

Extraction of Hydrocarbons from Seaweed Samples Using Sonication and Microwave-Assisted Extraction: A Comparative Study

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Abstract

A sonication method is compared with a microwave-assisted extraction method for recovering polycyclic aromatic hydrocarbons and aliphatic hydrocarbons from seaweed and acid humic samples. Different extracting solvents and adsorbents for the purification step are tested. For the sonication extraction, 10 g of the sample are extracted in an ultrasonic bath (60°C for 15 min with 20 mL of hexane). For the microwave-assisted extraction two steps are carried out, each step at 90°C under pressure in closed vessels with 20 mL of hexane for 10 min at 950 W. A clean-up step is performed for both extraction techniques. The results indicate that the recovery of hydrocarbons is dependent on both the extraction technique and the type of matrix. The most suitable technique appears to be sonication employing hexane as the extraction solvent. The recoveries obtained for aliphatic hydrocarbons are higher than those achieved for the polycyclic aromatic hydrocarbons, with values ranging within 81–109% and 40–76%, respectively.

Introduction

In recent years, there has been growing concern about the investigation of organic pollutants in a variety of environmental samples. Some of the most toxic and widely distributed include polycyclic aromatic hydrocarbons (PAHs), which are often found together with aliphatic hydrocarbons (AHs) characterized by the range C₁₅ to C₃₆ (1). PAHs exhibit carcinogenic, mutagenic, and estrogenic properties (2). They are currently ranked eighth on the hazardous substance list produced by the Agency for Toxic Substances and Disease Registry (3) and the Environmental Protection Agency (EPA).

Hydrocarbons enter the marine environment through natural pathways and anthropogenic processes (i.e., urban runoff, sewage disposal, and industrial effluents). Chronic pollution from these kinds of inputs affects marine organisms and should

be studied and quantitated (4). In coastal areas, PAHs are generally divided into three categories based upon diagnostic ratios: petrogenic, pyrogenic, and biogenic (5). Studying the hydrocarbon content of algae is of great importance because of its increasing presence in the markets as a new food. It has been estimated that human intake of PAHs from food is considerably higher than that from ambient air or drinking water (6).

The present study aimed to analyze two different extraction techniques [sonication and microwave-assisted extraction (MAE)] for the determination of PAHs and AHs in low contaminated seaweed samples collected off the Galician coast (Spain) because of the scarcity of contributions in this field.

Sonication extraction is widely employed in the cleaning of materials, extraction processes, and chemical processes (7). Some authors suggest this technique is less efficient than Soxhlet extraction (8); however, others report recovery efficiencies that are similar or better (9). Sonication offers the advantages of faster extraction times, relatively low cost, and small solvent volumes (8:10). This study analyzes the parameters of solvent type, solvent volume, and time of sonication in the ultrasonic extraction.

Another widely used extraction technique is MAE. It is used with environmental pollutants such as hydrocarbons, organochlorine pesticides, chlorinated pesticides and polychlorinated biphenyls, and dioxins and furans (11). MAE uses an organic solvent or a mixture of solvents to extract organic compounds from a solid matrix, which is irradiated by microwaves (12). The main advantages of this technique include reduced extraction time (typical sample preparation time for this technique is 10 min for extraction and 40 min for extract cooling, centrifugation, and extract concentration) and reduced solvent use (30 mL in the MAE, versus 300 mL in the Soxhlet extraction). Up to 12 samples can be extracted simultaneously within minutes (this study was performed with five samples being extracted simultaneously), resulting in increased sample throughput (13). The major limitation of MAE is that the solvent needs to be physically removed from the sample matrix prior to analysis (14). This study analyzes the parameters of solvent type, irradiation power, number of samples extracted per run, sample quantity, and matrix effects in MAE.

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To determine which is the most suitable technique for the analysis of several edible seaweed samples collected off the Galician coast, the recoveries obtained with sonication extraction are compared with those obtained by MAE for five PAHs included on the EPA Priority Pollutant List (anthracene, fluorene, fluoranthene, naphthalene, and pyrene) and six AHs (C₁₂, C₁₄, C₁₆, C₁₈, C₂₀, and C₂₈). Commercial humic acid was also analyzed because of its ability to interact with these organic compounds (15).

