

Analytical Head-space Supercritical Fluid Extraction Methodology for the Determination of Organochlorine Compounds in Aqueous Matrix

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The proposed head-space supercritical fluid extraction (SFE) methodology as an alternative to an existing conventional procedure was explored for the determination of organochlorine compounds in aqueous matrix. In this study, polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were utilized as target analytes. To enhance the recovery efficiency, the factors such as the CO₂ density, the extraction time, and the extraction mode were investigated. Furthermore, the analytical procedures and the results obtained were compared with those provided by the conventional method (the U.S. EPA method 8080). Under the optimized conditions, *i.e.*, a combination of static with dynamic SFE mode at 2,000 psi and 40 °C, the head-space SFE methodology gave equivalent or better to the conventional method in recovery efficiencies with clear advantages such as simple sample treatment and fast analysis time as well as reduced solvent and reagent consumption.

Key Words : Head-space SFE methodology, Organochlorine compounds, Conventional method, Static and dynamic mode

Introduction

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), classified into a group of persistent organic pollutants (POPs), are highly toxic chlorinated organic compounds. The toxicology of these compounds has been extensively studied in the subjects of lethality, inhibition of growth, immunotoxicity, and carcinogenicity.^{1,2} Although these compounds are now banned in most of countries, due to their widespread use for industrial and agricultural purposes they are still found in different environmental compartments such as atmosphere, water, soil, and sediment.³⁻⁶ They are slow to degrade in the environment and tend to accumulate in fatty tissues of aquatic organism such as fish through the food chain. As a consequence, human as a representative of fish consumers may also be badly affected. Therefore, there are increasing public concerns over the presence of these compounds in aquatic systems.

Although PCBs have been identified along with OCPs in aquatic systems, it is rare to find analytical methods for simultaneous analysis of such chlorinated organic compounds in the literature. Currently, the determination of various organochlorine compounds including PCBs and OCPs in water samples has been implemented according to the analytical procedures based on the conventional method (the U.S. EPA method 8080).⁷ However, it is well-known that these conventional methods require large volumes of solvents that are expensive and toxic, and are time consuming and quite labor intensive.⁸ In contrast, an ideal type of analytical methodology should be both rapid and inexpensive to perform, and should allow quantitative recovery of target analytes of interest without loss or degradation.

Besides, it should also generate a sample which is introduced into an instrumental analysis without requiring any additional clean-up and/or concentration steps.

Supercritical fluid extraction (SFE) has gained increasing attention as an alternative method which circumvents the analytical problems associated with sample preparations in analytical chemistry.⁹⁻¹¹ SFE presents most of the advantages necessary for an ideal analytical methodology as follows: 1) it is faster, easy to operate, and cost effective, 2) the typical solvent used, CO₂, is a chemically inert and inexpensive substance that is neither toxic nor flammable, and also has relatively low critical temperature ($T_c = 31.1$ °C) and pressure ($P_c = 73.8$ atm), 3) the solvent strength of the supercritical fluid can be altered by changing either the pressure or temperature of the fluid, and 4) it has the potential for reducing requirements for the clean-up and concentration of extracted analytes. Considerable data have been presented in the literatures, indicating that high recovery of organic analytes such as PAHs, PCBs, pesticides, dioxins, fats, food additives, and flavors can be achieved with SFE from a variety of solid samples including soil, sediment, fly ash, and food.¹²⁻¹⁵

For the last 20 years, the focus in analytical applications of SFE has gradually shifted from the solid to aqueous matrices. Except for an indirect route which involves the combination of solid-phase extraction (SPE) with SFE, most applications of SFE were based on direct extraction of a flowing liquid sample with supercritical CO₂ in a high pressure extraction cell. Currently, the different approaches employed for the direct SFE of a flowing liquid sample have been utilizing phase separation subsequent to extraction,^{16,17} electro-spraying fine aqueous droplets into supercritical

carbon dioxide,^{18,19} and countercurrent SFE.²⁰⁻²² These approaches for the direct SFE offer a particular benefit that allows larger sample volumes to be extracted. However, these techniques possess an inherent problem of water matrix itself being difficult to be isolated due to the relatively high solubility of water in supercritical CO₂, approximately 0.3%.²³⁻²⁵ Recently, this problem has been partially solved with the specially designed extraction cell, in which supercritical CO₂ is bubbled through the aqueous sample and the head space atmosphere (both supercritical CO₂ and extracted analytes) above liquid sample is removed.²⁶

