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Direct Immersion Solid-Phase Microextraction Analysis of Multi-class Contaminants in Edible Seaweeds by Gas Chromatography-Mass Spectrometry

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Abstract

The present work aimed at the development of a simple and accurate direct immersion-solidphase microextraction-gas-chromatography-mass spectrometry (DI-SPME-GC-MS) method for simultaneous determination of PAHs, PCBs, and pesticide residues in edible seaweeds. As the target contaminants possess a wide range of physical-chemical properties, multivariate experimental design was used for method optimization. In particular, two different methods were optimized and validated: one that allows for simultaneous determination of all targets, and an ad hoc method for determination of hydrophobic analytes, a class that often poses a challenge for extraction from food matrices. Optimum conditions suitable for simultaneous quantitation of all targeted compounds, namely buffer at pH=7.0, 20% acetone (v/v), 10% NaCl (w/w), 0.02% NaN_3, 60 min DI extraction at 55 °C, and 20 min desorption at 270 °C, afforded limits of quantitation (LOQs) in the range of 1-30 µg kg^{-1}, a wide linear range of 5-2000 µg kg^{-1}, the attainment of satisfactory determination coefficients (R^2>0.99) with no significant lack of fit ($p>0.05$) at the 5% level, and satisfactory accuracy and precision values. By modifying the extraction conditions to favor extraction of the most hydrophobic analytes (e.g. higher amount

of organic modifier and pH, and lower salt content) lower LOQs were obtained for these compounds ranging from 0.2-13.3 µg kg^{-1}. The established methods were then used for screening of commercial, edible dry seaweeds, with PCBs (≤16.0 ng g^{-1}) and PAHs (≤15.5 ng g^{-1}) detected in some samples. This method overcomes most challenges commonly encountered in dry sample analysis applications, and represents the first report of a DI-SPME method employing the matrix-compatible fiber for simultaneous multiclass and multiresidue analysis of seaweeds.

Keywords: Matrix-compatible SPME; Pesticides; Polycyclic aromatic hydrocarbons (PAHs); Polychlorinated biphenyls (PCBs); Multi-residue analysis; Dry seaweed

1. Introduction

Edible seaweeds, such as brown, green, and red seaweeds, among other varieties, represent a well-known source of sustenance, and are often considered a staple in many cuisines of Asian origin [1]. From a nutritional point of view, seaweeds are a low-calorie food, containing significant quantities of proteins, vitamins (A, E,C, and K), essential unsaturated fatty acids, and minerals, as well as bioactive compounds with known antioxidant, antimutagenic, and anticoagulant properties [2, 3]. Furthermore, seaweeds are a valuable source of dietary fiber; according to a previous study, an 8 g serving of dry seaweed can provide up to 12.5% of a person's daily fiber needs [4]. Indeed, the dietary value of edible seaweeds has prompted a large increase in their consumption as a healthy food worldwide in recent years, as well as led to the development of various seaweed-based industries [5].

However, seaweeds are inevitably exposed to the ubiquitous presence of organic pollutants such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and various pesticides, compounds that derive from both natural and anthropogenic sources, and are known to pose a health hazard [6, 7]. PAHs, which include a large group of over 200 different compounds, are categorized as compounds containing two or more fused benzene rings. Some of these compounds, such as benzo[a]pyrene, are known carcinogens, while others have been indicated as suspected carcinogens [6-9]. The U.S. Environmental Protection Agency (EPA-USA) considers 16 PAHs as priority organic pollutants; as such, these compounds have been extensively monitored [6, 10, 11]. PCBs are a family of compounds comprised of 209 chemically

related congeners that were widely used more than 25 years ago. Owing to their insulating and fire-retardant properties, PCBs were used in a variety of industrial applications, such as microscope oils, electrical insulators, capacitors, and electrical appliances [12]. They were also widely sprayed on dirt roads as a dust-control measure until some of the unintended consequences from their widespread use were unearthed, prompting their decreased use worldwide, as well as the establishment of CBC production bans in many countries. Indeed, exposure to PCBs has been implicated as a risk factor for "endocrine (hormone) disruption", which can lead to infertility, the development of certain types of cancer, and other hormone-related disorders [13]. Pesticides such as certain organophosphates (OP), carbamates (CAR), and pyrethroids (PYR) are widely employed in the agricultural and aquaculture industries as pest and disease control measures [14, 15]. Due to their widespread use, the potential human health hazards (neurotoxicity, among others) posed by these compounds have increasingly become a focus of public attention [16]. Owing to their hydrophobicity, PAHs, PCBs, and pesticides tend to associate to particulate matters; in this regard, within the context of our discussion, all three groups of compounds have been previously detected in seaweeds [16, 17]. Regulation (EC) No 396/2005 of the European Parliament only sets maximum residue levels (MRL) for some pesticides, ranging from 10 µg kg^{-1} to 50 µg kg^{-1} in edible seaweeds [18]. Alternatively, only scarce information is available on the limitation levels of PAH and PCB contaminants in seaweed. In view of the above, the development of e□ective extraction and enrichment techniques to determine the levels of the above pollutants present in edible seaweeds is of great interest.

Currently, most reports available in the literature addressing seaweed analysis have the detection of heavy metals as their focus. For determination of organic residues in seaweeds, liquid-liquid extraction, such as Soxhlet [19], pressured liquid extraction (PLE) [20], microwave-assisted extraction (MAE) [21], or matrix solid-phase dispersion (MSPD) [5] have been reported as methods of choice – approaches that unavoidably employ large volumes of organic solvents, and which require pre-concentration and clean-up steps. As a result, such methods are tedious, time-consuming, and not environmentally friendly. Conversely, solid-phase microextraction (SPME), which has been successfully applied towards analyses of organic contaminants in various matrices [22, 23], integrates sampling, extraction, concentration, and sample introduction into a single, low-solvent consuming and automatable step. Nonetheless, very few reports

mention the use of SPME for analysis of organic contaminants in dry seaweeds, especially for poorly volatile compounds, such as PAHs, PCBs, and pesticides.

Despite the numerous advantages presented by SPME, including its simplicity of operation, development of SPME methods for complex matrices, such as seaweeds, requires careful method optimization, including as a first step, the selection of mode of extraction. Since the majority of compounds studied in this work bear poor volatility, direct-immersion SPME (DI-SPME), in which the extraction phase is placed directly into contact with the sample, was selected as mode of extraction with aims to attain higher method sensitivity as well as better representativeness of analytes extracted from the seaweed matrix. The ideal features of a fiber coating for DI-SPME should be matrix compatibility, robustness, and good affinity towards the analytes of interest. Within this context, the analytes targeted in this work belong to three different chemical classes, and are characterized by widely different physiochemical properties, certainly posing a challenge for SPME analysis. A matrix-compatible coating (namely PDMS/DVB/PDMS) was developed with the purpose of enabling DI-SPME in complex matrices such as food commodities [24]. The robustness and endurance of this new coating were evaluated in various food samples, such as fruits [25-27] (i.e. grape, strawberries and avocado pulp) and raw blended vegetables[28] (i.e. spinach, tomato, and carrot), that present different analytical challenges such as water content, pigmentation, interfering matrix compounds, and vegetable texture. In all tested matrices, the PDMS/DVB/PDMS fiber showed excellent durability and robustness, allowing for over 100 consecutive extractions. Based on the above development, the currently presented work aimed at the optimization and development of a DI-SPME-GC-MS method for simultaneous analysis of multiresidue PAHs, PCBs, and pesticides in edible seaweeds. In order to achieve an accurate and robust analytical method by DI-SPME, multivariate approaches such as Plackett-Burman and Central Composite Design (CCD) were employed to screen and optimize the most relevant parameters a□ecting extraction e□ciency (such as pH, ionic strength, organic solvent content, sample temperature, and extraction time). As the studied compounds have a wide range of polarities, with most bearing high hydrophobicity, two optimized DI-SPME protocols — one aimed at broad-spectrum detection, and one targeting more hydrophobic compounds — were evaluated for their suitability towards the currently discussed application. An evaluation of the abovementioned DI-SPME conditions was carried out as a means to provide information to future users on how to tune their SPME method based on the physiochemical properties of the

targeted compounds. Despite the challenges encountered by this method, to the best of these authors' knowledge, this is the first report of a DI-SPME method using matrix-compatible fibers for multiclass and multiresidue analysis of edible seaweed. Further, the established method was successfully applied to the analysis of commercial samples.