Materials and Methods

Chemicals

Standards of aliphatic hydrocarbons: C₁₂, C₁₄, C₁₆, and C₁₈ were purchased from Aldrich (St. Louis, MO). C₂₀ was supplied by Supelco (Bellefonte, PA). C₂₈ was acquired from AnalytiCals Carlo Erba (Milan, Italy).

Standards of the PAHs anthracene and naphthalene were obtained from Carlo Erba (Milan, Italy). Fluorene, fluoranthene, and pyrene were supplied by Fluka (Buchs, Switzerland).

Residue-analysis grade n-hexane was from Merck (Darmstadt, Germany). Acetone and methanol were from Carlo Erba. Florisil, alumina, and silica were purchased from Aldrich. Gases (helium, air, and hydrogen) were supplied by SIAD (Bergamo, Italy).

Standards

A global solution of all AHs (1000 mg/L) was prepared in n-hexane. A global solution of PAHs (1000 mg/L) was prepared in methanol. A 10 mg/L solution of aliphatics was also prepared in acetone by appropriate dilutions of the one prepared in n-hexane.

Samples

The three brown seaweed samples chosen for this study were: *Undaria pinnatifida*, *Himantalia elongata*, and *Saccorhiza polyschides*, which were collected from different locations off the Galician Coast. A commercial humic acid sodium salt (Aldrich, St. Louis, MO) was also studied.

The *Undaria* sample was obtained dehydrated. The other two samples were supplied together and fresh, and then they were dried in the lab at 40°C in a ventilated oven until the constant weight was reached. These two samples were dealt with together, and both were referred to as brown algae. All three samples were powdered and homogenized before extraction was carried out.

Spiked matrix preparation

Spiked matrix samples were prepared as follows: 10 or 3 g of the matrix (depending on the extraction technique employed) were placed in a 15-mL vial. A suitable volume of standard solution was added (20 mg contaminant/kg of sample). The vial was hermetically closed with a Teflon-faced silicon top cap (Penicillin type) and stirred overnight. The extraction solvent was then added just before analysis. Tests for impurities were carried out by the extraction of blank matrices.

Ultrasonic extraction

Ultrasonic extractions were carried out following a modified version of the official method EPA 3550B (16) for extracting non-

volatile and semivolatile organic compounds from soil, sludge, and waste. Ten grams of each sample were weighed into 50-mL centrifuge tubes and spiked as previously described. The next day samples were extracted with 20 mL of n-hexane in an ultrasonic bath (Transsonic Digital Elma, Singen, Germany) for 15 min at 60°C. Extracts were stirred for 15 min (200 rpm). The supernatant was transferred to another tube and centrifuged at room temperature for 10 min at 2000 rpm. The supernatant obtained after this step then underwent the purification step.

MAE

MAE was developed using a Milestone MLS-1200 microprocessor-controlled microwave digestion system with the capacity to reach 1000 W power and store up to 100 10-step programs (Milestone, Bergamo, Italy). It was equipped with a 12-sample tray and 100-mL TFM fluoropolymer extraction vessels. The MAE method was carried out according to the Milestone Method FO-003-SEL (17).

A 3 g portion of each sample was weighed, spiked as previously described, and stirred overnight. The following day, 20 mL of n-hexane was added to the samples. The entire mixture was transferred to the Teflon-lined extraction vessels. Next, the vessels were hermetically closed and extractions were performed at 950 W for two 10 min steps. After extraction, the reactors were allowed to cool to room temperature before being opened. The supernatant was transferred to a 15-mL vial and then purified, as is described later.

Clean-up step

The clean-up of both extracts was carried out with activated florisil following the EPA method 3620B (18).

The florisil (1g) was weighed into a vial, and 2 mL of extract was added. The vial was closed and shaken vigorously. The supernatant was transferred to another vial and analyzed by a gas chromatography (GC)–flame ionization detector (FID).

