)anthracene, and d_{14} -benzo(*g,h,i*)perylene. Deuterated anthracene (d_{10} -anthracene) was employed as the instrument performance internal standard (IPIS).

For the APAHs, HPAHs and HHPACs, compound abbreviations consist of the substitution positions of alkyl groups and/or halogen atoms, followed by the substituent identity and the parent PAH structure. Abbreviations for unsubstituted PAHs are as follows: naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), 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), benzo[*g,h,i*]perylene (BgHiP), indeno[1,2,3-*c,d*]pyrene (Ind), and dibenz[*a,h*]anthracene (DahA).

Unsubstituted PAHs were purchased from AccuStandard Inc. (New Haven, CT, USA); APAHs, HPACs, and HHPACs were obtained from Chiron Chemicals (Trondheim, Trøndelag, Norway). The full list of abbreviations and details of HPAHs and their suppliers can be found in Table A1. The certified reference materials (CRMs) were two marine sediment samples (QPH121MS and QPH122MS) from the North Sea and/or Mediterranean and were purchased from Wepal-Quasimeme (Wageningen, GE, The Netherlands). The QPH122MS CRM contained small amounts of total petroleum hydrocarbon (0.256%) and was intentional fortified with PACs to assess our method's trueness, precision and measurement uncertainty. The QPH121MS CRM was employed for comparison studies between ASE and MBE and estimating measurement uncertainty due to its relative high concentration of total petroleum hydrocarbon (3.16%).

2.2. Microbead Extraction of Sediments

An accurately weighed aliquot of each CRM (1.00 ± 0.01 g) was transferred into 15 mL custom-engineered stainless steel microbead tubes containing 2.5 g of 2.8 mm zirconium oxide ceramic microbeads (Bertin Technologies, Montigny-le-Bretonneux, France), 4.0 g of silica gel, and 1.0 g of 5% deactivated alumina. Dichloromethane:ethyl acetate (DCM:EtOAc, 1:1, *v/v*; 8.0 mL) was added to each tube. Samples were spiked with the RIS and PAC working standard mixture 24 h prior to extraction and stored at 4 °C to allow equilibration.

Extractions were performed using a Precellys Evolution homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France) operated at 6800 rpm for eight extraction cycles of 20 s each, with 50 s intervals between cycles, resulting in a total extraction time of 8.5 min with 8.0 mL of extract. Following extraction, samples were centrifuged at 5000 rpm and 10 °C for 10 min. The supernatant was transferred to a 60 mL glass collection vial.

The extraction tubes were subsequently rinsed twice with 8.0 mL of DCM:EtOAc (1:1, *v/v*). Each rinse was vortex-mixed for 1 min and centrifuged under the same conditions, and the rinsates were combined with the initial extract. Residual moisture was removed by treatment with anhydrous sodium sulfate, after which the extract was transferred to a clean 60 mL vial using three hexane rinses (~2 mL each). The combined extract was concentrated to 1.0 mL under a gentle stream of ultra-high-purity nitrogen, fortified with the IPIS ($100 \text{ pg } \mu\text{L}^{-1}$), and stored at 4 °C in amber glass vials until analysis.

2.3. Method Validation of Microbead-Beating Extraction

Method validation was conducted in accordance with the Eurachem Guide *The Fitness for the Purpose of Analytical Methods*, which specifies performance characteristics including limits of detection, trueness (recovery), precision, and ruggedness [42]. Several extraction solvents were evaluated during preliminary method development using a representative subset of target PACs, and dichloromethane:ethyl acetate (1:1, v/v) provided the most effective overall extraction performance; this solvent system was therefore selected for the final method.

2.3.1. Detection Limits

Limits of detection (LOD) and quantification (LOQ) were determined by extracting approximately 1.0 g of the QPH122MS CRM ($n = 8$) fortified with 10 ng of PACs. The standard deviation of replicate signals (s_0) was calculated for each analyte, and the adjusted standard deviation (s_0') was obtained according to the Eurachem definition ($s_0' = s_0 / \sqrt{n}$). Analyte-specific LODs and LOQs were calculated as $3s_0'$ and $10s_0'$, respectively [42]. All LOD and LOQ values are reported on a mass-normalized basis.

2.3.2. Trueness

Trueness was evaluated using the QPH122MS CRM ($n = 8$) fortified with PACs at three concentration levels (10, 100, and 500 ng), corresponding to fortification solutions of 10, 100, and 500 $\text{pg } \mu\text{L}^{-1}$, respectively. Fortified samples were allowed to equilibrate for 24 h prior to extraction. Unfortified QPH122MS samples ($n = 8$) were also analyzed, and endogenous PAC concentrations were subtracted from fortified sample results to obtain net recoveries.

This CRM was selected in preference to surrogate matrices (e.g., hydromatrix or silica sand) because it is a representative sample of natural sediment matrices encountered in environmental monitoring. Method performance was further assessed using z-scores calculated according to Equation (1), where x represents the laboratory-measured value, X the assigned reference value, and $\hat{\sigma}$ the standard deviation for proficiency assessment [43].

$$z = \frac{x - X}{\hat{\sigma}} \quad (1)$$

2.3.3. Precision

Method precision was assessed as repeatability by extracting and quantifying PACs in the QPH122MS CRM fortified at 100 ng. Intraday precision was evaluated using eight replicate extractions performed within a 24 h period, while interday precision was determined using four replicate extractions conducted over three consecutive days. HorRat values were calculated using Equation (2), where an RSD_r represents the experimentally determined relative standard deviation of repeatability and PRSD_R corresponds to the predicted relative standard deviation derived from the Horwitz equation [44].

$$\text{HorRat} = \frac{\text{RSD}_r}{\text{PRSD}_R} \quad (2)$$

2.3.4. Ruggedness

Method ruggedness was evaluated by deliberately modifying selected extraction parameters, including solvent composition (DCM:EtOAc 70:30, v/v; $n = 4$), number of extraction cycles (two cycles; $n = 4$), and number of rinse steps (single rinse; $n = 4$). Paired, two-tailed Student's t -tests ($\alpha = 0.05$) were applied to assess the statistical significance of observed differences. Both CRMs were also extracted using a previously published PFE method [27], and results from the PFE and MBE methods were compared using Tukey's test and F-test ($\alpha = 0.05$).

2.3.5. Measurement Uncertainty

Combined measurement uncertainty (MU) was estimated by performing triplicate extractions of the QPH122MS CRM fortified with 100 ng of target analytes over two consecutive days. The uncertainty evaluation incorporated contributions from intermediate precision and analytical bias. Full details of the MU calculation approach are described elsewhere [45].