2 Experimental section

2.1 Materials and reagents

All employed solvents were of HPLC grade. Acetonitrile (ACN) and acetone were purchased from Fisher Scientific (Ottawa, ON, Canada). Sodium chloride and sodium azide were purchased from Sigma Aldrich (Oakville, Ontario, Canada). PDMS/DVB/PDMS (SPME-OC fiber assembly) 75 μm (Coating thickness includes 65 μm coating + 10 μm OC (overcoating)) fibers were purchased from Supelco (Bellefonte, PA, USA). Dry edible seaweeds (Wakame and Nori) were purchased at local markets in Waterloo(ON, Canada).

All standards were purchased from Sigma Aldrich (Oakville, ON, Canada), Accustandard (New Haven, CT, USA), and Cambridge Isotope Laboratories (Montreal, Quebec, Canada). With the exception of PCBs (Congeners Mix 3, 10 μg mL^{-1} in Isooctane), PAHs (Calibration Mix, 10 μg mL^{-1} in acetonitrile), lamda-cyhalothrin (100 μg mL^{-1} in acetonitrile), and (phenoxi-$^{13}C_6$)-cis-permethrin, all employed standards were of a purity higher than 94%. Detailed compound information for all analytes used in this work can be found in Table S1of Supporting ISInformation.

Individual solutions of pesticide standards were prepared in acetonitrile at 10 mg mL-1. Internal standard (naphthalene-d8, benzo[a]anthracene-d12, Acenaphthene-d10, Phenanthrene-d10, Chrysene-d12, Benzo[a]pyrene-d12, PCB 30, PCB 103, PCB 169, and (diethyl-D10)-chlorpyrifos) solutions were prepared at 1 mg mL-1 in acetonitrile. All standard solutions were stored in a freezer at -30 °C.

For method development steps, a series of mixed calibration solutions, ranging from 0.01 to 10 ng μL-1 (for PAHs and PCBs; seven levels) and 0.1 to 100 ng μL-1 (for pesticides; seven levels), were prepared during method development to calculate amounts (in ng) extracted by SPME for each analyte. Liquid injections of calibration solutions were carried out in triplicate.

148 During the method validation steps, spiking standard mixtures were firstly prepared
149 containing target analytes at 1000 μg mL-1 (for pesticides), 10, 1 and 0.1 μg mL-1 (for all
150 analytes), and internal standards were prepared at 5 μg mL-1. Aliquots of the above mentioned
151 mixtures were spiked into blank seaweed samples to obtain related concentration levels required
152 for each validation part.

153 2.2 Instrumentation

154 Seaweed samples were grinded with the use of a Salton grinder CG1451 (Montreal,
155 Quebec, Canada). Sample pH was measured with a Metter Toledo MP220 (Schwerzenbach,
156 Switzerland) pH meter. For homogenization of samples, a Benchmark BenchMixer^{TM} (Edison,
157 NJ, USA) was employed.

158 GC-MS analysis was performed with an Agilent 7890B gas chromatography coupled to a
159 5977A mass spectrometer (Agilent Technologies, Mississauga, ON, Canada), equipped with a
160 Gerstel MultiPurpose Sampler (GERSTEL, Linthicum, MD, USA). Chromatographic separation
161 was performed in an HP-5 MS column (30 m × 250 μm × 0.25 μm). Helium was employed as
162 carrier gas, with a column flow at 1.0 mL min^{-1}. The oven temperature program was initially set
163 at 70 ˚C for 10 min, then ramped at 20 ˚C min^{-1} to 200 ˚C for 8 min. Next, it was ramped at
164 10 °C min^{-1} to 260 °C for 8 min, and then ramped at 10 ˚C min^{-1} to 300 ˚C, at which point it was
165 held for 8 min, resulting in a total run time of 38 min. The injector (equipped with a deactivated
166 glass liner for SPME, 0.75mm i.d.) was maintained at 270 °C in splitless mode (the split valve
167 was opened for after 20 min in case of SPME desorption and after 1.0 min for liquid injections).
168 For the single quadrupole MS, the operational conditions were as follows: the transfer line, ion
169 source, and MS Quad temperature were 280 °C, 230 °C, and 150 °C, respectively; the fixed
170 electron energy (EI), 70 eV; the mass range, m/z 50-400; acquisition rate, 50 Hz; detector
171 voltage, 1338 V. Retention times, as well as the selected quantifier ions obtained in the above
172 GC-MS conditions are presented in SI Table S2.
173

174 2.3 Preparation of spiked seaweed samples

175 Dry organic edible seaweed samples (previously analyzed for the absence of target
176 analytes) were grinded into powder and transferred to a glass container. In order to demonstrate
177 the suitability of the proposed method for different types of edible seaweed, a mixture of Nori

and Wakame (1:1, w/w) was used as matrix during SPME method optimization. 30 g of mixed seaweed powder was weighed into a 250 mL glass jar, after which 40 mL of acetonitrile spiked with the multi-class analytes were added. The mixture was then left to homogenize overnight under agitation conditions so as to allow for sufficient analyte-matrix binding to occur. Spiked samples were dried under N_2 flow in a fume hood and stored at +4 ºC in refrigerator for 2 days prior to extraction to simulate typical interaction conditions between seaweed and the target compounds.

Spiking concentrations were carefully selected to guarantee enough sensitivity for all analytes during the optimization processes (see SI Table S3). Blank seaweed samples were prepared in the same manner as described above, with the exception that no standard was added. All the samples needed for matrix matched calibration were prepared at according to the abovementioned protocol properly adjusting the amount of spiked analytes and internal standards.

2.4 SPME procedure

Preliminary tests in spiked seaweed sample were performed by adjusting desorption temperature and time. As the data indicated (not shown), desorption temperature at 270 °C and desorption time of 15 min yielded no significant carryover.

0.25 g spiked seaweed sample and 8 mL dispersive solution were placed into a 10 mL glass vial, and thoroughly vortexed for 1h prior to extraction. For initial SPME method development, deionized water was added as dispersive solution. After 1 h mixing, a 1 min pre-extraction incubation of the sample was performed at 40 °C in the agitation unit at 500 rpm, followed by a 30 min direct immersion extraction. Following extraction, the fiber was rinsed for 15 s in deionized water, and then desorbed for 15 min at 270 °C.

For Plackett-Burman and CCD experiments, all SPME parameters were set as described above, with the exception of the composition of the employed dispersive solutions, which was varied to correspond to each experimental condition, in accordance with the experimental matrix.

Two SPME methods were optimized, one for simultaneous determination of all targeted analytes (method#1), and a second one (method#2) optimized ad hoc for the most hydrophobic

analytes (LogP>5.2). For method #1, optimized sample preparation conditions were: buffer at pH=7.0, containing 20% acetone (v/v), 10% NaCl (w/w) and 0.02% NaN_3. Method #2 utilized a buffer with pH=10.0, containing 40% acetone (v/v) and 0.02% NaN_3, aiming at enhanced extraction of the more hydrophobic compounds. For both protocols the following procedure was followed: after 1 h mixing, samples were incubated for 5 min at 55 °C in the agitation unit at 600 rpm, followed by 60 min and 30 min direct immersion extractions, for method #1 and method #2 respectively. Following extraction, fibers were rinsed for 25 s in an acetone-water (2:8, v/v) solution, and then desorbed for 20 min at 270 °C.