GC–FID analysis

A Varian CP-3800 GC equipped with an Autosampler 8200 and a FID was used. The GC was fitted with a Phenomenex ZB-5 capillary column (30 m × 0.25-mm i.d., 0.25- μ m film thickness). Helium was used as the carrier gas with a flow rate of 0.8 mL/min. The injection port and detector temperatures were set at 280°C and 300°C, respectively. The column temperature was programmed as follows: 2 min at 50°C, ramped to 140°C at 40°C/min, raised to 240°C at a rate of 8°C/min, and then to 280°C at a rate of 2°C/min, isothermal at 280°C for 1 min.

Results and Discussion

Calibration

Identification of the analytes was based on comparison of retention times with those obtained from standard solutions. Calibration curves were constructed using diluted solutions with a range concentration (0.5–20 mg/L). The curves consisted of a plot of peak area versus concentration. Table I summarizes the calibration curve data. In all cases, the curves were shown to be linear, with correlation coefficients higher than 0.99 for all compounds.

Extraction yields and efficiency of the tested extraction procedures

Table II shows the results obtained by both extraction techniques. Recovery data for AHs were higher than those obtained

Table I. Linearity and Calibration Curves Data

Analyte	Slope	Intercept	Linearity (r^2)
C ₁₂	7687	-1099	0.9998
Naphthalene	6235	-1556	0.9999
C ₁₄	6576	-1041	0.9998
C ₁₆	6590	-1461	0.9999
Fluorene	4407	-1474	0.9998
C ₁₈	5507	-1390	0.9998
Anthracene	3371	-1225	0.9997
Fluoranthene	3249	-834	0.9998
Pyrene	3945	-1012	0.9998
C ₂₀	3863	-899	0.9995
C ₂₈	2898	-798	0.9987

Table II. Recovery Experiments Obtained with Both Extraction Methods

Analyte	Sonication (%Recoveries)			MAE (%Recoveries)		
	Undaria	Humic acid	Brown algae	Undaria	Humic acid	Brown algae
C ₁₂	90.7	97.5	93.5	83.9	50.4	62.5
Naphthalene	66.3	76.2	70.0	61.5	36.1	48.6
C ₁₄	91.1	98.6	95.6	84.5	49.2	61.4
C ₁₆	89.0	97.8	97.4	78.6	45.4	60.9
Fluorene	50.5	60.0	56.0	49.9	30.2	39.1
C ₁₈	81.1	87.8	87.7	82.3	45.6	53.5
Anthracene	46.8	58.1	52.6	83.7	28.4	35.9
Fluoranthene	42.6	44.0	45.4	33.8	0.6	31.4
Pyrene	40.4	49.0	46.1	34.6	16.2	30.5
C ₂₀	87.8	105.6	97.2	81.4	58.6	59.8
C ₂₈	93.7	101.5	109.3	90.5	27.4	61.9

Table III. Recovery Experiments Obtained with Other Adsorbents in Undaria Sample

Analyte	Sonication (%Recoveries)			MAE (%Recoveries)	
	Florisil	Silica	Alumina	Silica	Alumina
C ₁₂	32.55	24.60	59.95	51.94	58.53
Naphthalene	28.69	14.01	42.76	35.48	48.16
C ₁₄	29.90	23.17	97.92	51.40	67.29
C ₁₆	27.96	22.12	57.75	50.76	51.01
Fluorene	24.15	10.20	32.32	31.97	43.22
C ₁₈	22.92	18.71	50.50	47.56	46.45
Anthracene	19.90	9.29	19.84	33.52	39.86
Fluoranthene	20.59	8.19	20.71	28.89	44.13
Pyrene	16.81	8.31	22.00	25.45	33.96
C ₂₀	20.70	20.26	54.00	49.05	49.83
C ₂₈	20.27	22.32	58.03	60.21	57.10

for the PAHs in all cases. Using sonication, the recovery results arranged in descending order were as follows: humic acid > brown algae > Undaria pinnatifida. Using MAE, the recovery results arranged in descending order were as follows: Undaria pinnatifida > brown algae > humic acid. It appears that MAE was only suitable for the extraction of these compounds in Undaria samples. The results indicated that sonication was the extraction technique of choice.