In this study, we adopted a newly proposed extraction approach, which is called head-space SFE, as an alternative system for SFE of aqueous samples. The performance of the head-space SFE methodology was demonstrated by its application in the recovery of the multi-component PCBs and OCPs frequently encountered in aquatic media. Operating factors affecting the recovery efficiency of target analytes, such as critical temperature and pressure, extraction time, and static and dynamic extraction mode were investigated. Furthermore, analytical procedures as well as the experimental results obtained using the head-space SFE methodology were also compared to the conventional method (the U.S. EPA Method 8080), which is commonly used for the determination of organochlorine compounds in aqueous matrices.

Experimental Section

Materials. Neat PCB congeners (IUPAC # 77, 96, 114,

126, 176) with certified purity of 99+% by GC/FID & GC/MS, organochlorine pesticides (lindane, heptachlor, aldrin, dieldrin, endrin and 4,4'-DDT), and an internal standard (pentachlorobenzene) in solutions of known concentration were obtained from Accustandard (New Haven, CT, USA). A standard solution containing selected PCB congeners and organochlorine pesticides was prepared in isooctane at concentration of 1,000 ppm level. All solvents (acetone, dichloromethane, *n*-hexane, isooctane, MTBE) used in this study were of HPLC-grade (Mallinckrodt Baker, Inc., NJ, USA). The CO₂ of 99.999% ultra high purity, purchased from MS Gas Co., Ltd. (Busan, Korea) was used for supercritical fluid extracting solvent.

SFE equipment. An integrated bench-scale SFE equipment designed for this study is shown in Figure 1. The extraction vessel, the surge tank, and the impinger cold trap were all made of stainless steel in a science machine shop, Korea. Their internal volume was approximately 137 mL, 317 mL, and 92 mL, respectively. Carbon dioxide was pressurized by an air driven gas booster (Maxpro Technologies, Inc., Erie, PA, Germany) and then delivered into an extraction vessel *via* a surge tank. The surge tank was used to dampen any sudden pressure surge of the compressed CO₂ before entering the extraction vessel. The gas booster was connected to the extraction vessel with a stainless steel tube (o.d.: 1/4 in.). The temperature in the extraction vessel was regulated by a heater equipped with a temperature controller and monitored with a thermometer, using a T-type thermocouple inserted into a thermowell which extends deep into the extraction vessel. The pressure in the extraction vessel was controlled by adjusting the back-pressure regulator