2.5 Optimization by experimental design

Aiming to optimize the analysis of 41 contaminants in seaweeds, a Plackett-Burman experimental design (PBD) was selected to screen significant independent variables (factors) impacting analysis. Following the identification of the most significant factors (p-value < 0.05), a response surface methodology (RSM), namely central composite design (CCD) 2^4, was used for optimization. Data were processed through the software Statistica 13.0 (TIBCO® Statistica™, CA, USA).

For PBD screening, evaluated factors and respective ranges included the following: Extraction time (15 – 45 min), Salt content (0 – 20 %), Organic solvent (0 – 20 %, w/w), Temperature (30 – 60 ºC), Stirring rate (250 – 600 rpm), Incubation time (5 – 15 min) and pH (4 – 10), as shown in SI Table S4. Factor ranges were selected according to preliminary tests.

For CCD experiments, extraction temperature (ºC), organic solvent (mL), salt content (%), and pH were evaluated. A total of 30 experiments were performed: eighteen in the factorial points (-1, 1), eight in the axial points (-2, 2), and four in the central point (0). The studied factors and their associated ranges can be found in SI Table S5.

3 Results and discussion

3.1 Evaluation of binding time as a factor in the extraction of target analytes from spiked seaweeds

Significant differences in extraction results were observed between spiked seaweed samples submitted to extraction at the same experimental conditions, but at different time intervals after preparation of samples. According to Burford [29], "freshly" spiked analytes have little time to interact with the sample matrix. As such, sufficient equilibration (binding) time between spiked analyte and matrix should be allocated so as to attain samples that can be considered representative of real contamination scenarios. To investigate optimum binding time for analytes spiked on seaweeds, SPME was carried out at different binding periods (at +4 ºC in refrigerator) of the spiked sample, namely at 24, 48, and 72 hours following spiking of analytes. The results are shown in Supporting Information Figure S1. For most target analytes, extraction amounts were observed to decrease from 24 h to 48 h, but remain relatively unchanged as binding time surpassed the 48 hour mark. Therefore, in order not to introduce errors associated with insufficient binding time, "freshly" prepared spiked samples were allowed to equilibrate with the spiked analytes at least 48 h prior to extraction.

3.2 Sample preparation optimization

3.2.1 Evaluation of sample to water ratio

Unlike most fresh vegetable and fruit matrices, small amounts of dry seaweed can expand to large volumes once introduced to water. In DI-SPME analysis, when 10 mL vials are used for sampling, care should be exercised to prepare total sample volumes. The volumes should be large enough to enable the full immersion of the SPME fiber, but do not exceed 9 mL so as to avoid contamination of the fiber holder due to spilling, that can result into contamination of the GC injector. Moreover, samples should not be so dense as to incur SPME fiber breakage during DI extraction in agitation conditions. As such, careful optimization of the seaweed-to-water ratio was carried out, taking into account the abovementioned requirements, prior to SPME method development. Results showed that when 0.25 g seaweed and 8 mL water were used, the sample solution enabled suitable conditions for DI-SPME.

3.2.2 Degradation study of analytes in sample solution at room temperature

Envisioning the application of the proposed SPME to routine analysis, and thus foreseeing the preparation of large amounts of samples that will inevitably be queued for analysis on an autosampler rack, the currently presented work included an evaluation of whether any

degradation of analytes in the sample matrix occurred at room temperature. This assessment constituted an important step in method development, as it allowed for proper tuning of the sample preparation strategy, and thus circumvented the production of unreproducible and unreliable data sets. For this assessment, after carrying out sample preparation as per procedures previously outlined in this work (Sections 2.3 and 2.4), samples were placed in an autosampler tray at room temperature. DI-SPME was then carried out under the following conditions: immediately after vortexing (0 h), and after 6, 12, 24, and 48 h (n=3 for each set). Any potential loss and/or degradation of analytes was assessed by evaluating variations in their extracted amounts. According to the obtained results, a decreasing trend was observed for most analytes from 12 h to 48 h (data not shown). Of note, pressure build up into the vial was noticed since the septum of the sample vial was observed to 'bloat' after being placed at room temperature for 24 h; thus, the authors presume that biodegradation may have taken place during the investigated waiting time.

Based on previous reports [30, 31], the dispersion solution was enriched with 0.02% sodium azide (NaN_3) so as to prevent bacterial growth from occurring in the matrix media. The abovementioned set of experiments was repeated to verify the efficacy of the bacterial growth inhibitor added to the dispersion solution. Related results regarding the target analytes, namely pesticides, PCBs, and PAHs, are shown in Figure 1(a-c). For almost all analytes, extraction amounts remained relatively stable within 48 h, indicating that addition of 0.02% NaN_3 effectively prevented any form of analyte degradation/loss. Therefore, the prepared seaweed samples that included addition of 0.02% NaN_3 were able to stand as long as 48 h at room temperature prior to be submitted to extraction. Moreover, a comparison of extracted amounts of each targeted analyte by DI-SPME, with or without addition of 0.02% NaN_3, was also carried out (immediately after vortexing) so as to investigate the effect of addition of NaN3 on the extraction of target analytes. The attained results revealed comparable analyte extraction amounts for both extraction conditions, confirming that addition of NaN_3 does not significantly affect analyte extraction (Supporting information Figure S2).

3.2.3 Optimization of pre-desorption rinsing of fiber coatings

As optimization of a pre-desorption rinsing step constitutes a crucial step in the development of a reliable DI-SPME protocol, preliminary studies were performed using spiked

seaweeds in order to select the most suitable rinsing conditions. According to a previous study, acetone showed good capability in removing oily residues from coating surfaces [27]. Considering that seaweed, as a complex matrix, contains abundant unsaturated fatty acids, proteins, and pigments, among other possible interfering constituents, acetone was selected as organic solvent for the rinsing step. Moreover, five different ratios of acetone to water were tested as rinsing solutions, namely water, acetone-water (1:9, v/v), acetone-water (2:8, v/v), acetone-water (1:1, v/v) and acetone-water (9:1, v/v). For each rinsing solution, three different rinsing times, 5 s, 15 s and 25 s, were tested. The tested procedures are summarized in Figure S3 (Supporting Information). Results were compared in terms of average extraction amounts of analytes, as well as in terms of SPME fiber and glass liner cleanness. Although longer rinsing times may afford cleaner fibers, extended fiber exposure to the rinsing solution may also lead to loss of analytes extracted onto the fiber. Thus, when optimizing this parameter, a suitable compromise must be made between extraction sensitivity and effective cleaning of the coating surface.

In the current work, best results were achieved when rinsing time was increased to 25 s for all rinsing solutions, with the exception of acetone-water (9:1, v/v), for which a rinsing time of 15 s was deemed as most suitable. Detailed comparisons of data pertaining to these experiments can be found in Supporting Information Figure S4 (a-e).

While addition of acetone in the rinsing solution can aid in the removal of co-extracted matrix macro-components, it may also act as an additional phase, competing with the SPME coating for the partition of analytes by inducing their back-extraction from the coating and into the rinsing solution [32]. In view of this, extraction results pertaining to the three rinsing times tested for each rinsing solution were compared in terms of analyte loss, with results shown in Figure 2. As part of the optimization of rinsing time for each solution, SPME coatings and GC injection port glass liner inserts were also inspected in terms of cleanliness, either visually or by microscope, after every 9 extractions and injections. Accumulation of matrix components onto the coating surface could be clearly observed in microscope photos of fibers that were rinsed with solutions containing water; in this regard, the cleanliness of the fiber coatings was observed to be positively correlated with the amount of acetone added to the solution, with higher percentages of acetone yielding cleaner fibers (Supporting Information Figure S5).

In summary, a mixture of acetone-water (2:8, v/v) for the pre-desorption rinsing step and 25 s as rinsing time were chosen as optimal parameters to minimize the deposition of matrix residues on the coating, while also enabling suitable extraction efficiencies for the studied analytes.

