Supercritical fluid extraction of synthetic organochlorine compounds in submerged aquatic plants

Phillip R. McEachern and Gregory D. Foster

Department of Chemistry, George Mason University, Fairfax, VA 22030 (USA)

ABSTRACT

Supercritical carbon dioxide was used to extract nine synthetic organochlorine compounds (SOCs) from tissues of the aquatic plant *Hydrilla verticillata* in an off-line supercritical fluid extraction configuration. Lyophilized plant tissues (50 to 100 mg) were fortified with the SOCs at a level of 5 to 10 mg/kg and subjected to supercritical carbon dioxide extraction at 50°C and 38 MPa for 15 min. Mass recoveries of the SOCs amended to plant tissues averaged between 89 and 109%. When Florisil was present in the outlet end of the extraction vessel for the purpose of direct in-line clean-up during supercritical carbon dioxide extraction, mass recoveries of the amended SOCs were markedly lower for four of the nine analytes.

INTRODUCTION

During the past decade, the explosion in biomass of submerged aquatic vegetation (SAV) throughout the tidal freshwater reach of the Potomac river near Washington, DC, USA, particularly by the vascular plant *Hydrillu verticillata* Royle [1], has prompted interest in the role of SAV in influencing the environmental behaviour and biogeochemical cycling of synthetic organic compounds (SOCs) in the lower Potomac river aquatic food web. The collective SAV abundance in the Potomac river during the spring and summer growing seasons creates a sizable reservoir of plant lipids into which hydrophobic organic compounds may partition from contaminated water and sediments. To address this issue, it has become necessary to measure refractory organochlorine contaminants bioaccumulated in *Hydrilla* tissues.

The recent application of supercritical carbon dioxide extraction (SCDE) in trace analysis has been reported for a wide variety of analytes and matrices -- including volatile food and fragrance constituents [2–4], pesticides in soils and tissues [5–9], hydrocarbons in marine sediments [10], and air pollutants collected on polyurethane foam sorbents [11] -- clearly demonstrating the benefits and usefulness of this extraction technique. The supercritical fluid state of matter has unique physical properties which are now being exploited in environmental trace analysis to lessen the need for using large amounts of subcritical organic solvents, such as dichloromethane. Supercritical fluids generally have low viscosities (e.g., 10^{-3}–10^{-4} g/cm·s), moderate densities (e.g., 0.348 g/cm^3), and high diffusivities (e.g., 10^{-3}–10^{-4} cm^2/s) enabling very efficient solubilization and mass transfer processes to rapidly isolate trace organics from solid matrices such as sediments, solid sorbents, and tissues. It was the overall intent of this study to assemble a laboratory-built SCDE apparatus and develop methods by which SOCs could be extracted from *Hydrillu* tissues in an off-line collection mode for subsequent analysis by using gas chromatography-electron-capture detection (GC-ECD).

Correspondence to: G. D. Foster, Department of Chemistry, George Mason University, Fairfax, VA 22030, USA.

EXPERIMENTAL

Plant sample preparation

Hydrilla verticillata specimens were collected with a rake, wrapped in clean aluminium foil, and frozen upon return to the laboratory until further processing. *Hydrilla* tissue was prepared for analysis by lyophilization in a VirTis Unitrap II freeze dryer (The VirTis Company, Gardiner, NY, USA) utilizing a dry ice/2-propanol slush as a pump oil fume trap to prevent sample contamination. The freeze dried sample was then dry homogenized in a Waring blender with a stainless-steel cup, transferred to a clean beaker, and placed in a desiccator until analysis. Care was taken when sub-sampling to obtain a consistent range of particle sizes when placing them into the SCDE extraction vessel. Plant tissue sample sizes ranged from 50 to 100 mg.

A 3-g portion of lyophilized, homogenized *Hydrilla* tissue was Soxhlet extracted with dichloromethane for 24 h to determine the percent weight of extractable lipids in the plant samples. The dichloromethane extract was subsequently evaporated to dryness and the residue, regarded as extractable lipids, was measured gravimetrically.