The combined method uncertainty (U_C) was calculated by combining the uncertainty associated with method bias (U_{bias}) and the uncertainty arising from intermediate precision (U_{IP}) according to Equation (3).

$$U_C = \sqrt{U_{bias}^2 + U_{IP}^2} \quad (3)$$

The uncertainty associated with intermediate precision (U_{IP}) was estimated using analysis of variance (ANOVA) [46]. Intermediate precision was determined by combining the uncertainty due to repeatability (U_r) and the between-day variability, expressed as the between-group uncertainty (U_{BG}), as shown in Equation (4).

$$U_{IP} = \sqrt{U_r^2 + U_{BG}^2} \quad (4)$$

The uncertainty due to repeatability (U_r) was estimated as the square root of the mean square error (MSE) obtained from the ANOVA:

$$U_r = \sqrt{MSE} \quad (5)$$

The between-group uncertainty was calculated using Equation (6), where MSG is the mean square of groups and n is the number of replicate measurements performed on a single day.

$$U_{BG} = \sqrt{(MSG - MSE)/n} \quad (6)$$

The uncertainty associated with bias (U_{bias}) was calculated by combining the uncertainty of the laboratory bias estimate ($U_r/\sqrt{n}$) and the uncertainty of the certified reference material (U_{CRM}), as shown in Equation (7) [42], where n is the number of replicate measurements performed on a single day.

$$U_{bias} = \sqrt{U_{CRM}^2 + \frac{U_r^2}{n}} \quad (7)$$

2.4. Gas Chromatography Tandem Mass Spectrometry Conditions

Analyses were performed using an Agilent 7890 gas chromatograph coupled to an Agilent 7000C triple quadrupole mass spectrometer (Agilent Technologies, Inc, Santa Clara, CA, USA) equipped with an electron ionization (EI) source. Separations were achieved using an Agilent J&W HP-5ms Ultra Inert column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m), with helium as the carrier gas at a constant flow rate of 1.2 mL min^{−1}. A 1 μ L aliquot of extract was injected in splitless mode at 250 °C using a PAL RSI 85 autosampler.

Compound-specific oven temperature programs were applied. For PAHs, APAHs, HPACs, and HHPACs, the oven was held at 60 °C for 1 min, ramped to 120 °C at 35 °C min^{−1}, increased to 220 °C at 14 °C min^{−1}, raised to 260 °C at 3 °C min^{−1} and held for 5 min, then increased to 300 °C at 10 °C min^{−1}, and finally to 310 °C at 50 °C min^{−1}. For HPAHs, the oven program consisted of an initial hold at 60 °C for 1 min, followed by ramps to 210 °C at 35 °C min^{−1}, 260 °C at 2 °C min^{−1}, and 300 °C at 10 °C min^{−1} with a 5 min hold, before a final increase to 325 °C at 50 °C min^{−1} with a 5.5 min hold. The transfer line

and ion source temperatures were both maintained at 320 °C. Ultra-high-purity nitrogen was used as the collision gas at 60 psi. Quantification and confirmation ions, as well as multiple reaction monitoring (MRM) transitions, are reported elsewhere [27,28,45].

3. Results and Discussion

The overall performance of the MBE method was assessed by intentionally fortifying the QPH122MS CRM with 117 PACs at three levels and extracting them using MBE. The results are shown in Table A2. It should be stated that the certified concentrations of the analytes in the unspiked material (presented in Table 1) ranged from 1.8 to 14.9 ng g⁻¹. We subtracted the measured ion signals of each analyte in the spiked material from the certified values to obtain meaningful estimates of the performance of the MBE method. As emphasized earlier, employing a certified sediment reference material was important for these studies, as opposed to surrogates like diatomaceous earth or silica sand, since the resulting extract more closely represents that of an authentic environmental sediment sample.

With the exception of 6-n-Propyl-Chr, LODs/LOQs for all 117 of our target analytes were all $\leq 6.5/21$ ng g⁻¹ and ranged from 0.3/1.1 ng g⁻¹ (Benzo[*b*]naphtho[2,3-*d*]furan) to 4.0/13.4 ng g⁻¹ (BgHiP). Group-specific LODs/LOQs ranges were as follows: PAHs, 0.6/1.9 ng g⁻¹ (Ant) to 4.0/13.4 ng g⁻¹ (BgHiP); APAHs, 0.4/1.5 ng g⁻¹ (1,2,6,9-Tetramethyl-Phe) to 0.1/20.2 ng g⁻¹ (6-n-Propyl-Chr); HPAHs, 0.5/1.6 ng g⁻¹ (2,7-Br₂-Phe) to 2.0/6.5 ng g⁻¹ (2-Br-Fle); HPACs, 0.3/1.1 ng g⁻¹ (Benzo[*b*]naphtho[2,3-*d*]furan) to 2.4/8.1 ng g⁻¹ (Dibenz[*c,h*]acridine); and HHPACs, 0.5/1.8 ng g⁻¹ (2-Br-quinoline) to 1.9/6.2 ng g⁻¹ (3,6-Br₂-carbazole).

The trueness of the extraction method was assessed by comparing measured concentrations of PACs in fortified QPH122MS CRM samples with their nominal spike levels (10, 100 and 500 ng g⁻¹, *n* = 8 in each case). For PAHs at the 10 ng g⁻¹ spiking level, measured values fell within the criteria set by Association of Official Agricultural Chemists (AOAC) of 60–115% [44], except for Ant (57.1%). At the 100 and 500 ng g⁻¹ levels, all PAHs recoveries met AOAC criteria. For APAHs, recoveries ranged from 81.5 to 130.8%, 86.5 to 129.1%, and 84.4 to 153.0% at the three respective fortification levels. Similarly, HPAH recoveries ranged from 57.8 to 134.6%, 68.2 to 116.4%, and 60.7 to 131.8%, while HPAC recoveries ranged from 44.9 to 114.2%, 45.3 to 121.6%, and 48.1 to 164.3%. For HHPACs, recoveries ranged from 60.7 to 115.9%, 70.0 to 120.5%, and 75.2 to 138.7% at the 10, 100, and 500 ng g⁻¹ levels, respectively. Deviations from AOAC recovery guidelines were most pronounced at the lowest fortification level and for structurally complex or highly substituted PACs, reflecting increased analytical variability at concentrations approaching the method detection limits and the inherent heterogeneity of the sediment matrix. Importantly, recoveries at medium and high fortification levels were consistently within or close to recommended acceptance ranges, indicating that matrix effects were minimal under typical analytical conditions.