3.3 Optimization of SPME parameters

The Plackett-Burman (PBD) experimental design is generally employed in method development to identify the most important factors affecting a given process without consideration given to the interaction effects between and among the evaluated factors. As such, PBD designs are often used as a screening approach in cases where the analysis of a given matrix may involve a high number of factors. As part of method development, once significant factors are identified via PBD, a response surface methodology should be subsequently employed in order to fully optimize the process [33].

Results were evaluated by taking into account the extracted mass of each analyte under study. Due to the substantial chemical diversity of the targeted analytes, Pareto charts obtained from the design were evaluated for each chemical class (pesticides, PAHs and PCBs), while analyte response was divided within each class according to compound hydrophobicity (LogP < 4, 4 < logP < 5, logP > 5). An examination of the obtained Pareto charts, which can be found under Supporting Information Figures S6-S8, demonstrated that extraction time and stirring rate were positive significant factors for all classes and subgroups of analytes considered. In light of these results, the stirring rate was set at the maximum tested value, 600 rpm (chosen to enable fast agitation and preserve SPME coatings from mechanical damage), with further investigations of the entire extraction time profile for each analyte carried out under this optimized condition (Section 3.4). While incubation time, namely the period of time that samples spend in the heater/agitator prior to extraction, is generally a more important factor to consider for headspace SPME applications — as it is needed to establish equilibrium between the sample and its headspace — in DI-SPME, the incubation period can be employed to control the temperature of samples prior to extraction. The results obtained from the Pareto charts (Figures S6-S8) revealed that for most analyte groups and classes, incubation time did not significantly affect the extraction performances; as for analytes for which incubation significantly affected extraction, the highest responses were obtained at short incubation periods. As such, incubation time was set

at 5 min (the lowest level tested in the PBD) for further experiments. While addition of organic solvent as a matrix modifier can promote the release of analytes originally bound to the sample matrix — and thus not available for extraction via SPME — on the other hand, any added organic solvent can act as a competing extraction phase with the SPME coating, diminishing the extractable amount of certain analytes. As evident in Figure S6, addition of organic solvent did not positively affect the recovery of the least hydrophobic analytes (logP<4), which are less likely to bind to matrix constituents, and could thus be directly affected by addition of a competing partition phase. Conversely, for compounds with logP>4, addition of organic solvent was shown to yield a significant positive effect on extracted amounts. This effect can be related to two possible phenomena: the shifting of the binding equilibria established within the matrix toward the free forms of analytes belonging to this class (logP>4), and the stabilization of these analytes in the dispersive solution, which avoids loss of analytes due to poor solubility in pure aqueous media. Extraction temperature does affect the diffusivity of analytes into the sample media, and may have an effect on the partition coefficient of the analytes into the SPME coating. In analyses of complex matrices, temperature may also play a role in the binding equilibria established with matrix components; thus, an attentive optimization of this parameter is required so as to ensure temperature has a positive effect on extraction. In the current work, temperature was revealed to be positively significant for all classes and subgroups, with the exception of pesticides with 4<LogP<5. As such, optimization of this parameter was further carried out by CCD. Varying the ionic strength of the sample by addition of salts can promote the extraction of certain analytes via the salting-out effect; however, in the presence of binding media, variation of the ionic strength can shift binding equilibria toward the bound form of analytes, reducing recoveries by SPME. Considering that an interaction effect exists between ionic strength adjustments and media temperature, this parameter was further optimized by CCD. Sample pH adjustments play a significant role in SPME analysis, as certain SPME coatings are only capable of extracting molecular species in their neutral form. Moreover, the sorption of pyrene into some dissolved hydrophobic organic matters commonly found in the marine environment, such as humic acid (HA) and fulvic acid (FA), among others, has been previously shown to be strongly pH-dependent. As pH increases, an obvious decreasing trend can be observed for the partition coefficients of pyrene binding to HA and FA [34]. An examination of the Pareto Charts (Figures S6-S8) revealed that pH played a significant role in the recovery of different chemical classes,

yielding both a positive and negative significant effect; as such, this parameter was also further optimized via CCD.

Following the successful identification of parameters significantly influencing targeted analyte response, a multivariate optimization of these variables was carried out with aims of selecting parameters capable of yielding maximum signal response.

Response surface methodology (RSM) has been widely employed in the optimization of analytical chemistry processes [35-38]. Its widespread application in analytical chemistry lies in the ability of RSM to allow for optimization of various parameters via few experiments, as well as in its capability of not only providing information regarding the individual influences of significant factors, but information pertaining to the interactions occurring among these parameters as well. In addition, a suitable prediction mathematic model can be applied to determine the response inside the range studied for each factor, andit is worth highlighting that this can be achieved by only using experimental design approaches [37, 39].

For this purpose, a Central Composite Design (CCD) was employed for optimization of extraction temperature (°C), acetone addition (%, v/v), pH, and salt addition (% of NaCl). All other parameters were kept constant, as follows: stirring rate set at 600 rpm; pre-incubation time of 5 min; extraction time set at 45 min.

The 41 contaminants evaluated were distributed in classes of hydrophilic and lipophilic compounds due to their diverse affinities for the SPME coating, as already described in previous work [40]. However, in contrast to the abovementioned work [40], the currently presented work included an evaluation of the response of each compound, as opposed to an evaluation of the sum of signals pertaining to the targeted analytes. When only the sums of analyte signals are considered, individual information regarding each compound can be missed, especially in cases where there is high intensity variability among the targeted analytes. As an example, an increase in the total response (sum) does not always correspond to an increased response for a given analyte, as variations in independent variables (factors) can significantly enhance responses analytes while negatively affecting the responses of others. Moreover, the effect of a given independent variable on the response of a given analyte is strictly related to the chemical properties of said analyte. Thus, in cases where many compounds are being studied, dividing them into groups based on their chemical properties may constitute a feasible firsthand approach

to analysis. However, when grouping compounds in such a manner, one should not interpret the sum of their response as a single response corresponding to the whole group. When such an approach is carried out, individual information regarding each compound will likely be missed, enforcing the necessity of individually evaluating compounds, even if they are firstly divided into groups for convenience of analysis.

In view of the above, as a firsthand approach to an evaluation of the influences of individual factors on analyte response, the 41 contaminants under study were separated into three groups based on their Log P: highly lipophilic (Log P $\geq$ 5.6), lipophilic (3.46 $\leq$ Log P < 5.6), and hydrophilic compounds (Log P < 3.46). Log P ranges were selected based on the similarity of significant factors among compounds ($p < 0.05$); in other words, categorization of compounds into groups was based on the similarity of their behavior in relation to the evaluated factors. A detailed discussion regarding the effect of the individual variables for each group of analytes will be carried out next.

Using the Derringer & Suich's desirability function approach, which can maximize the overall desirability (multiple response) based on controllable factors, optimized conditions for each specific group of analytes [41, 42] based on their Log P were attained, as well as a suitable compromise of all conditions for simultaneous analysis of all targeted analytes. Seeing as most targeted analytes under study are characterized by a lipophilic nature, the conditions optimized for simultaneous analysis of all compounds mainly favored an enhancement of response for lipophilic compounds. As overall desirability is obtained by combining individual desirabilities via their geometric mean, general method response decreases significantly for hydrophilic analytes for which best conditions differ from the desired conditions for analysis of lipophilic compounds (which represent the majority of the targeted analytes) [37, 41, 42]. To solve this issue, the final conditions for analysis of all targeted analytes were selected by slightly displacing the values of the independent variables to also favor hydrophilic compounds.

Experimental values attained using the selected conditions were then compared to values obtained through a mathematical model of predicted values. The relative standard deviation (RSD) obtained for all compounds presented less than 5% variation, indicating that the equation was well adjusted. The determination coefficient (R^2) ranged from 0.668 to 0.925, and the lack-

of-fit test yielded non-significant results ($p > 0.05$), confirming a good fit for all compounds [37, 41, 42].