Apparatus

A schematic diagram of the SCDE setup is shown in Fig. 1. The supercritical state was attained and maintained through the use of a Perkin-Elmer Series 10 high-performance liquid chromatography pump (Perkin-Elmer, Norwalk, CT, USA) which had been modified for low-temperature liquid flow by the addition of a custom-designed pump-head heat exchanger according similar specifications reported by Simpson *et al.* [12] for supercritical fluid chromatography. Liquid carbon dioxide was pumped first (Fig. 1) through a 4-m section of 1/16 in. (1 in. = 2.54 cm) (O.D.) stainless-steel tubing (f) which itself was immersed in a constant-temperature water bath held at 50°C (e). Supercritical carbon dioxide then entered a flow-through pressure gauge [g; Alltech, Deerfield, IL, USA, 6000 p.s.i. (1 p.s.i. = 6894.76 Pa) full scale] in route to the extraction vessel (h), also positioned in the water bath. An SSI on/off valve (Alltech) followed the extraction vessel (i), to which was connected a low dead volume stainless-steel union fitted with an internal reducer (Suprex, Pittsburgh, PA, USA), 1/32 in. gland
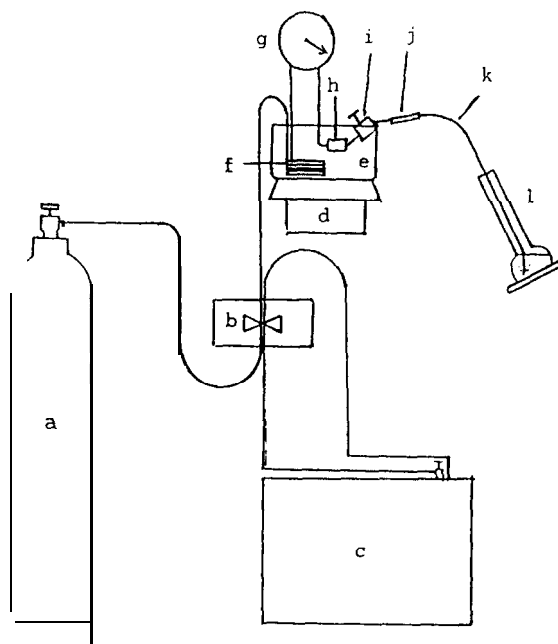

Fig. 1. Schematic diagram of apparatus used for supercritical carbon dioxide extraction: (a) CO, source; (b) CO, pump; (c) recirculating chiller for pump head cooling; (d) hot plate/stirrer; (e) large Pyrex crystallization dish; (f) heat exchange coil; (g) pressure gauge; (h) extraction vessel; (i) on/off valve; (j) low dead volume connector; (k) fused-silica restrictor; and (l) collection flask.

(Suprex), and 1/32 in. Vespel ferrule (Suprex); this arrangement was used to connect 20-22 μm (I.D.) fused-silica restrictor tubing (Polymicro Technologies, Phoenix, AZ, USA) to the SSI valve. The lengths of restrictors used were *ca.* 30–35 cm.

The extraction vessel (Fig. 2) was constructed by fitting a Swagelock low dead volume 1/16 in. to 1/4 in. stainless-steel reducing union to a Swagelock stainless-steel 1/16 in. to 1/4 in. fractional tube to fractional tube stub (Washington Valve and Fitting, Rockville, MD, USA). It was necessary to modify the vessel to utilize a 1/4 in. diameter, 0.062 in. thick, 0.5 μm porosity stainless-steel frit (Alltech) by grinding 1/8 in. from the fractional tube stub cylinder, giving an internal volume of about 0.31 ml.

Extraction and collection

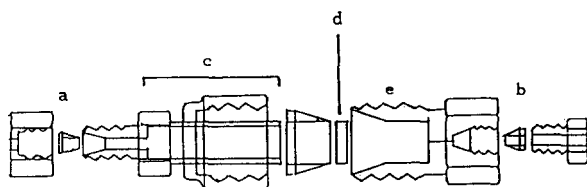The carbon dioxide used for SCDE was SFE grade and was acquired from Scott Specialty Gases

Fig. 2. Exploded view of supercritical fluid carbon dioxide extraction vessel: (a) inlet, stainless-steel 1/16 in. fractional tube and ferrules; (b) outlet, stainless-steel 1/16 in. male nut and ferrules; (c) Swagelock stainless-steel 1/16 in. fractional tube to 1/4 in. fractional tube stub; (d) 1/4 in. diameter, 0.5 μm pore size stainless-steel frit; and (e) Swagelock stainless-steel 1/4 in. to 1/16 in. low dead volume reducing union.