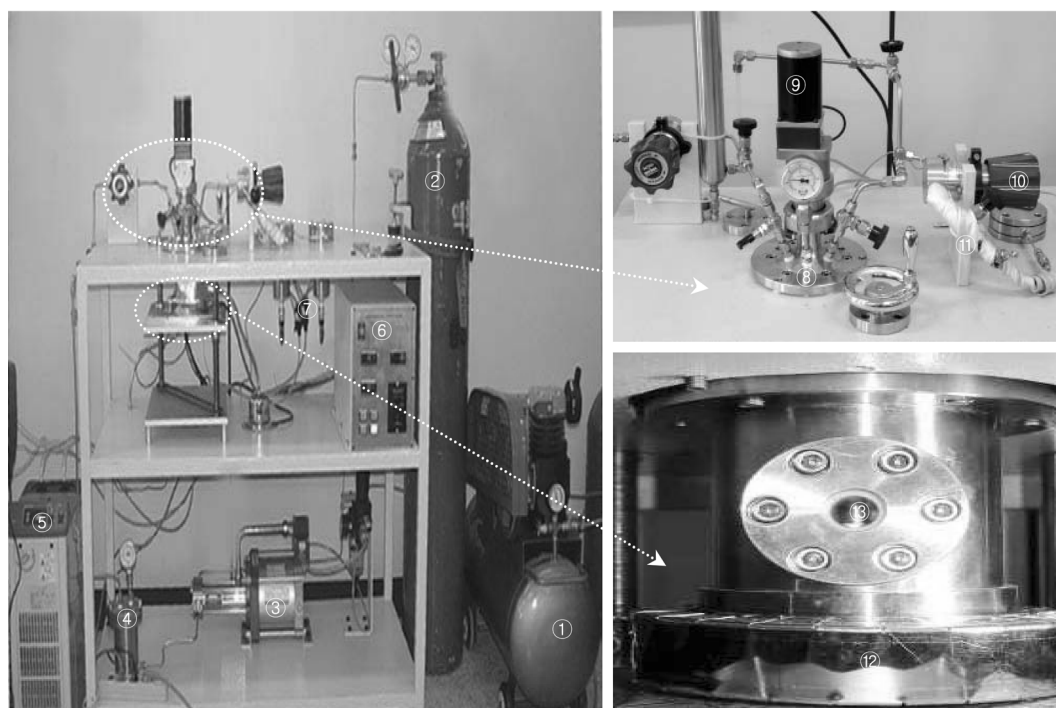


Figure 1. Schematic diagram of the head-space supercritical fluid apparatus. ① Compressor; ② CO₂ reservoir; ③ Gas booster; ④ Surge tank; ⑤ Temperature circulator; ⑥ Temperature controller; ⑦ Impinger cold trap; ⑧ Head-space extraction vessel; ⑨ Magnetic stirrer; ⑩ Back pressure regulator; ⑪ Heater & needle valve; ⑫ Heater; ⑬ Sapphire window.

(BPR, Tescom, model 26-1721) and monitored electronically by a pressure transducer. A sapphire window was attached in the middle of the extraction vessel to allow the observation of the phase behavior. The extraction vessel was equipped with a mechanical agitator to achieve equilibrium more rapidly.

A stainless steel transfer line (1/16 in. i.d.) was attached to the outlet of the extraction vessel to maintain the pressure inside the extraction vessel during the extraction run and inserted into a serial cold trap, where the effluents (CO₂ and extracted analytes) were collected. The part of transfer line was heated by applying an electrical current to elevate the temperature of the effluents and prevent it from freezing closed during the fluid expansion process. The solvent traps were cooled by circulating a -5 °C water/ethylene glycol mixture. The flow rate of depressurized CO₂ was controlled with a micrometering valve and measured using a digital flow check (Alltech, Deerfield, IL, USA) with an accuracy of ± 0.1 mL/min.

Analytical procedure of the head-space SFE methodology. The extraction vessel was filled with 100 mL of deionized water and then 100 μL of standard solution was spiked. Once the temperature of the extraction vessel has reached a predetermined value, CO₂ was charged into the extraction vessel via a surge tank up to the desired operating pressure. All SFEs were run in a static and a combination of static with dynamic mode for a given time at selected conditions of temperature (40 °C) and different pressures (1,200, 1,500, and 2,000 psi). During the extraction, the temperature was kept constant to investigate the effects of pressure which has the biggest effect on CO₂ density and

also on the diffusion coefficient.²⁷ Under the operating temperature and pressures listed above, the approximate densities of pure CO₂ calculated from an equation of state were 0.30, 0.64, and 0.76 g/mL, respectively.²⁸

Analytes were extracted from the head-space above the water sample inside the extraction vessel and collected by bubbling the depressurized CO₂ through 20 mL of isooctane in two consecutive cold traps. The latter solvent trap was served to ensure extracted analytes collected in the former solvent trap. During the extraction, the solvent traps were kept at a temperature of -5 °C. The depressurization of CO₂ was performed through a 150 °C heated transfer line. The flow rate of CO₂ through the transfer line was approximately 2 mL/min. After a given extraction period, the solution was withdrawn from the solvent traps and then diluted up to an exact final volume of 1 mL by using a rotary evaporator and a gentle stream of N₂.