In sum, optimal conditions were obtained for the simultaneous analysis of all targeted compounds, as well as for each of the three groups described above. The development of different optimization processes, including a process for the simultaneous analysis of all studied compounds as well as optimizations targeted at specific groups, can be very useful in a variety of applications. As the analyst can easily select conditions that lead to optimal method performance for a specific group of contaminants of interest, or select conditions that allow for the simultaneous analysis of different classes of pollutants characterized by a wide range of physicochemical properties. Thus, the currently presented methods can be easily tailored to various analytical goals.

Optimum conditions for analysis of highly lipophilic compounds were determined as 80 ºC extraction temperature, 40 % organic solvent, 5 % salt content, and pH 10 (Figure S9). Conditions for analysis of medium lipophilicity compounds included 30 ºC extraction temperature, 20 % organic solvent, 0 % salt content, and pH 4 (Figure S10). For hydrophilic compounds, optimum conditions were 30 ºC extraction temperature, 0 % organic solvent, 20 % salt content, and pH 4 (Figure S11). Lastly, optimized conditions for the simultaneous determination of all targeted analytes were 55 ºC extraction temperature, 20% organic solvent, pH 7, and 10 % salt content (Figure S12).

This approach provided a powerful analytical tool, as based on the focus of the study, applying optimized conditions for a specific group of analytes can enable the attainment of lower limits of quantification in compliance with MRLs imposed by regulatory agencies. However, it is important to highlight here that the focus of this work centered on the simultaneous evaluation of all studied contaminants in a single analytical run; consequently, only optimized conditions pertaining to the whole group of contaminants were employed for further method validation within the scope of this work.

3.4 Evaluation of the extraction time

Based on the CCD results, extraction times varying from 5 min to 120 min were studied under the optimized conditions for all analytes (method #1), and for hydrophobic analytes (method#2).

The results obtained by method#1 are shown in Figure 3. As can be seen, for most analytes with LogP<4.5, equilibrium was reached within 30 min, while equilibrium of analytes with 4.5<LogP<5.2, necessitated an extraction period of 60 min, Lastly, analytes with LogP> 5.2 necessitated an equilibration time equal to or longer than 90 min. Thus, an extraction time of 60 min was selected as a compromise between method sensitivity and practicality of method throughput.

As discussed in section 3.3.2, a higher extraction temperature of 80 ˚C was deemed as most suitable for most hydrophobic compounds. However, a decrease in the extracted amounts for certain pesticides, such as cerpermethrin and cyfluthrin, was observed for extractions carried out at 80 ˚C. To investigate this phenomenon, spiked seaweed samples were extracted at three temperatures, 55 ˚C, 67 ˚C, and 80 ˚C, The extracted peaks in the above three conditions were compared with those attained from spiked water. As shown in Figure 4, peak shapes for the two isomers of cerpermethrin and cyfluthrin started to change at 67 ˚C, with new peaks with different retention times observed at 80 ˚C. Such a phenomenon can be assumed to occur due to the degradation of these compounds at a high temperature. A similar result was obtained in past research[43], where it was assessed that the hydrolysis of cypermethrin in aqueous solutions was accelerated by high temperatures.

Therefore, the extraction time profile for hydrophobic analytes with LogP>5.2 was set at a lower temperature of 55 ˚C, while all other parameters obtained from the CCD (pH=10, 40% acetone (v/v)) remained as previously stated. As can be seen from results shown in Figure 5, equilibrium was reached at 60 min for almost all PAHs, pesticides, and some PCBs, such as PCB 18, PCB 28, etc., although equilibration for most hydrophobic PCBs could only be reached after 90 min.

3.4 Method validation

3.4.1 Selection of internal standard

For method validation, quantitation of multi-class analytes was carried out with the use of fortified internal standards (IS) in seaweed samples so as to compensate for matrix effects, drifts in instrumental responses, as well as losses during sample preparation steps. As commonly established, a suitable IS should be characterized by physicochemical properties that are very similar to that of the analyte under study, while also allowing for sufficient separation from said analyte via chromatography. In SPME, a suitable IS should also be able to mimic the partition of the analyte toward the extraction phase as well as its partition to any other competing phase [26]. To date, the most accurate and simplest method for quantification is the use of an isotopically labeled internal standard. However, due to the prohibitive cost of some isotopically labeled analytes, congeners of analytes or compounds meeting the above requirements are often selected as alternative IS.

Based on previous reports, (phenoxi-$^{13}C_6$)-cis-permethrin was used as IS for PYR, while (diethyl-D_{10})-chlorpyrifos was used as IS for the remaining pesticides [44]. Naphthalene-d_8, benzo[a]anthracene-d_{12}, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and benzo[a]pyrene-d_{12} were used as IS for PAHs [45, 46]. However, for PCBs, due to the existence of chlorine isotopes in PCB compounds, perdueterated PCBs do not show enough mass shift to overcome the chlorine isotope patterns, while 13-C labeled PCB congeners are prohibitively expensive [47]. Therefore, PCB congeners (isomers), which are not reported as main contaminants in aquatic products and can be fully separated from all other PCB congeners in the GC conditions used for this work, were selected as internal standards for PCBs (e.g PCB 30, PCB 105, and PCB 169).

3.4.2 Linearity and Limits of Quantitation

Linearity was evaluated by matrix-matched calibration curves, using relative area versus IS area. Preparation of the spiked samples at different calibration levels was performed as reported in section 2.1. Calibration levels were set at 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 ng g-1, while the concentration of the internal standards was 100 ng g-1. Limits of quantification (LOQ) were calculated using the signal-to-noise method. For these calculations, peak-to-peak noise values around each analyte retention time were measured, and concentrations of a given analyte that would yield a signal equal to ten times that of the signal-to-noise ratio (10 S/N) were established as the LOQ value for said analyte. Results obtained for method #1 in DI-SPME mode for all analytes are shown in Table 1.

Generally, the coefficient of determination (R^2) of a calibration equation is used to evaluate linearity of calibration curves. However, the intercept term of the calibration equation might be influenced by the baseline of the spectrometer and the nature of the reference sample. As extra terms in an equation will always improve the fit to a straight line, simply determining how well a straight line is fitted to the data does not always provide reliable information regarding the linearity of the calibration curve over the entire calibration range studied. Therefore, in addition to evaluating the coefficient of determination, a lack-of-fit statistical test was also employed in the present work to confirm linearity for all analytes. The overall lack-of-fit of the calibration equation, excluding the intercept term, was then compared with the replicate error (analysis of variance, ANOVA), with effects deemed statistically significant at a 95% confidence level ($p < 0.05$). The attained results revealed no significant effects for all analytes, meaning that the intercept term was not important. Thus, R^2 could be used to evaluate linearity without consideration given to other interference factors. As can be seen from Table 1, the method showed good linearity for all analytes in the entire calibration range selected for each analyte, with $R^2 > 0.995$.

Excellent LOQs were achieved with the proposed method. For all PCBs, LOQs lower than or equal to 13.3 ng g^{-1}were achieved, while LOQs lower than or equal to 10.0 ng g^{-1} were achieved for all PAHs. LOQs of 20.0 ng g^{-1} or lower were achieved for most pesticides, with the exceptions of LOQs corresponding to more hydrophilic pesticides (such as propoxur and carbaryl), owing to the low affinity of these compounds toward the fiber coating, as well as to their impaired GC responses and of LOQs of some highly hydrophobic pesticides, such as resmethrin, cyfluthrin, cypermethrin, and deltamethrin, among others ($20.0 < LOQs \leq 33.0$ ng g^{-1}). Despite the toxicity of the above contaminants, which may pose a hazard to both human health and the environment at high enough quantities, regulation for these contaminants in edible seaweed is still very limited. While the EU Pesticide Database [48] regulated the maximum residue levels (MRLs) for some pesticides in seaweeds, no clear limit information was found for PCBs and PAHs in seaweed matrix. The attained LOQs of the currently discussed method (method #1) for all pesticides were lower than the MRL set for seaweeds in the EU Pesticide Database, with the exceptions of carbaryl, resmethrin and flucythrinate, for which the LOQ was a bit higher than the MRL (Table 1).