TABLE I

ORGANOCHLORINE ANALYTES USED IN THIS STUDY AND THEIR IMPORTANT PHYSICOCHEMICAL PROPERTIES

Analyte	pS_w^a	$\log K_{ow}^b$	Ref.[c]	GC peak No.[d]
Aldrin	7.4	na	13	4
a-Chlordane	6.9	6.0	14	7
y-Chlordane	6.9	6.0	14	6
4,4′-DDE	7.5	5.7	14	8
Dieldrin	7.2	5.4	14	9
y-HCH (Lindane)	6.3	3.8	14	3
Hexachlorobenzene	6.9	5.5	15	2
Oxychlordane	na[e]	na		5
Pentachlorobenzene	6.3	5.2	15	1

[a] Inverse logarithm of molar water solubility.
[b] Logarithm of n-octanol-water partition coefficient.
[c] Physical property literature reference.
[d] Gas chromatography peak reference number for Figs. 3 and 4.
[e] na indicates parameter not available.

(Plumsteadville, PA, USA). During preliminary off-line SCDE, a pressure of 38 MPa (ca. 5500 p.s.i.) was maintained in the extraction vessel throughout the extraction cycle and extractions were performed in the dynamic mode. SCDE was performed for 15 min with a steady-state liquid carbon dioxide flow of 0.25 ml/min registered at the pump (corresponding to cu. 12 extraction vessel turnover volumes of equivalent liquid carbon dioxide).

Approximately 3 ml of n-hexane (Burdick & Jackson, Waukeegan, WI, USA) was placed in a 5-ml volumetric flask (Fig. 11) to collect the extracted analytes from decompressing carbon dioxide. The fused-silica restrictor (Fig. lk) was immersed directly in the collection solvent and was held in place with the aid of a paper clip. Mirex was added as a surrogate standard to the flask prior to extraction to evaluate the retention of the analytes collected in n-hexane during carbon dioxide sparging. Holdup of analyte in the restrictor tube was evaluated (i) by performing SCDE using clean, empty extraction vessels immediately following a sample extraction with the same restrictor used in the sample extraction, and (ii) by analyzing n-hexane rinses of the restrictor tube after sample extractions. Isodrin was added to the solution after SCDE as an internal injection standard and contents in the collection flask were diluted to volume with n-hexane.

SCDE performance

The synthetic organochlorine compounds used to evaluate SCDE of *Hydrilla* tissue are shown in Table I along with their important physicochemical properties. Pure standards of each of these compounds along with isodrin and mirex were obtained from the U.S. EPA Pesticides and Industrial Chemicals Repository (Research Triangle Park, NC, USA) in neat form.

All general purpose glassware was scrupulously cleaned with nitric acid solution, rinsed in distilled water and burned overnight at 450°C, stored covered by burned aluminum foil, and rinsed repeatedly with hexane before use. Volumetric glassware was soaked in nitric acid solutions for 12 h, rinsed and sealed with foil, and hexane rinsed repeatedly prior to use. Granular anhydrous sodium sulfate (J. T. Baker, Haupauge, NJ, USA) was used as the matrix for the initial optimization of SCDE conditions. Finely cut glass wool was placed within the extraction vessel along with Florisil sorbent and the sample for the evaluation of in-line clean-up. Sodium sulfate, Florisil, and glass wool were burned at 450°C overnight to remove any trace organic residues.

Initial optimization experiments involved spiking sodium sulfate housed in the extraction vessel with a hexane solution containing the nine analytes − the hexane was allow to evaporate from the extraction vessel before SCDE was initiated − and sequentially extracting the sample over 15-min intervals, with the collection flask and solvent renewed at each interval, for a total of 60 min (4 fractions). In addi-

tion, SCDE of spiked sodium sulfate was performed under 23, 30 and 38 MPa pressure regimes.