Gas chromatographic analysis. A gas chromatograph (Trace GC 2000, Italy) equipped with an electron capture detector (ECD) and a 30 m × 0.25 mm, 0.25 μm 5% phenylpolysiloxane Zebron ZB-5 column (Torrance, CA) was used for separation and quantification of extracted analytes. The GC oven program was the following: initial temperature 170 °C, retained for 2 min, then increased at a rate of 5 °C to 230 °C, retained for 2 min, then increasing at a rate of 5 °C to 300 °C, and finally retained for 10 min. The injection was performed in a splitless mode. Pentachlorobenzene, used as an internal standard, was added to the final extracted analytes before GC/ECD analysis and response factors for extracted analytes were calculated from four concentration levels of the external standards.

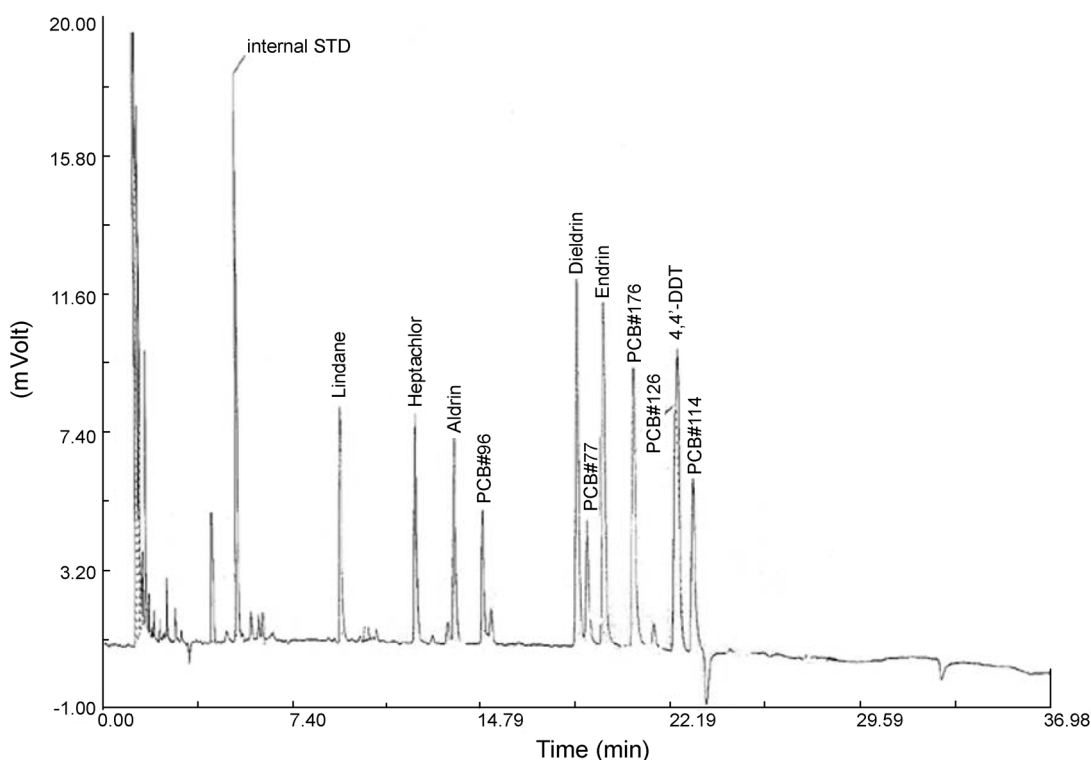


Figure 2. GC chromatogram obtained with the standard solution containing PCBs and OCPs.

Results and Discussion

PCBs and OCPs spiked on water. A small portion of standard solution containing 5 PCBs and 6 OCPs was initially spiked into a 100 mL distilled water at a 100 μg concentration level. Prior to the analyses of PCBs and OCPs in water by means of the U.S. EPA method 8080 and the head-space SFE methodology, a GC chromatogram of the standard solution, added pentachlorobenzene as an internal standard, is shown in Figure 2. Here it is observed that PCBs and OCPs can be detected with greatest sensitivity using an electron capture detector (ECD).