Table 1. Trueness, precision and z-scores for analysis for selected PACs in QPH121MS and -122MS ($n = 8$ in both cases) standard reference materials.

Analyte	QPH121MS						QPH122MS					
	Certified Reference Value (ng g ⁻¹) ^a	Uncertainty ^a	Measured Concentration (ng g ⁻¹)	Trueness (%)	Precision (RSD %)	z-Score	Certified Reference Value (ng/g) ^a	Uncertainty ^a	Measured Concentration (ng g ⁻¹)	Trueness (%)	Precision (RSD %)	z-Score
Acenaphthene	31.3	8.61	14.3	45.6	11.3	−2.0	0.9	0.3	0.43	48.2	20.3	−1.6
Acenaphthylene	26.1	7.75	31.4	120.3	5.6	0.7	n/a	n/a	0.50	n/a	n/a	n/a
Anthracene	110	26.2	73.0	66.4	5.9	−1.4	1.8	0.6	1.56	88.0	4.4	−0.4
Benz(a)anthracene	201	42	183.7	91.4	5.7	−0.4	5.0	1.6	3.72	73.8	19.1	−0.8
Benzo(a)pyrene	202	42.4	199.4	98.7	6.5	−0.1	4.4	1.4	2.86	65.1	14.9	−1.1
Benzo(b)fluoranthene	223	62.8	206.7	92.7	8.7	−0.3	9.7	3.2	13.49	138.8	26.4	1.2
Benzo(ghi)perylene	199	48.6	196.9	99.0	2.5	0.0	6.0	1.6	4.56	75.7	9.7	−0.9
Benzo(k)fluoranthene	100	24.4	97.1	97.1	8.0	−0.1	4.3	1.3	3.10	72.5	28.8	−0.9
Chrysene	233	46.6	168.8	72.4	5.2	−1.4	5.8	1.9	6.00	102.7	13.6	0.1
Dibenz(a,h)anthracene	33	8.93	49.6	150.4	4.9	1.9	1.4	0.4	1.50	108.6	23.0	0.3
Fluoranthene	399	83.1	346.6	86.9	8.4	−0.6	14.9	3.3	10.06	67.5	13.4	−1.5
Fluorene	50.5	13.5	31.9	63.1	14.6	−1.4	1.5	0.5	1.15	77.4	47.7	−0.7
Indeno(1,2,3-c,d)pyrene	156	40.8	155.5	99.7	2.9	0.0	6.9	1.8	5.85	84.7	8.0	−0.6
Naphthalene	208	56.3	87.0	41.8	21.8	−2.2	4.8	1.6	2.69	55.6	34.8	−1.4
Phenanthrene	371	78	225.1	60.7	8.7	−1.9	9.9	2.3	6.97	70.8	10.7	−1.3
Pyrene	430	77.4	353.9	82.3	9.6	−1.0	10.1	2.8	7.13	70.5	17.6	−1.1
Chysene+Triphenylene	233	46.6	242.6	104.1	3.9	0.2	6.6	1.3	7.22	109.5	13.0	0.5
C1-Nap	534	107	222.4	41.6	18.2	−2.9	5.8	0.9	3.81	65.5	13.3	−2.3
C1-Phe+Ant	569	114	281.7	49.5	10.6	−2.5	n/a ^b	n/a ^b	n/a	n/a	n/a	n/a
2-Me-Phe	132	26.5	69.4	52.6	9.8	−2.4	n/a ^b	n/a ^b	n/a	n/a	n/a	n/a

^a Numbers in bold are certified values as provided by the proficiency testing organizer, while the rest are referenced values. ^b No assigned value offered by the CRM provider.

The precision of the MBE method, expressed as a relative standard deviation (RSD), was evaluated using eight replicate extractions over 24 h (intraday) and 12 replicate extractions over 3 consecutive days (interday) of the QPH122MS CRM spiked at three fortification levels. For PAHs, intraday RSDs ranged from 4.6% (Chr) to 24.4% (BghiP), while interday values ranged from 11.4% (Ace) to 29.9% (Pyr). Only two PAHs at a 10 ng g^{-1} spike level exceeded the AOAC precision criteria of 22% [44], namely BghiP (24.4%) and DahA (28.2%). Intraday RSDs for the other two spiking levels of PAHs were consistently <7%. For HPAHs, intraday precisions were <10% across analytes, while interday values ranged from 9.0% (2,7-Cl₂-Fle) to 52.1% (2,3,9,10-Br₄-Ant). For APAHs, most intraday RSDs were <20%, with the exceptions of 6-n-Propyl-Chr (36.3%), 5-Methyl-Chr (26.1%) and 9-Ethyl-Fle (20.3%). Irrespective of spiking level, the intraday precision for HPACs and HHPACs were <20% for all of our target analytes. Collectively, these results indicate that while increased variability is observed for certain analytes at low spiking levels, the overall precision of the MBE method is consistent with accepted performance expectations for complex sediment matrices.

According to the AOAC, acceptable HorRat(r) values are between 0.3 and 1.30 [44]. As shown in Table A2, only four of our target analytes fell outside this acceptable range, with all the PAHs and HHPACs meeting the criterion. The specific analytes that failed to meet the criterion were 9-Ethyl-Fle (2.09), 6-methyl-BaP (1.56), 9-Cl-Fle (1.57) and dibenz[*c,h*]acridine (1.47).

The method's ruggedness was tested by deliberately changing key parameters of method and comparing the recoveries of the analytes to the standard method. Reducing a rinse cycle or adding an extraction cycle resulted in a statistically significant difference (Student *t*-test, $p < 0.05$) in fewer than 10% and 25% of analytes, respectively. However, a solvent change to DCM:EtOAc (70:30, *v/v*) had a more substantial impact, causing a significant statistical difference (Student *t*-test, $p < 0.05$) in 30 of the analytes.

We then performed a side-by-side comparison of the MBE method with a previously validated PFE method for PAC analysis [27]. The results shown in Figures 1 and 2 are based on triplicate analyses of both CRM materials. For the higher concentrated QPH121MS sample, the MBE method performed well, with 13 out of 17 certified analytes showing no statistical difference ($p < 0.05$) in recovery when compared to the ASE method. The MBE method also performed comparably to the ASE method for the lower-concentration unspiked QPH122MS sediment sample, with no statistical difference ($p < 0.05$) for more than 80% of the certified analytes.