Extraction performance involving actual samples of **Hydrilla** were performed in several stages. First, 50–100-mg samples of lyophilized **Hydrilla** tissue were loaded into the extraction vessel and SCDE was performed with and without the analytes amended to the sample. Secondly, Florisil was placed in the extraction vessel along with the tissue to minimize plant lipid carryover into the collection flask (*i.e.,* clean-up); the materials were loaded into the extraction vessel in the order of **Hydrilla,** glass wool (a neutral separator), and Florisil. Blanks were extracted with only glass wool and Florisil present. The analytes were amended to the samples at a concentration of 5-10 mg/kg per component. All SCDE experiments were performed either in triplicate or quadruplicate.

GC *analysis*

All GC analyses were performed on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a ^{63}Ni electron-capture detector. The gas chromatograph was fitted with a 30 m × 0.25 mm I.D., 0.25 μm film thickness, SE-54 fused-silica capillary column (Alltech). Samples were introduced into the gas chromatograph via the split-less mode of a split/splitless injector system. The GC injector and detector were maintained at 250 and 300°C, respectively. Helium was used as the carrier gas and its linear velocity through the column was 35 cm/s at 100°C, and 5% methane in argon was used as make up gas for the detector and its flow was maintained at 60 ml/min. Separations on the GC were temperature programmed with an initial temperature of 50°C (1 min hold), ramped from 50°C to 120 °C at a rate of 10°C per minute (with a 1-min hold at 120°C), then ramped again at a rate of 4°C per minute to 280°C (9.5 min final hold) for a total run time of 60 min. Analyte recoveries were obtained directly from auto-quantitation routines programmed in a Hewlett-Packard 3396 recording integrator.

RESULTS AND DISCUSSION

SCDE optimization

Extraction temperature was partially decided on by conforming to a temperature close to that com-

monly used in other SCDE experiments [16]. However, the most important factors in selecting the extraction temperature were based on the following considerations: (a) 50°C is safely above the 31°C critical temperature of carbon dioxide; (b) 50°C was a convenient temperature to use with the constant temperature water bath; and (c) it was desirable to keep the solubilization power of carbon dioxide at a maximum with working pressures less than 40 MPa by optimizing density. Although extraction temperature is known to be an important variable in SCDE, its effect on the target analyte recoveries was not studied in the experiments described herein.

Initial experiments involving SCDE of analyte-spiked anhydrous sodium sulfate demonstrated that recoveries were essentially independent of applied pressure. Therefore, 38 MPa was used subsequently in all SCDE experiments because it was known this pressure was well above any pressure-limited extraction condition, and this represented the highest density state for supercritical carbon dioxide (0.90 g/ml at 50°C and 38 MPa) that was feasible to use in the lab-built system.

Extraction of the target analytes from anhydrous sodium sulfate at 38 MPa and 50°C was essentially complete during the first 15 min as evidenced by Fig. 3. All of the analytes extracted during the second 15 min were present at levels below quantitative analysis. Because anhydrous sodium sulfate was considered to be an inert surface, the results obtained from the optimization experiments were thought to reflect the most ideal SCDE conditions from which a starting point for plant tissue analysis was derived.