U.S. EPA method 8080. The U.S. EPA method 8080 has been used for the determination of various organochlorine compounds in water. A flow schematic, described in Figure 3, shows the analytical procedures of the method. As can be seen from Figure 3, this method involves various sample preparations such as the addition of HgCl_2 and NaCl , pH adjustment, liquid-liquid extraction, clean-up as well as concentration steps, previous to the GC/ECD analysis.

The analytical procedure was repeated five times to determine both the mean recoveries and the standard deviations. Table 1 details the experimental results obtained for the determination of above listed PCBs and OCPs in water according to the U.S. EPA method 8080, along with their standard deviations (SDs). Values of the mean recovery and the standard deviation were expressed as percentage. As stated in Table 1, the mean recoveries of each analyte were

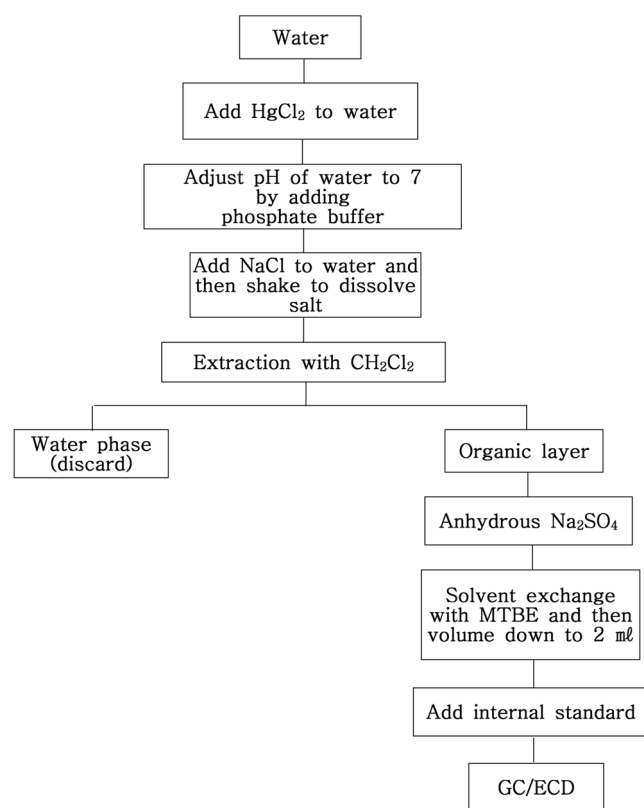


Figure 3. Flow schematic of the U.S. EPA method 8080 for the analysis of organochlorine compounds in water.

Table 1. The % mean recoveries of PCBs and OCPs in water obtained using the U.S. EPA method 8080

Organochlorine compounds ^a	U.S. EPA method 8080 $X \pm SD^b$
Lindane	92.8 \pm 0.9
Heptachlor	93.6 \pm 1.0
Aldrin	90.1 \pm 3.3
PCB (# 96)	88.6 \pm 4.1
Dieldrin	89.0 \pm 4.3
PCB (# 77)	86.8 \pm 5.8
Endrin	90.0 \pm 3.9
PCB (# 176)	84.1 \pm 4.8
4,4'-DDT	80.3 \pm 2.1
PCB (# 126)	79.8 \pm 5.5
PCB (# 114)	82.3 \pm 3.7
Average for all compounds	87.1 \pm 3.6

^aEach individual organochlorine compounds is identified by its retention time of GC/ECD. ^bStandard deviations (SD) were based on the results of analysis averaged from five trials.

ranged from approximately 80% to 93% with the SDs of 0.9-5.8%. In addition, average mean recovery and standard deviation for all analytes were 87.1% and 3.6%, respectively. In general, both the mean recovery and the standard deviation are important factors when an analytical method is evaluated. It can be considered that a method is acceptable when the recovery and the standard deviation are over 80% and less than 10%, respectively. From the data of the results obtained, it confirms that the U.S. EPA method 8080 is generally reliable, even though this method is typically composed of a long and tedious analytical procedures.

Application of the head-space SFE based methodology.