To assess the fitness for purpose of the MBE method, we analyzed the two CRMs that contained substantially different analyte concentrations. The z-score, a widely accepted metric for evaluating the quality of analytical data, was used to compare our measured values to the certified values. By definition, a z-score shows how close measured values agree with the assigned values. According to the standard criteria, a z-score of $|z| \leq 2.0$ indicates satisfactory performance, while a value of $|z| \geq 3.0$ suggests unsatisfactory performance. Intermediate z-scores of $2.0 < |z| < 3.0$ imply a questionable performance [43]. In general, the z-scores for both sediment CRMs are similar for all the compounds. For the higher-concentration CRM, QPH121MS, all 17 certified compounds had z-scores smaller than 3, and 82% of these showed satisfactory performance. Similarly, for the lower-concentration CRM, QPH122MS, all analytes except C1-Nap had satisfactory z-scores. Overall, the consistent and favorable z-scores for both CRMs demonstrate that the MBE method is reliable for its intended purpose over a wide range of analyte concentrations.

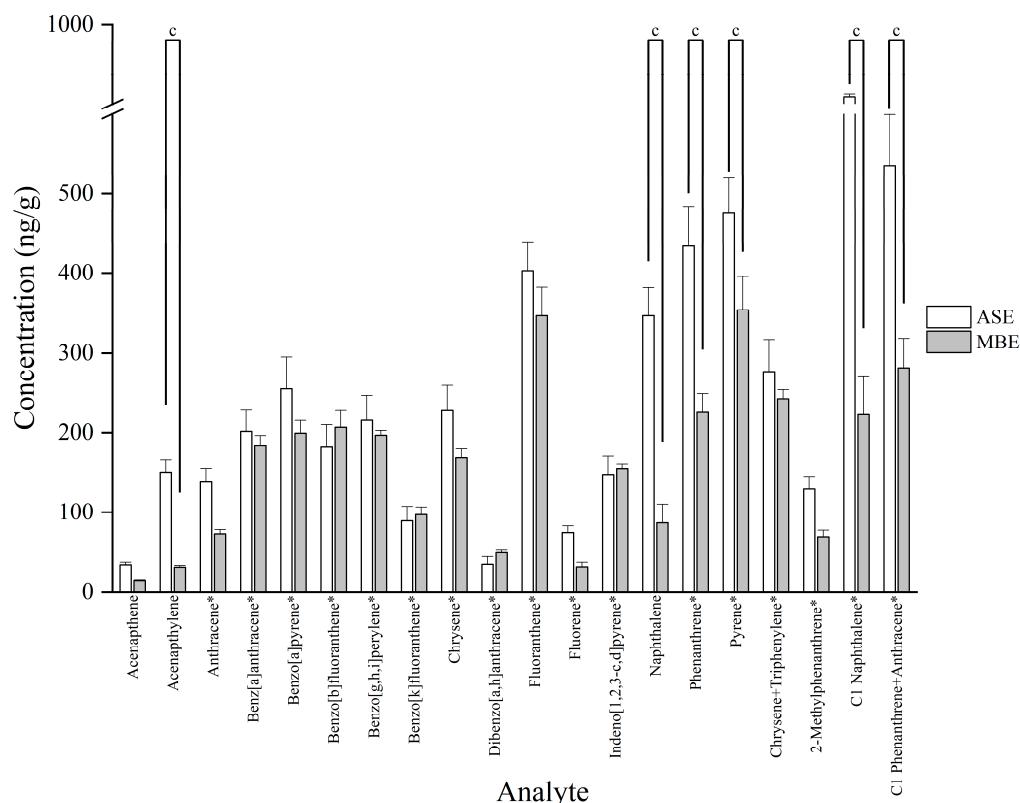


Figure 1. Results of Tukey's test for QPH121MS (c: $p \leq 0.001$, certified analytes were denoted by *).

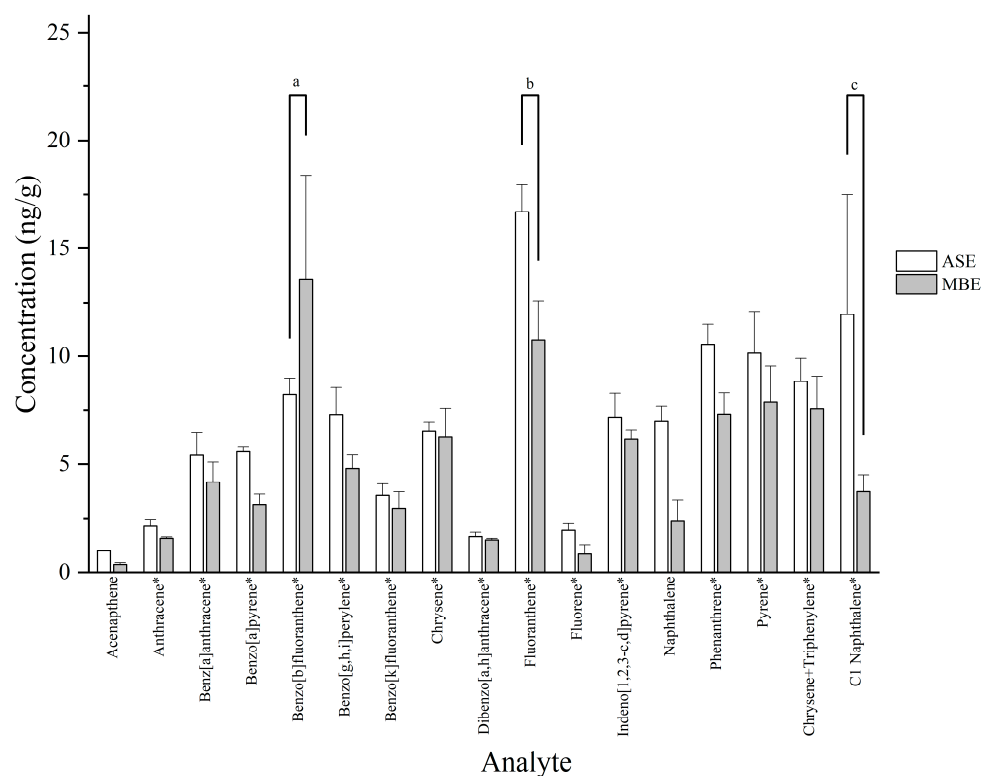


Figure 2. Results of Tukey's test for QPH122MS (a: $p \leq 0.05$, b: $p \leq 0.01$, c: $p \leq 0.001$, certified analytes were denoted by *).