The analytes studied in the present work belong to three different classes of compounds encompassing a wide range of polarities, with the majority of studied compounds characterized by hydrophobicity. In order to evaluate the sensitivity of the DI-SPME method for detection of these hydrophobic compounds in seaweeds, as well as provide additional information regarding method selection based on the physiochemical properties of compounds of interest, linearity and LOQ were also evaluated for the optimized DI-SPME conditions (method #2) targeted at the analysis of hydrophobic compounds (Log P>5.2). Results shown in Table 2. Excellent linearities for all hydrophobic analytes were achieved in their own linear range. Furthermore, LOQs for all pesticides obtained in this optimized condition were much lower those attained via method#1 (LOQs≤13.3 ng g^{-1}), satisfying the LOQ requirements set by EU regulation [48].

Since MRLs for PCBs and PAHs in seaweed have yet to be established by regulatory agencies, the LOQs achieved in the proposed work were compared with recent publications reported for GC-based residue analyses in seaweeds. To the best of our knowledge, no reports on the simultaneous detection of all PCBs and PAHs studied in the current work have been published to date; thus, different papers, focusing on either PCBs or PAHs, were used for comparison [49, 50] (Table 3). As can be clearly surmised by comparing results in Table 3, the majority of LOQs for PCBs and PAHs achievable via either one of the two optimized DI-SPME conditions were similar or lower than those previously reported. Although LOQs for some PAHs, such as naphthalene and aceaphthyene, were slightly higher than previously reported LOQs, the attained values are nonetheless still satisfactory, particularly in view of the wide spectrum analytical capabilities of the method herein discussed.

3.4.3 Accuracy and precision

The spiking standard mixtures, as well as the spiked seaweed samples used in during steps were prepared according to the procedure described in section 2.3. Precision was studied by performing repeatability (intra-day precision) and reproducibility (inter-day precision) studies. Repeatability was determined via analysis of seaweed samples spiked at four concentrations: 15, 50, 100, and 500 ng g^{-1}. Data from five analyses for each concentration level performed in the same day were used for calculations (n=5), with the data expressed as relative standard deviations (RSD %). For reproducibility measurements, all seaweed samples were spiked at the same concentration levels as abovementioned and submitted to analysis. Data from three analyses for each concentration level performed in three nonconsecutive days were calculated,

589 and the reproducibility was expressed as RSD %. As presented in Table 4, good results were
590 obtained for analytes studied in this work, with attained RSDs for repeatability and
591 reproducibility lower than 22.3% and 25.5%, respectively.

592 To evaluate the accuracy of present method, the mean relative recovery of the analyte was
593 assessed by fortifying blank seaweed samples at the four concentration levels above mentioned,
594 considering both inter- and intra-day measurements. The spiked samples were quantified using
595 the matrix matched calibration curves. For most analytes, accuracy ranged from 60 to 125%
596 (Table 4).

597 Since the present work had as central focus the simultaneous analysis of multiresidues
598 characterized by a wide range of polarities, precision and accuracy for DI-SPME method #2,
599 which was developed to specifically target hydrophobic analytes, were not investigated in this
600 work.

601 **4 Analysis of real samples**

602 Five different commercially available edible seaweed sample types, including natural dry
603 nori and wakame, as well as different flavored seaweed snacks, were bought from a local
604 supermarket in Waterloo, ON, Canada. The validated DI-SPME-GC-MS (method #1) was used
605 for analysis of the above real samples. The attained results are shown in Table 5. Analysis
606 showed that concentrations of the three classes of residues were below the LOQs in four samples.
607 However, one pesticide (tetramethrin, 8.3 ng g^{-1}), one PCB (PCB 18, 16.0 ng g^{-1}), and two PAHs
608 (phenanthrene, 15.5 ng g^{-1} and fluoranthene, 5.2 ng g^{-1}) were detected in a dry Wakame sample.
609 As a matter of concern, it should be noted that the above detected compounds, which are widely
610 employed in household or industrial applications, are well-known to have toxic effects on both
611 humans and the environment; as such, their presence in edible foodstuffs should be given careful
612 attention.

613 **5 Conclusion**

614 For the first time, a matrix-compatible PDMS/DVB/PDMS coating was applied to DI-
615 SPME of multiclass residue analysis in dry seaweeds, enabling the simultaneous quantitation of
616 41 analytes belonging to three different chemical classes. The validated method yielded good
617 accuracy, precision, and sensitivity. In view of the satisfactory performance of the method, as

well as its green and automated nature, the proposed method can be considered as appropriate for the detection of pesticides, PCBs, and PAHs in seaweeds. Furthermore, the method was successfully applied to the determination of multiresidues in commercial dry seaweeds.

Although the DI-SPME method developed for wide spectrum coverage of analytes (method#1) failed to yield a satisfactory enough performance in the detection of some highly hydrophobic pesticides (e.g., cyfluthrin, cypermethrin, etc.), better results were achieved via the optimized condition focused on hydrophobic analytes with LogP>5.2. Therefore, using this work as reference, analysts can select the appropriate DI-SPME conditions for a given application based on the physiochemical characteristics of the compounds of interest, as well as the regulated requirements of detection of said analyte(s). A limitation of the currently presented method pertains to the relatively poor LOQs obtained for hydrophilic pesticides (e.g., carbaryl and propoxur). In this regard, further investigations will be carried out in the future, particularly in view of the development of alternative coating chemistries with enhanced affinity toward polar analytes.

The currently presented method overcomes most of the common challenges associated with dry sample analysis. Compared with previous reports, which have mainly employed liquid-liquid extraction, the proposed SPME method integrates sampling, extraction, concentration, and sample introduction into a single, low-solvent consuming and automatable step, presenting a much simpler and greener approach to analysis. Moreover, by using the matrix-compatible PDMS/DVB/PDMS coating and thoroughly optimizing the DI-SPME conditions, higher sensitivity and better representativeness of analytes were achieved, affording a especially suitable method for the analysis of hydrophobic compounds in dry seaweed matrix. The current work represents a first report of a DI-SPME method utilizing matrix-compatible fibers for simultaneous multiclass and multiresidue analysis of seaweeds.

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Figure Captions

Fig.1 Extracted amount of (a) pesticides, (b) PAHs and (c) PCBs from spiked samples, containing 0.02% of NaN_3, held in the autosampler rack at r.t. at different waiting times prior SPME extraction.

Fig. 2 Comparison of DI-SPME results for extractions carried out under the optimal rinsing times for each rinsing solution.