Comparison of both methods by general parameters

The most appropriated methodology for the extraction of a certain class of compounds should strike a balance between considerations, such as extraction yields and selectivity, time of extraction, consumption of solvents, and use of temperature and pressure discharges.

Both techniques presented the advantage of providing short extraction times, but sonication proved to be faster despite being followed by a centrifuge step. This happened because in MAE the samples reached a higher temperature, and all sample vessels had to remain unopened until atmospheric pressure was reached (~ 30 min) to ensure that the more volatile PAHs, such as naphthalene, were not lost.

Any reduction in the use of organic solvents was advisable. However, there was no difference between the two techniques in the volume of n-hexane used; thus, this parameter was not a determinant. The same was true for the clean-up step because both techniques required purification.

Effect of solvent and other adsorbents

To see if recoveries could be improved, a different solvent was tested. A mixture of n-hexane-acetone (60:40) was selected to increase the solubility of the most polar compounds.

The MAE method was changed to Milestone EN-003-SEL (19), which employed 2 mL less of extraction solvent, and it required that the second step of the microwave program to be 10 min longer.

For the purpose of verifying if florisil was retaining the PAHs, alumina and silica were tested as alternative adsorbents for the purification step.

Because of the fact that acetone deactivates adsorbents, the purification step was carried out as follows: 2 mL of the extract were transferred to a vial and water was added in order to drag along the acetone. After shaking, the aqueous phase was removed and sodium sulphate was added. The supernatant was transferred to a vial with 1 g of adsorbent, and 1 mL of hexane was added.

Tables III and IV show the recovery results. It was not possible to carry out these experiments with the brown algae because of a lack of samples. The extract of humic acid obtained by MAE was not enough to carry out the purification with alumina, so Table IV only presents results for silica.

In spite of what happened, better results were obtained with MAE than with sonication when using a hexane-acetone mixture.

With respect to the adsorbent, silica seems to be a good adsorbent for the analysis of AHs; however, it retains aromatic hydro-

Table IV. Recovery Experiments Obtained with Other Adsorbents in Humic Acid Sample

Analyte	Sonication (%Recoveries)		MAE (%Recoveries)
	Silica	Alumina	Silica
C ₁₂	55.11	55.11	126.51
Naphthalene	20.08	39.50	62.77
C ₁₄	54.09	54.16	131.29
C ₁₆	50.89	49.36	123.76
Fluorene	11.00	15.27	43.43
C ₁₈	44.02	40.69	111.45
Anthracene	11.61	5.74	61.15
Fluoranthene	8.89	8.54	37.38
Pyrene	12.84	12.77	37.28
C ₂₀	59.55	49.71	110.21
C ₂₈	68.87	47.50	187.42

carbons. For *Undaria* samples, alumina yields better recoveries than silica or florisil in sonication extraction, and it yields similar recoveries to silica in MAE. For humic acid samples, alumina provides similar recoveries to silica in sonication.

Conclusion

When employing hexane as the extraction solvent, the best results for both groups of hydrocarbons were found with sonication extraction. In all cases, better recoveries were obtained for AHs than for aromatic hydrocarbons.

Using a hexane–acetone mixture instead of hexane did not improve the results. However, the hexane–acetone mixture achieved better recoveries with MAE than with sonication.

For AHs, the results obtained with florisil or silica were similar, though for the PAHs, florisil seemed to be the most suitable.

In the authors' opinion, the best protocol is ultrasonic extraction with hexane followed by clean-up with florisil.

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References

1. E. Vázquez Blanco, P. López Mahía, S. Muniategui Lorenzo, D. Prada Rodríguez, and E. Fernández Fernández. Optimization of microwave-assisted extraction of hydrocarbons in marine sediments: comparison with the Soxhlet extraction method. *Fresenius' J. Anal. Chem.* **366**: 283–88 (2000).
2. J.D. Berset, M. Ejem, R. Holzer, and P. Lischer. Comparison of dif-

ferent drying, extraction and detection techniques for the determination of priority polycyclic aromatic hydrocarbons in background contaminated soil samples. *Anal. Chim. Acta* **383**: 263–75 (1999).