The comparison between the combined measurement uncertainties derived from the MBE method and the certified uncertainties reported for each analyte in the CRM demonstrated no statistical differences (see Table 2). This implies that our combined

uncertainties, which encompasses the total variability in the MBE method, including sample preparation, extraction efficiency, instrumental analysis and data processing, fall within the range established for the CRM. Specifically, the MBE method does not introduce additional sources of uncertainty beyond those already accounted for in the CRM certification process. This provides strong evidence that the precision of the MBE approach is consistent with established reference standards, further supporting its applicability for routine application in sediment analysis.

Table 2. Combined measurement uncertainty (MU) of the microbead beating extraction method for selected PACs in the fortified QPH122MS ($n = 3$) standard reference material.

Analyte	Grand Mean QPH122MS (ng g ⁻¹)	U _{IP} ^a	Certified QPH122MS Value (ng g ⁻¹)	Sd of Certified QPH122MS	Bias ^b	U _{bias} ^c	95% Confidence Interval for Bias ^d		Statistical Significance	Practically Acceptable (Y/N)	Combined MU QPH122MS ^e
							Lower Limit	Upper Limit			
Acenaphthene	0.359	0.211	0.889	0.289	−0.530	0.304	−1.138	0.078	No	No	0.370
Acenaphthylene	0.500	0.131	n/a	n/a	0.500	0.058	0.383	0.616	Yes	No	0.143
Anthracene	1.557	0.079	1.770	0.574	−0.213	0.575	−1.363	0.937	No	Yes	0.580
Benz(a)anthracene	3.721	0.862	5.040	1.640	−1.319	1.685	−4.688	2.050	No	Yes	1.892
Benzo(a)pyrene	2.863	0.523	4.400	1.430	−1.537	1.449	−4.435	1.361	No	Yes	1.541
Benzo(b)fluoranthene	13.488	14.041	9.720	3.160	3.768	7.029	−10.291	17.827	No	Yes	15.702
Benzo(ghi)perylene	4.564	0.518	6.030	1.600	−1.466	1.617	−4.700	1.767	No	Yes	1.698
Benzo(k)fluoranthene	3.102	1.073	4.280	1.280	−1.178	1.367	−3.913	1.556	No	Yes	1.738
Chrysene	5.999	0.955	5.840	1.900	0.159	1.947	−3.736	4.054	No	Yes	2.169
Dibenz(a,h)anthracene	1.499	0.417	1.380	0.445	0.119	0.482	−0.846	1.083	No	Yes	0.637
Fluoranthene	10.055	1.504	14.900	3.290	−4.845	3.358	−11.561	1.871	No	Yes	3.679
Fluorene	1.153	0.632	1.490	0.483	−0.337	0.560	−1.456	0.782	No	Yes	0.844
Indeno(1,2,3-c,d)pyrene	5.851	0.559	6.910	1.770	−1.059	1.788	−4.634	2.516	No	Yes	1.873
Naphthalene	2.689	1.048	4.840	1.570	−2.151	1.638	−5.427	1.126	No	Yes	1.945
Phenanthrene	6.973	7.026	9.850	2.260	−2.877	3.871	−10.618	4.864	No	Yes	8.022
Pyrene	7.125	1.447	10.100	2.780	−2.975	2.854	−8.683	2.734	No	Yes	3.200
Chrysene+Triphenylene	7.219	1.112	6.59	1.32	0.629	1.411	−2.192	3.450	No	Yes	1.796
C1-Nap	3.812	0.622	5.820	0.873	−2.008	0.916	−3.840	−0.176	Yes	Yes	1.107

^a The uncertainty from intermediate precision was estimated using the one-way ANOVA table. ^b Bias was calculated as the difference between the laboratory obtained value for the QPH122MS and the certified QPH122MS value. ^c The uncertainty of the bias was determined by combining the uncertainty from the laboratory estimate of the bias and the uncertainty from the certified reference. ^d The 95% confidence interval for the bias includes lower and upper limits, which were estimated as Bias $\pm 2 \times U_{bias}$. ^e The uncertainty listed with each compound for QPH122MS is the expanded uncertainty about the mean, with a coverage factor of 2. The combined uncertainty includes uncertainty of bias and intermediate precision.

While the present study employed marine sediment certified reference materials for validation, the MBE method relies on a general extraction mechanism involving mechanical disruption of the sediment matrix and solvent-assisted desorption, which is not inherently limited to a specific matrix type. Consequently, the approach is expected to be applicable to other fine-grained sediment types, including freshwater sediments. Nevertheless, matrices with substantially different characteristics, such as high organic matter content or coarse grain size, may require further optimization and validation prior to routine application.

Reproducibility and method transferability are supported using standardized, commercially available homogenization equipment and extraction materials. Although solvent composition was identified as a sensitive parameter during ruggedness testing, consistent performance was achieved when the optimized solvent ratio was maintained, indicating that adherence to defined method conditions enables reliable inter-laboratory implementation.

4. Conclusions

In conclusion, the validation results demonstrate that the MBE method is a suitable and efficient technique for extracting PACs from sediments. Its method performance characteristics consistently met or exceeded accepted criteria, and it provides significant advantages over conventional extraction techniques by requiring substantially less solvent and offering markedly shorter extraction times. The incorporation of dispersant within the MBE tubes enables in situ clean-up, further simplifying and streamlining the overall

sample preparation workflow. In comparison with conventional extraction and clean-up methods such as Soxhlet extraction (24–48 h per sample) or PFE (~30 min per sample), the MBE approach required substantially less time, completing the extraction in under 15 min. From an operational perspective, implementation of the MBE method in routine environmental monitoring laboratories does not necessitate additional quality control procedures beyond those commonly applied in PAC analysis. The use of isotopically labelled internal standards, procedural blanks, and certified reference materials remains sufficient to ensure data quality. Additionally, this work could certainly be adapted for the extraction of other persistent organic pollutants in sediments. Ultimately, the MBE technique will enhance the capabilities of environmental laboratories to generate faster, more reliable data while minimizing their environmental impact and maintaining good data quality objectives.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. List of HPAHs.