797 Fig. 3 Extraction time profiles of a) PAHs, b) PCBs and c) pesticides for DI-SPME method
798 carried out under optimized conditions for simultaneous analysis of all targeted compounds

799 Fig. 4 Chromatographic profiles showing the variations in extraction amounts of cyfluthrin and
800 cypermethrin for extractions carried out at different temperatures

801 Fig. 5 Extraction time profile of a) PAHs, b) PCBs and c) pesticides, obtained under optimized
802 conditions for highly hydrophobic compounds

Table 1. Linearity, R^2, and LOQs for all targets extracted under optimized DI-SPME conditions for simultaneous analysis of all analytes under study

Compound	Linear range/ng g^{-1}	R^2	LOQ/ ng g^{-1}	MRL[a]/ ng g^{-1}
2-phenylphenol	20-2000	0.997	16.7	50
Propoxur	50-2000	0.994	26.2	
Chlorpyrifos-methyl	1-2000	0.996	1.0	
Carbaryl	20-2000	0.995	29.2	10
Chlorpyrifos-ethyl	5-2000	0.995	5.0	
Piperonyl butoxide	2-2000	0.998	1.4	
Resmethrin	50-2000	0.997	25.4	10*
Tetramethrin	10-2000	0.995	6.2	
Lamda-cyhalothrin	20-2000	0.994	13.3	20*
Permethrin	10-2000	1.000	8.5	50*
Cyfluthrin	50-2000	0.993	30.0	20*
Cypermethrin	50-2000	0.995	30.0	50*
Flucythrinate	20-2000	0.994	20.0	10*
Fenvalerate	20-2000	0.998	13.4	20*
Deltamethrin	50-2000	0.993	33.0	50[ψ]
PCB 18	10-2000	0.997	10.0	
PCB 28	5-2000	0.999	5.0	
PCB 52	5-2000	0.999	4.0	
PCB 44	2-2000	0.999	1.7	
PCB 101	5-2000	0.999	5.0	
PCB 149	5-2000	0.993	5.0	
PCB 118	5-2000	0.999	2.5	
PCB 153	10-2000	0.995	7.8	
PCB 138	5-2000	0.995	5.0	
PCB 180	10-2000	0.998	6.0	
PCB 170	10-2000	0.998	6.0	

Table 1 (*Continued*)

Compound	Linear range/ng g^{-1}	R^2	LOQ/ ng g^{-1}	MRL[a]/ ng g^{-1}
Naphthalene	10-2000	0.999	10.0	
Acenaphthyene	10-2000	0.999	7.8	
Acenaphthene	20-2000	1.000	13.3	
Fluorene	10-2000	1.000	10.0	
Phenanthrene	5-2000	0.998	3.4	
Anthracene	5-1000	0.999	2.6	
Fluoranthene	2-2000	1.000	2.0	
Pyrene	5-2000	0.999	2.2	
Benzo(a)anthracene	2-2000	1.000	1.8	
Chrysene	5-2000	1.000	3.3	
Benzo(b)fluoranthene	1-2000	1.000	1.0	
Benzo(k)fluoranthene	1-2000	1.000	1.0	
Benzo(a)pyrene	1-2000	1.000	1.0	
Indeno(1,2,3-cd)pyrene	1-2000	0.999	1.0	
Dibenz(ah)anthrene	1-2000	0.999	1.0	
Benzo(ghi)perylene	1-2000	0.997	1.0	

Note: "*"- sum of all isomers; "ψ"- sum of cis-isomers

"a"- EU Pesticide Database. http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN

Table 2. Linearity, R^2, and LOQs for hydrophobic compounds with logP>5.2

Compound	Linear range/ng g^{-1}	R^2	LOQ/ ng g^{-1}
Benzo(a)anthracene	1-2000	0.999	0.7
Chrysene	1-2000	0.999	1.0
Benzo(b)fluoranthene	1-2000	0.999	0.4
Benzo(k)fluoranthene	1-2000	0.998	0.2
Benzo(a)pyrene	1-2000	0.999	0.6
Indeno(1,2,3-cd)pyrene	1-2000	0.999	0.7
Dibenz(ah)anthrene	1-2000	0.995	0.5
Benzo(ghi)perylene	1-2000	0.999	0.6
PCB 18	1-2000	0.999	0.4
PCB 28	2-2000	1.000	1.6
PCB 52	1-2000	1.000	0.8
PCB 44	1-2000	1.000	1.0
PCB 101	2-2000	1.000	1.5
PCB 149	1-2000	0.999	0.6
PCB 118	1-2000	1.000	0.8
PCB 153	1-2000	1.000	0.9
PCB 138	1-2000	0.999	0.8
PCB 180	1-2000	0.999	1.0
PCB 170	1-2000	0.999	0.8
Resmethrin	5-2000	0.998	4.0
Lamda-cyhalothrin	10-2000	0.998	6.0
Permethrin	5-2000	0.999	4.6
Cyfluthrin	20-2000	0.997	19.2
Cypermethrin	20-2000	0.998	13.3
Flucythrinate	10-2000	0.999	7.8
Fenvalerate	5-2000	0.999	4.0
Deltamethrin	10-2000	0.998	5.4

Table 3. Comparison of LOQs obtained in present work versus previously reported LOQs for PAHs and PCBs

Compound	LOQ/ ng g^{-1}			Compound	LOQ/ ng g^{-1}		
	Present work (a)	Present work (b)	Paper [37]		Present work (a)	Present work (b)	Paper [36]
Naphthalene	10.0		0.6	PCB 18	10.0	1.4	
Aceaphthyene	7.8		0.1	PCB 28	5.0	1.6	8
Acenaphthene	13.3		0.5	PCB 52	4.0	0.8	6.3
Fluorene	10.0		0.2	PCB 44	1.7	1.0	
Phenanthrene	3.4		0.4	PCB 101	5.4	1.5	5.7
Anthracene	2.6		0.5	PCB 149	5.0	0.6	
Fluoranthene	2.0		0.4	PCB 118	2.5	0.8	0.62
Pyrene	2.2		0.6	PCB 153	7.8	0.9	10
Benzo (a) anthracene	1.8	0.7	0.9	PCB 138	5.0	0.8	
Chrysene	3.3	1.0	0.7	PCB 180	6.0	1.0	6.9
Benzo (b) fluoranthene	1.0	0.4	1.0	PCB 170	6.0	0.8	
Benzo (k) fluoranthene	1.0	0.2	0.5				
Benzo (a) pyrene	1.0	0.6	0.8				
Indeno (1,2,3-cd)pyrene	1.0	0.7	0.2				
Dibenz (ah) anthracene	1.0	0.5	0.8				
Benzo (ghi) perylene	1.0	0.6	0.7				

Note: “a” means optimized DI-SPME conditions for simultaneous extraction of all targeted analytes;

“b” means optimized DI-SPME conditions for hydrophobic analytes with logP>5.