SCDE of Hydrilla tissue

Efficient isolation of the target analyses amended to *Hydrilla* tissue was obtained after 15 min of extraction at 50°C and 38 MPa as shown in Fig. 4 and Table II. Less than 0.5% of the amended analytes were recovered during a second 15 min extraction of the same sample (see Fig. 4b). The third and fourth 15 min extraction intervals over the cumulative 60 min extraction showed similar chromatograms to that displayed in Fig. 4b. Chromatograms of blank extractions (with and without the presence of Florisil and glass wool) were nearly identical to the chromatogram shown in Fig. 4b, with the only exception from Fig. 4b being the absence of the
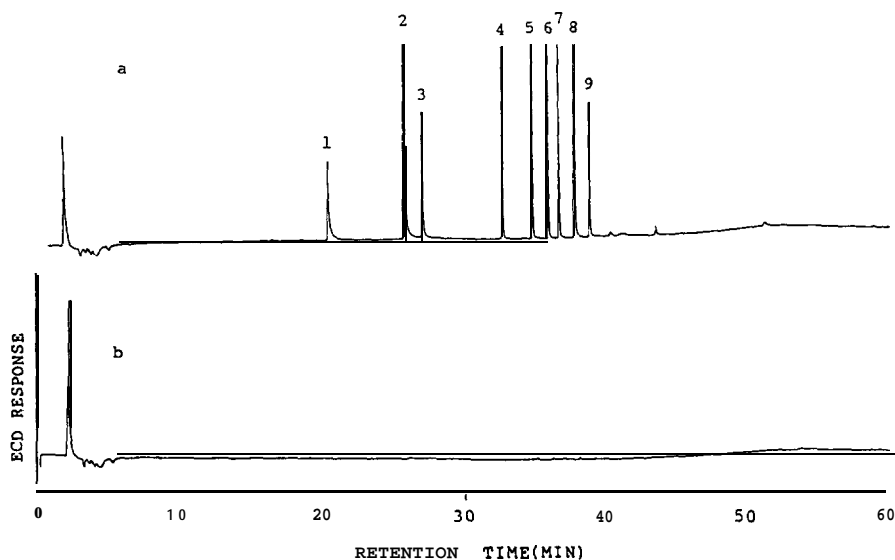
Fig. 3. Chromatograms showing optimization results from supercritical carbon dioxide extraction of analyte-amended (10 mg/kg per component) sodium sulfate: (a) first 15 min period of extraction; and (b) second 15 min period of extraction. GC peak designations arc listed in Table I.

barely visible analyte peaks (isodrin and mirex excluded) in the blank chromatograms.

Recoveries of amended analytes in *Hydrilla* tissues are shown in Table II. Mean recoveries ranged from 89% to 109% for SCDE carried out on amended *Hydrilla* tissue alone, and the R.S.D. values for the same extractions varied from 11.8 to 20.4%. Mirex recoveries were greater than 85% in
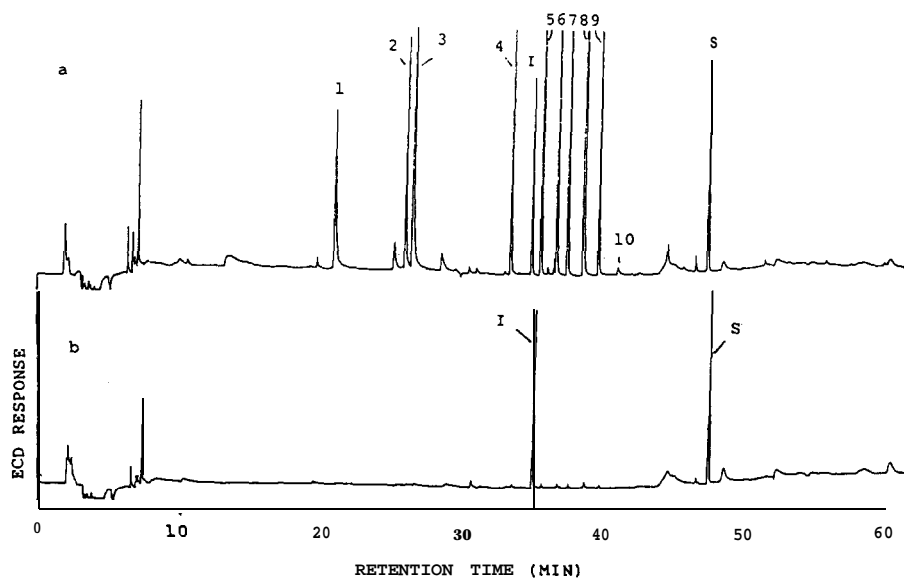

Fig. 4. Chromatograms showing supercritical carbon dioxide extraction of analyte-amended (10 mg/kg per component) *Hydrilla* tissue: (a) first 15 min period of extraction; and (b) second 15 min period of extraction. GC peak designations are listed in Table I. Isodrin internal standard (I) and mirex surrogate standard (S) peaks are also labeled in both chromatograms.