IUPAC Name	Acronym	CAS Number ^a	Source	Address
1,2-dibromoacenaphthylene	1,2-Br ₂ -Any	13019-33-5	Sigma Aldrich	St Louis, MO, USA
1,4-dichloroanthracene	1,4-Cl ₂ -Ant	66259-12-9	Sigma Aldrich	St Louis, MO, USA
1,5,9,10-tetrachloroanthracene	1,5,9,10-Cl ₄ -Ant	82843-47-8	Sigma Aldrich	St Louis, MO, USA
1,6-dibromopyrene	1,6-Br ₂ -Pyr	27973-29-1	Matrix Scientific	Columbia, SC, USA
1-bromoanthracene	1-Br-Ant	7397-92-4	Tokyo Chemical Industries	Tokyo, Japan
1-chloroanthracene	1-Cl-Ant	4985-70-0	Sigma Aldrich	St Louis, MO, USA
2,3,9,10-tetrabromoanthracene	2,3,9,10-Br ₄ -Ant	82843-47-8	Sigma Aldrich	St Louis, MO, USA
2,7-dibromofluorene	2,7-Br ₂ -Fle	16433-88-8	Sigma Aldrich	St Louis, MO, USA
2,7-dibromophenanthrene	2,7-Br ₂ -Phe	62325-30-8	Tokyo Chemical Industries	Tokyo, Japan
2,7-dichlorofluorene	2,7-Cl ₂ -Fle	7012-16-0	Toronto Research Chemicals	Toronto, ON, Canada
2-bromofluorene	2-Br-Fle	1133-80-8	Sigma Aldrich	St Louis, MO, USA
2-chlorofluorene	2-Cl-Fle	2523-44-6	Matrix Scientific	Columbia, SC, USA
3-bromofluoranthene	3-Br-Flu	13438-50-1	Tokyo Chemical Industries	Tokyo, Japan
3-bromophenanthrene	3-Br-Phe	715-50-4	Sigma Aldrich	St Louis, MO, USA
4-bromobenz[<i>a</i>]anthracene	4-Br-BaA	61921-39-9	Tokyo Chemical Industries	Tokyo, Japan
4-bromopyrene	4-Br-Pyr	1732-26-9	Tokyo Chemical Industries	Tokyo, Japan
5,6-dibromo-1,2-dihydroacenaphthylene	5,6-Br ₂ -Ana	19190-91-1	Matrix Scientific	Columbia, SC, USA
5-bromoacenaphthene	5-Br-Ana	2051-98-1	Sigma Aldrich	St Louis, MO, USA
9-bromo-1,5-dichloroanthracene	9-Br-1,5-Cl ₂ -Ant	201406-34-0	Sigma Aldrich	St Louis, MO, USA
9-chlorofluorene	9-Cl-Fle	6630-65-5	Matrix Scientific	Columbia, SC, USA

^a CAS number = Chemical Abstracts Service registry number.

Table A2. Method performance characteristics of the MBE method for the analysis of PACs determined by fortifying the QPH122MS standard reference material at three levels ($n = 8$ at each fortification level: 10, 100, and 500 ng g^{−1}).