Table 4. Precision and accuracy of the proposed method

Compound	Intra-day accuracy (%)				Intra-day precision (RSD %)				Inter-day accuracy (%)				Inter-day precision (RSD %)			
	15 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	500 ng g^{-1}	15 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	500 ng g^{-1}	15 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	500 ng g^{-1}	15 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	500 ng g^{-1}
2-phenylphenol	/	113.7	105.5	96.4	/	5.8	7.2	13.5	/	113.5	101.4	88.9	/	8.8	14.4	2.2
Propoxur	/	44.8	77.2	102.4	/	17.0	3.7	2.7	/	52.9	92.5	122.7	/	14.1	1.8	3.3
Chlorpyrifos-methyl	63.3	119.8	87.2	95.6	22.3	12.0	7.1	7.2	63.7	92.9	79.4	128.0	20.2	19.5	16.4	7.4
Carbaryl	/	107.9	113.7	93.7	/	13.5	8.6	6.9	/	96.7	96.5	96.4	/	13.2	16.4	10.9
Chlorpyrifos-ethyl	105.0	143.3	75.2	95.6	2.7	20.8	6.7	7.2	137.9	95.9	100.0	95.6	21.7	17.0	8.9	6.4
Piperonyl butoxide	117.5	53.4	95.0	109.1	20.0	14.5	1.0	7.0	140.8	76.2	86.5	97.8	16.7	21.7	18.2	5.6
Resmethrin	/	97.3	98.7	93.1	/	16.1	6.6	6.9	/	74.8	102.9	95.7	/	3.2	1.1	1.7
Tetramethrin	83.2	101.6	109.6	125.5	11.9	8.8	3.7	10.2	91.9	88.1	107.9	99.7	11.9	24.7	4.3	17.0
Lamda-cyhalothrin	107.8	103.1	96.3	103.0	8.6	3.2	4.0	4.7	100.9	106.0	92.6	100.1	12.5	12.5	9.3	2.3
Permethrin	78.0	101.7	101.2	103.1	15.8	4.5	4.6	4.6	79.2	100.9	99.2	99.9	16.9	9.0	6.4	1.2
Cyfluthrin	/	87.9	103.1	113.5	/	10.2	8.5	8.8	/	93.3	101.2	100.0	/	14.1	7.8	5.7
Cypermethrin	/	100.4	110.3	114.2	/	10.8	8.2	11.3	/	110.3	97.0	106.3	/	21.2	18.1	2.1
Flucythrinate	/	106.9	106.9	109.8	/	6.0	6.6	3.7	/	96.1	102.0	99.9	/	15.9	8.9	5.6
Fenvalerate	87.4	109.7	112.6	110.6	16.9	13.9	11.5	3.8	84.6	99.2	100.8	99.9	22.8	12.3	9.5	6.2
Deltamethrin	/	96.5	95.2	112.3	/	14.3	8.0	4.6	/	91.4	84.9	100.8	/	15.2	15.9	5.6
PCB 18	106.0	102.6	98.2	88.1	6.2	4.9	1.0	1.6	99.8	107.1	98.3	100.0	11.6	8.6	3.3	7.7
PCB 28	79.7	99.8	93.6	83.2	14.6	1.8	2.9	3.1	77.5	104.7	93.3	100.2	20.6	14.1	6.2	11.0
PCB 52	75.1	102.4	98.2	93.6	6.7	2.9	1.7	3.3	72.4	105.4	100.2	99.9	7.3	14.4	4.9	5.5
PCB 44	64.2	103.6	100.4	97.4	12.3	2.1	2.1	4.2	65.4	104.1	102.2	99.8	13.4	12.7	4.6	4.3
PCB 101	103.0	99.6	89.2	84.4	7.2	4.7	5.9	5.2	91.2	97.3	88.7	100.7	25.5	7.2	5.4	11.3
PCB 149	71.0	101.0	114.6	106.2	6.1	7.9	4.3	4.5	61.2	97.1	113.4	99.5	20.2	13.7	5.7	5.8
PCB 118	106.8	101.3	90.5	82.5	10.4	0.9	5.6	5.2	96.3	110.9	91.5	100.2	16.1	17.6	8.8	11.9
PCB 153	73.1	104.0	108.9	97.1	8.9	3.5	3.2	2.8	70.5	99.3	108.1	99.7	17.8	12.8	4.9	3.5

Table 4 (*Continued*)

Compound	Intra-day accuracy (%)				Intra-day precision (RSD %)				Inter-day accuracy (%)				Inter-day precision (RSD %)			
	15 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	500 ng g^{-1}	15 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	500 ng g^{-1}	15 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	500 ng g^{-1}	15 ng g^{-1}	50 ng g^{-1}	100 ng g^{-1}	500 ng g^{-1}
PCB 138	65.2	104.0	110.3	100.4	4.9	5.8	3.2	3.0	61.7	100.0	109.6	99.6	18.7	15.2	4.9	3.1
PCB 180	75.0	98.6	99.3	86.4	11.7	2.1	0.7	2.2	74.0	104.2	99.3	100.0	10.4	20.5	4.7	8.9
PCB 170	87.0	103.8	100.6	90.3	10.7	0.5	0.6	1.9	80.5	106.2	100.6	99.9	21.1	13.4	3.4	6.5
Naphthalene	85.6	103.5	89.8	99.7	21.8	5.3	8.3	10.1	86.1	108.5	88.2	102.6	16.7	9.8	12.8	9.9
Acenaphthyene	110.9	111.4	91.7	101.4	4.3	0.7	0.9	1.1	109.9	116.7	97.7	106.1	5.3	14.0	9.4	5.0
Acenaphthene	87.6	105.6	95.8	100.4	5.6	1.8	1.4	3.9	87.3	101.9	96.2	104.8	18.5	8.0	5.3	6.7
Fluorene	88.9	104.6	97.7	100.0	3.2	4.6	2.2	0.8	89.1	109.3	98.8	102.0	7.6	15.8	4.1	9.2
Phenanthrene	93.4	105.5	97.0	100.9	2.4	3.7	1.1	0.7	94.1	102.9	98.6	106.5	20.5	15.3	1.1	3.5
Anthracene	96.5	102.9	97.9	100.7	5.8	1.4	1.8	0.9	96.7	103.7	100.0	105.4	7.5	13.7	2.3	3.9
Fluoranthene	99.7	109.7	94.0	101.9	2.8	1.1	3.7	3.7	98.9	109.2	96.8	105.9	13.5	18.8	3.4	4.4
Pyrene	94.8	109.3	94.4	101.6	3.4	1.7	4.0	4.0	95.0	110.0	97.1	105.8	6.7	14.6	3.7	5.4
Benzo(a)anthracene	95.9	108.8	94.1	100.3	6.4	2.6	1.2	0.7	96.2	107.6	96.0	103.1	7.1	10.9	3.7	1.6
Chrysene	104.8	110.7	92.7	99.8	2.7	2.4	1.6	1.9	104.8	110.8	95.4	103.3	2.7	12.8	3.5	2.7
Benzo(b)fluoranthene	80.6	114.5	91.4	100.7	4.0	2.4	1.3	1.4	80.6	118.1	94.1	104.2	6.7	18.5	5.5	3.4
Benzo(k)fluoranthene	69.4	109.3	96.1	100.5	6.7	5.9	1.8	0.9	70.1	116.6	98.0	103.2	5.1	17.3	3.6	5.0
Benzo(a)pyrene	79.1	110.6	94.0	100.3	4.4	0.9	1.3	1.0	77.6	116.9	96.4	103.1	12.2	17.6	1.4	1.6
Indeno(1,2,3-cd)pyrene	58.6	113.2	92.9	101.6	14.0	3.0	1.0	3.4	58.1	123.7	96.9	103.8	20.4	20.1	2.8	4.3
Dibenz(ah)anthrene	58.8	110.1	97.2	101.5	14.4	3.4	3.7	2.4	57.9	118.2	100.0	105.0	23.1	20.0	2.9	6.8
Benzo(ghi)perylene	88.0	122.6	84.7	102.5	11.7	1.0	1.2	1.7	86.6	113.7	90.0	105.2	18.4	20.4	10.5	5.9

Table 5. Detection results for commercial edible seaweed samples

Compound	Detection amount (ng g^{-1})				
	Dry Nori-W	Dry Wakame-Guanqun	Dry Wakame-WE1PAC	Seaweed snack-Paido	Seaweed snack-Paido
PCB 18	<LOQ	<LOQ	16.0	<LOQ	<LOQ
PCB 52	n.d	<LOQ	<LOQ	<LOQ	<LOQ
PCB 44	n.d	<LOQ	n.d	n.d	n.d
PCB 149	n.d	<LOQ	n.d	n.d	n.d
Naphthalene	n.d	n.d	n.d	<LOQ	n.d
Acenaphthene	<LOQ	<LOQ	<LOQ	n.d	n.d
Fluorene	n.d	n.d	<LOQ	n.d	n.d
Phenanthrene	n.d	<LOQ	15.5	n.d	n.d
Anthracene	<LOQ	<LOQ	<LOQ	n.d	n.d
Fluoranthene	n.d	n.d	5.2	n.d	n.d
Pyrene	<LOQ	<LOQ	n.d	n.d	n.d
Chrysene	n.d	<LOQ	<LOQ	n.d	n.d
2-phenylphenol	n.d	n.d	n.d	<LOQ	<LOQ
Chlorpyrifos-methyl	n.d	<LOQ	<LOQ	n.d	n.d
Resmethrin	n.d	<LOQ	n.d	n.d	n.d
Tetramethrin	n.d	n.d	8.3	n.d	n.d
Permethrin	<LOQ	n.d	<LOQ	n.d	n.d
Cyfluthrin	n.d	n.d	<LOQ	n.d	n.d
Fenvalerate	n.d	n.d	n.d	n.d	n.d