3. ATSDR, CERCLA, 2003. CERCLA Priority List of Hazardous Substances. <http://www.atsdr.cdc.gov/clist.html>. Date Accessed: September 14, 2006.
4. N. Venturini and L.R. Tommasi. Polycyclic aromatic hydrocarbons and changes in the trophic structure of polychaete assemblages in sediments of Todos os Santos Bay, Northeastern, Brazil. *Mar. Poll. Bull.* **48**: 97–107 (2004).
5. M.D. Fang, C.L. Lee, and C.S. Yu. Distribution and source recognition of polycyclic aromatic hydrocarbons in the sediments of Hsinta Harbour and adjacent coastal areas, Taiwan. *Mar. Poll. Bull.* **46**: 941–53 (2003).
6. A. Barranco, R.M. Alonso-Salces, A. Bakkali, L.A. Berrueta, B. Gallo, F. Vicente, and M. Sarobe. Solid-phase clean-up in the liquid chromatographic determination of polycyclic aromatic hydrocarbons in edible oils. *J. Chromatogr. A* **988**: 33–40 (2003).
7. L.M. Assis, J.J. Silva Pinto, and F.M. Lanças. Comparison among different extraction methods (PFE, SFE, Sonication, Soxhlet) for the isolation of organic compounds from coal. *J. Microcolumn Sep.* **12**: 292–301 (2000).
8. T.F. Guerin. The extraction of aged polycyclic aromatic hydrocarbon (PAH) residues from a clay soil using sonication and a Soxhlet procedure: a comparative study. *J. Environ. Monit.* **1**: 63–67 (1999).
9. V. Pino, J.H. Ayala, A.M. Afonso, and V. González. Ultrasonic micellar extraction of polycyclic aromatic hydrocarbons from marine sediments. *Talanta* **54**: 15–23 (2001).
10. P.A.P. Pereira, J.B. De Andrade, and A.H. Miguel. Determination of 16 priority polycyclic aromatic hydrocarbons in particulate matter by HRGC-MS after extraction by sonication. *Anal. Sci.* **17**: 1229–31 (2001).
11. Y.Y. Shu, R.C. Lao, C.H. Chiu, and R. Turle. Analysis of polycyclic aromatic hydrocarbons in sediment reference materials by microwave-assisted extraction. *Chemosphere* **41**: 1709–16 (2000).
12. H. Budzinski, M. Letellier, S. Thompson, K. LeMenach, and P. Garrigues. Combined protocol for the analysis of polycyclic aromatic hydrocarbons (PAHs) and polychlorobiphenyls (PCBs) from sediments using focussed microwave-assisted (FMW) extraction at atmospheric pressure. *Fresenius' J. Anal. Chem.* **367**: 165–71 (2000).
13. V. Lopez-Avila, R. Young, and W.F. Beckert. Microwave-assisted extraction of organic compound from standard reference soils and sediments. *Anal. Chem.* **66**: 1097–1106 (1994).
14. J.R. Dean and G. Xiong. Extraction of organic pollutants from environmental matrices: selection of extraction technique. *Trends Anal. Chem.* **19**: 553–64 (2000).
15. Y.P. Chin, G.R. Aiken, and K.M. Danielsen. Binding of pyrene to aquatic and commercial humic substances: the role of molecular weight and aromaticity. *Environ. Sci. Technol.* **31**: 1630–35 (1997).
16. Environmental Protection Agency 3550B, 1996. Ultrasonic Extraction. Revision 2, December. Washington, D.C. <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3550b.pdf>. Date accessed: September 14, 2006.
17. Milestone FO-003-SEL. "Total oil from palm fiber". Milestone, Microwave Laboratory Systems, Shelton, CT (1999).
18. Environmental Protection Agency 3620B, 1996. Florisil Clean-up. Revision 2, December. Washington, D.C. <http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3620b.pdf>. Date accessed: September 14, 2006.
19. Milestone EN-003-SEL. "Polynuclear aromatic hydrocarbons (PAHs) from sludges". Milestone, Microwave Laboratory Systems, Shelton, CT, 1999.