Compound	Calibration Solution Level						Interday Precision (RSD %)	LOD (ng/g)	LOQ (ng/g)	HorRat
	Low		Medium		High					
	Trueness (%)	Precision (RSD %)	Trueness (%)	Precision (RSD %)	Trueness (%)	Precision (RSD %)				
PAHs ^a										
Nap	101.1	8.0	103.3	2.8	109.7	1.0	13.7	1.3	4.4	0.59
Ace	91.2	6.3	102.5	2.6	109.1	2.6	11.4	0.9	3.1	0.51
Acy	98.1	8.5	103.9	4.4	105.4	1.8	21.7	1.3	4.5	0.98
Fle	100.8	11.7	104.1	3.8	112.6	1.9	16.0	1.9	6.3	0.64
Phe	90.2	11.4	103.2	3.7	114.5	4.6	14.0	1.7	5.6	0.59
Ant	57.1	6.1	96.2	6.7	113.3	3.7	25.8	0.6	1.9	1.14
Flu	87.2	9.1	96.4	3.1	112.2	2.4	11.8	1.3	4.3	0.40
Pyr	62.1	20.4	104.4	5.3	111.0	4.7	29.9	2.0	6.8	1.25
BaA	97.2	7.3	109.7	6.4	113.2	6.0	24.1	1.2	3.9	0.94
Chr	82.0	4.6	96.9	3.5	120.9	3.0	21.2	0.6	2.1	0.62
BbF	94.2	16.8	95.6	2.3	92.8	2.1	20.4	2.6	8.5	0.82
BkF	108.7	7.2	110.2	2.5	117.1	1.5	18.8	1.3	4.2	0.46
BaP	96.5	15.5	103.3	2.5	111.9	2.3	15.1	2.4	8.1	0.55
Ind	87.3	9.4	106.2	4.1	109.6	3.9	13.3	1.3	4.4	0.51
DahA	102.7	24.0	104.6	3.4	111.1	2.1	15.1	4.0	13.3	0.64
BghiP	101.4	24.4	106.7	2.7	111.0	2.4	20.1	4.0	13.4	0.78
APAHs ^a										
1,7-Dimethyl-Phe	117.1	10.6	104.5	3.1	98.4	2.3	10.0	2.0	6.7	0.43
1,8-Dimethyl-Phe	96.9	3.6	97.3	2.4	95.6	2.2	9.4	0.6	1.9	0.37
1-Methyl-Nap	101.7	3.9	100.1	4.4	111.2	3.8	13.3	0.6	2.1	0.59
1-Methyl-Phe	109.6	6.8	91.4	6.8	111.0	3.9	39.9	1.2	4.0	1.17
2,6-Dimethyl-Phe	103.3	7.6	102.8	2.9	95.7	2.1	12.8	1.3	4.3	0.50
2-Methyl-Nap	118.3	4.0	108.7	4.9	123.7	4.2	14.5	0.8	2.6	0.61
2-Methyl-Phe	116.6	7.2	103.3	8.7	108.7	4.8	23.2	1.4	4.5	0.92
3,6-Dimethyl-Phe	98.1	3.9	100.4	3.4	98.6	2.6	15.2	0.6	2.0	0.44
3-Methyl-Phe	109.9	6.7	107.7	5.1	123.2	4.9	17.3	1.2	4.0	0.76
9-Methyl-Phe	100.9	6.1	102.8	6.6	118.0	3.1	20.6	1.0	3.3	0.89
5-Methyl-Chr	87.4	26.1	113.9	4.8	125.8	5.9	22.8	3.7	12.3	0.92
6-Ethyl-Chr	91.3	6.7	106.7	6.0	111.6	6.7	23.9	1.0	3.3	0.90
1,4-Dimethyl-Nap	106.6	3.4	106.9	2.7	113.8	3.3	10.9	0.6	1.9	0.45
1,3-Dimethyl-Phe	111.8	7.2	100.8	4.9	96.7	2.4	13.8	1.3	4.4	0.59
6-n-Propyl-Chr	103.0	36.3	126.4	5.4	116.4	3.6	31.5	6.1	20.2	1.09
2,3,5-Trimethyl-Nap	106.4	5.1	107.6	6.5	117.6	5.6	16.9	0.9	2.9	0.76
1,2,6-Trimethyl-Phe	108.4	6.7	111.1	5.2	123.9	4.2	15.6	1.2	3.9	0.55
6-n-Butylchrysene	81.5	19.6	95.8	1.9	90.8	3.0	22.8	2.6	8.6	0.86
1,4,6,7-Tetramethyl-Nap	97.9	4.9	97.5	3.2	101.0	3.7	11.9	0.8	2.6	0.48
1,2,6,9-Tetramethyl-Phe	107.5	2.5	116.5	3.3	124.0	4.0	19.0	0.4	1.5	0.73
Retene	106.2	5.3	109.5	5.1	113.7	4.0	13.3	0.9	3.1	0.58
6-Methyl-BaP	104.5	6.4	92.1	7.9	124.1	4.7	62.5	1.1	3.6	1.56
1-Methyl-Fle	105.9	8.0	114.1	7.7	126.9	4.0	23.4	1.4	4.6	0.99
1-Methyl-Pyr	112.4	5.5	113.9	2.2	129.0	3.7	14.0	1.0	3.4	0.58
7,10-Dimethyl-BaP	106.2	10.9	95.4	6.1	114.1	4.9	29.1	1.9	6.3	1.07
9-Ethyl-Fle	111.1	20.3	86.5	12.4	84.4	8.2	49.2	3.7	12.2	2.09
1-Ethyl-Pyr	103.8	10.7	98.4	6.5	100.6	3.7	15.3	1.8	6.0	0.63
9-n-Propyl-Fle	93.5	8.1	96.0	2.9	102.6	1.5	10.2	1.2	4.1	0.22
1-n-Propyl-Pyr	128.4	7.5	125.6	3.9	146.6	4.1	27.0	1.6	5.2	0.93
9-n-Butyl-Fle	101.5	3.8	105.6	4.3	110.9	2.7	11.4	0.6	2.1	0.51
1-n-Butyl-Pyr	130.8	8.8	129.1	3.9	153.0	4.8	21.5	1.9	6.2	0.68
HPAHs ^a										
1,2-Br ₂ -Any	72.9	7.2	94.5	3.7	91.1	4.8	42.2	0.9	2.8	0.46
1,4-Cl ₂ -Ant	102.2	6.6	107.5	3.0	119.0	3.5	13.3	1.1	3.6	0.56
1,5,9,10-Cl ₄ -Ant	91.8	7.5	94.8	2.5	97.0	2.1	26.7	1.1	3.7	1.15
1,6-Br ₂ -Pyr	95.0	4.8	108.8	1.9	98.6	1.4	28.8	0.7	2.5	0.64
1-Br-Ant	96.9	5.7	105.0	1.4	109.5	2.6	12.1	0.9	3.0	0.54
1-Cl-Ant	94.6	4.4	100.5	3.6	93.9	2.1	12.6	0.7	2.3	0.51
2,3,9,10-Br ₄ -Ant	62.8	7.1	68.2	7.5	60.7	8.5	52.1	0.7	2.4	0.85
2,7-Br ₂ -Fle	99.2	8.1	104.8	5.3	121.4	3.3	23.6	1.3	4.4	0.78
2,7-Br ₂ -Phe	89.8	3.2	95.9	2.1	91.4	2.0	26.2	0.5	1.6	0.48
2,7-Cl ₂ -Fle	101.3	7.5	101.3	1.2	100.9	2.5	9.0	1.2	4.1	0.32
2-Br-Fle	134.6	9.0	106.4	2.5	112.2	3.2	14.0	2.0	6.5	0.57
2-Cl-Fle	94.2	5.0	99.7	4.1	104.8	2.9	13.5	0.8	2.6	0.45
3-Br-Flu	91.4	4.5	102.4	3.9	95.5	2.6	19.6	0.7	2.2	0.57
3-Br-Phe	95.0	6.2	102.4	3.0	99.1	3.7	13.5	1.0	3.2	0.57
4-Br-BaA	96.8	9.4	108.4	5.6	118.3	4.9	16.8	1.5	4.9	0.63
4-Br-Pyr	90.9	6.8	108.0	3.5	109.4	2.6	14.0	1.0	3.3	0.39
5,6-Br ₂ -Ana	95.9	6.1	99.7	4.8	119.1	3.5	25.0	1.0	3.2	0.87
5-Br-Ana	69.6	5.6	70.4	4.4	72.6	3.1	10.2	0.6	2.1	0.40
9-Br-1,5-Cl ₂ -Ant	57.8	5.1	75.4	5.2	91.3	3.2	30.8	0.5	1.6	0.68
9-Cl-Fle	105.2	7.3	116.4	5.5	131.8	3.8	36.2	1.2	4.1	1.57

Table A2. Cont.