TABLE II

RECOVERIES OF ORGANOCHLORINE ANALYTES FROM HYDRILLA TISSUE USING OFF-LINE SUPERCRITICAL CARBON DIOXIDE EXTRACTION WITH AND WITHOUT FLORISIL SORBENT

Analyte	*Hydrilla* alone[a]		*Hydrilla* + glass wool + Florisil[b]	
	Mean recovery (%)	R.S.D. (%)	Mean recovery (%)	R.S.D. (%)
Aldrin	87.2	14.3	86.8	3.4
a-Chlordane	95.0	13.5	99.3	5.2
y-Chlordane	98.6	11.8	101	6
4,4'-DDE	89.8	12.9	73.0	19.3
Dieldrin	109	15	55.9	45.0
γ-HCH (Lindane)	90.9	20.4	67.0	7.5
Hexachlorobenzene	94.6	18.7	X8.5	2.8
Oxychlordane	104	12	106	5
Pentachlorobenzene	101	14	86.7	5.4

[a] $n = 4$.
[b] $n = 3$.

all of the experiments, indicating that the collection technique employed did not "purge out" the analytes isolated in n-hexane. Furthermore, there was no evidence of analyte holdup in the restrictor during a spiked tissue SCDE experiment.

Extractable lipids constitute a formidable interference problem in the conventional analysis of SOCs in tissues [17], and a lipid clean-up must usually be undertaken prior to GC-ECD analysis. The lipid content of lyophilized **Hydrilla verticillata** tissues was found to be 2.1% (w/w). Because it can be expected that lipids and other plant products will co-extract with the SOC analytes, a small amount of Florisil was incorporated into the extraction vessel in an attempt to fractionate interfering materials from the analytes during SCDE.

Substantial reductions in recoveries were observed for γ-hexachlorocyclohexane (HCH), 4,4'-DDE, dieldrin, and pentachlorobenzene when glass wool and Florisil were present in the extraction vessel (Table II), presumably due to the strong interaction of these analytes with the polar Florisil sorbent. Interestingly, the R.S.D. values for most of the analytes were consistently lower in samples with attending glass wool and sorbent with the exception of 4,4'-DDE and dieldrin. Dieldrin showed much lower recoveries and much higher variability in recoveries with Florisil present in the extraction vessel. None of the analyte physical properties shown

in Table I seem to correlate with the observed lower recovery effect. It has recently been stated, however, that supercritical fluid extraction efficiency is best correlated with the dipole moments of the supercritical fluids [18].

Florisil was selected in this study for in-line clean-up because this sorbent is used extensively for conventional column chromatography clean-up in pesticide analysis. Sorbents other than Florisil have yet to be evaluated in the SCDE of **Hydrilla** tissues. The in-line deployment of Florisil in SCDE would ideally minimize the need to conduct further clean-up in tissue analysis, substantially streamlining sample preparation procedures. In addition, in-line clean-up will be essential to the development of directly coupled SCDE-GC in the analysis of SOCs in tissues. It is important to note that one major observation concerning these particular samples was that the collected products from **Hydrilla** alone in the extraction vessel gave the n-hexane in the receiving flask a distinct yellow color, whereas those of **Hydrilla** with glass wool and Florisil in the extraction vessel showed a virtually no visible color in the collection solution. It is possible that the co-extraction of pigments or other natural plant substances may have accounted for the relatively higher R.S.D. values of the samples where Florisil and glass wool were not present, but further investigation was not attemped.

The SCDE procedure described herein would provide a detection limit of 0.5 mg/kg (estimated as an analyte response greater than three times the standard deviation of the baseline signal determined from replicate blank analyses) for the organochlorine analytes if it is used without any further modification. Unfortunately, this is well above 0.001 mg/kg detection limits that are typically available in more conventional tissue analysis methods [18]. Lowering detection limits for the SCDE technique to make it more applicable to field studies could, in fact, be accomplished in several ways, including (a) increasing the sample size, (b) concentrating the n-hexane collection solvent to a small volume, and (c) coupling SCDE with GC in an on-line (i.e., directly coupled) configuration. It is strategy (a) and (b) above that is presently being pursued in developing a method for analyzing SOCs in *Hydrilla* tissues in our laboratory. The results of this study suggest that SCDE has the potential to replace subcritical solvents, when Soxhlet extraction is used for example, in the isolation of synthetic organochlorine contaminants from plant tissues.

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