Compound	Calibration Solution Level						Interday Precision (RSD %)	LOD (ng/g)	LOQ (ng/g)	HorRat
	Low		Medium		High					
	Trueness (%)	Precision (RSD %)	Trueness (%)	Precision (RSD %)	Trueness (%)	Precision (RSD %)				
HPACs ^a										
Quinoline	72.0	3.8	64.8	8.9	79.9	5.6	21.1	0.4	1.5	0.37
Isoquinoline	53.3	16.4	45.3	11.3	68.1	8.2	30.7	1.4	4.7	0.57
Indole	84.6	7.2	98.9	6.4	111.8	3.9	21.6	1.0	3.3	0.61
2-Methylquinoline	78.1	5.1	87.7	6.8	104.5	7.1	41.9	0.6	2.1	0.78
4-Methylbenzothiophene	90.7	4.2	95.2	6.0	100.0	2.9	21.6	0.6	2.0	0.79
3-Methylisoquinoline	47.5	9.4	50.1	9.1	68.4	7.0	13.5	0.7	2.4	0.61
2,3-Dimethylbenzothiophene	95.0	10.1	99.9	5.9	108.9	3.2	18.1	1.6	5.2	0.74
4-Phenylpyridine	62.5	13.4	53.5	9.1	82.4	9.0	33.6	1.4	4.5	0.73
Dibenzofuran	114.2	7.8	95.3	5.9	109.1	2.8	18.5	1.4	4.8	0.67
2-Methyldibenzofuran	97.7	3.0	93.2	4.1	96.8	4.9	11.9	0.5	1.6	0.53
Benzo[<i>h</i>]quinoline	106.2	3.8	104.8	4.0	113.3	3.6	8.6	0.7	2.2	0.34
Acridine	44.9	7.9	46.0	4.1	48.1	4.2	6.7	0.6	1.9	0.28
9-Methylcarbazole	95.3	5.6	101.0	1.9	109.4	4.0	8.4	0.9	2.9	0.28
Carbazole	109.5	10.7	107.0	3.2	114.2	5.7	13.8	1.9	6.3	0.57
3-Methylbenzo[<i>f</i>]quinoline	73.1	11.3	70.1	7.5	82.8	3.4	19.3	1.3	4.5	0.75
2-Methylacridine	101.1	6.8	100.0	7.1	134.8	3.8	21.5	1.1	3.7	0.68
3,6-Dimethylcarbazole	102.3	8.8	106.3	5.2	96.2	4.9	18.2	1.5	4.9	0.77
Benzo[<i>b</i>]naphtho[2,3- <i>d</i>]furan	94.2	2.1	95.9	4.2	90.6	4.2	15.7	0.3	1.1	0.71
3-Tert-butyl-9H-carbazole	60.2	9.7	100.6	6.5	107.5	5.3	22.2	1.0	3.2	0.79
9-Phenylcarbazole	111.9	6.7	116.2	8.1	114.6	4.0	17.9	1.2	4.0	0.68
7,8,9,10-Tetrahydro-benzo[<i>b</i>]naphtho[2,3- <i>d</i>]thiophene	102.8	5.9	109.8	5.7	112.8	5.4	18.0	1.0	3.3	0.72
Benz[<i>c</i>]acridine	105.0	3.3	110.0	6.6	124.2	5.5	20.0	0.6	1.9	0.76
11H-Benzo[<i>a</i>]carbazole	100.4	5.4	95.0	3.4	94.1	4.3	11.9	0.9	2.9	0.40
7H-Benzo[<i>c</i>]carbazole	98.2	14.0	94.6	4.9	91.8	5.5	16.8	2.2	7.4	0.64
6-Methylbenzo[<i>b</i>]naphtho[2,1- <i>d</i>]thiophene	91.8	2.1	90.4	5.6	90.9	6.8	10.1	0.3	1.0	0.36
Dinaphtho[1,2- <i>b</i> ;1',2'- <i>d</i>]furan	85.7	11.3	95.7	8.4	91.7	5.9	24.6	1.6	5.2	0.76
Dibenz[<i>c,h</i>]acridine	76.0	19.8	96.7	11.8	109.0	4.6	36.0	2.4	8.1	1.47
13(H)-Dibenzo[<i>a,i</i>]carbazole	66.5	11.1	115.2	4.2	164.3	4.8	59.8	1.2	4.0	0.87
Benzo[<i>b</i>]naphtho[1,2- <i>d</i>]thiophene	106.4	9.5	118.6	7.3	127.3	4.6	26.0	1.6	5.5	1.17
Benzo[<i>b</i>]naphtho[2,3- <i>d</i>]thiophene	111.9	8.8	121.6	6.0	138.7	5.6	27.8	1.6	5.3	1.09
Dibenzothiophene	102.8	6.1	107.8	1.9	112.7	1.5	15.7	1.0	3.4	0.59
2-Methyldibenzothiophene	104.6	3.7	110.1	3.3	116.8	2.5	10.5	0.6	2.1	0.42
1,2-Dimethyldibenzothiophene	110.4	3.9	114.2	3.5	120.3	2.1	8.4	0.7	2.3	0.34
4-n-Propyldibenzothiophene	106.5	8.1	115.4	1.0	116.8	2.4	13.8	1.4	4.7	0.38
4,6-Diethyldibenzothiophene	98.7	4.8	102.4	2.3	109.9	3.5	5.8	0.8	2.6	0.25
HHPACs ^a										
2,3-Br ₂ -thiophene	86.8	5.0	89.8	3.4	84.6	5.4	14.6	0.7	2.4	0.50
5-Cl-benzothiophene	98.4	6.9	104.7	5.7	102.4	3.7	14.5	1.1	3.7	0.54
2-Cl-quinoline	83.9	5.8	92.5	4.9	94.0	3.8	13.9	0.8	2.6	0.47
2-Br-benzothiophene	69.7	6.3	74.1	4.1	75.2	3.7	12.2	0.7	2.4	0.49
2,3-Cl ₂ -benzothiophene	87.1	4.7	89.7	5.6	92.8	4.5	7.5	0.7	2.2	0.31
2-Br-quinoline	93.4	3.5	95.5	4.7	101.6	4.6	11.1	0.5	1.8	0.44
5-Cl-indole	86.9	6.8	91.0	5.2	103.4	3.2	10.7	1.0	3.2	0.19
3-Br-isoquinoline	98.6	5.7	104.9	4.4	123.0	4.3	13.4	0.9	3.0	0.49
5-Br-indole	94.0	7.2	102.4	6.6	122.6	6.8	21.3	1.1	3.6	0.91
2,3-Br ₂ -benzothiophene	83.2	6.4	88.1	9.1	86.9	2.9	12.8	0.9	2.9	0.50
9-Cl-acridine	60.7	9.1	70.0	7.5	98.7	4.1	15.4	0.9	3.0	0.65
2-Br-dibenzothiophene	115.9	9.1	120.5	5.4	138.7	4.3	22.2	1.7	5.7	0.91
3,6-Cl ₂ -9H-carbazole	104.1	4.6	102.9	4.6	119.4	3.7	19.6	0.8	2.6	0.72
2,8-Br ₂ -dibenzothiophene	105.6	5.4	106.2	8.0	116.0	3.5	20.6	0.9	3.1	0.91
3,6-Br ₂ -carbazole	80.1	14.3	92.0	3.9	93.2	2.7	12.3	1.9	6.2	0.49

^a Each sub-class of compound is formatted in bold and underlined.

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