The performance of the proposed head-space SFE methodology was assessed by determining the recovery efficiencies of organochlorine compounds from spiked water. An experimental design for this methodology is introduced into a flow schematic, given in Figure 4. In the initial studies of developing the head-space SFE methodology, variables affecting SFE such as CO_2 density, extraction time, and extraction mode (static and dynamic) were considered. Static SFE is normally performed by pressurizing the extraction vessel and extracting the analytes from the sample without outflow of the supercritical fluid during a given equilibrium time. Whereas, dynamic SFE is a type where supercritical fluid is continually passed over the sample to be extracted.

A repeatability experiment ($n = 5$) was undertaken on each set of SFE condition. Previous to SFE of target analytes from water sample, blank experiments were done in a manner identical to that used for samples. Blank extracts (generated by performing SFE of an empty extraction vessel) showed no significant impurities in CO_2 being used as SFE solvent.

Table 2 shows the mean recoveries and the standard deviations of individual PCB and OCP in water obtained using the head-space SFE methodology with a static mode only. With a 10-min static SFE mode at 40 $^\circ\text{C}$ and 1,200 psi ($d = 0.30$ g/mL), significantly low mean recoveries ranging

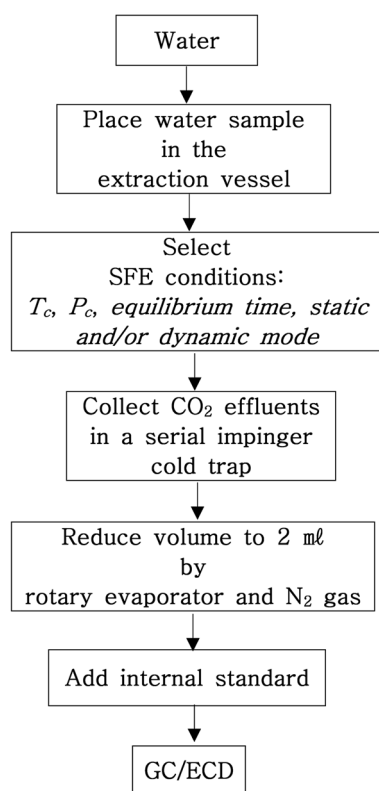


Figure 4. Flow schematic of the head-space SFE methodology for the analysis of organochlorine compounds in water.

from 33% to 50%, as shown in Figure 5, were observed for PCBs and OCPs with SDs < 11%. At 40 °C and 1,500 psi ($d = 0.64$ g/mL), a 30-40% increase of the recovery efficiency was obtained, compared to the condition as stated above. However, when 40 °C and 2,000 psi ($d = 0.76$ g/mL) condition was used, the mean recoveries of each analyte were increased up to approximately 2-fold times, showing the SDs for corresponding analytes < 5%. For instance, the

mean recoveries of lindane, endrin, and PCB #126 were 32.9, 34.8, and 34.2% respectively at a density of 0.30 g/mL (40 °C and 1,200 psi). Whereas, at a density of 0.76 g/mL (40 °C and 2,000 psi), the mean recoveries of above-mentioned analytes were 57.8, 70.3, and 63.3%. The results clearly demonstrate that increasing the density is much more effective for raising the recovery efficiency due to the higher diffusion of supercritical CO₂ through water. Nevertheless, since such recoveries are below 80%, the proposed head-space SFE methodology performed by a static mode only is still unsatisfactory for adopting as an alternative analytical method for the determination of organochlorine compounds in water.

To further investigate the effect of the SFE mode on the recovery efficiency of each analyte, a combination of static with dynamic mode was evaluated and compared with a static SFE mode only as well as the U.S. EPA method 8080. Table 3 shows the experimental results obtained from the combined static/dynamic SFE modes at constant temperature and different pressures. With a combination of static with dynamic SFE mode at 1,200 psi (40 °C), the mean recoveries of each analyte were in the range from 60% to 82%, and the values of standard deviation were found to be approximately 7% or less. Such a combined static/dynamic SFE mode yielded the mean recoveries nearly identical to those achieved using a static extraction mode only at 2,000 psi (40 °C). For example, with the combined static/dynamic SFE mode at pressure of 1,200 psi (40 °C), the average recovery for all analytes was 71.0%, while the average recovery for corresponding analytes was 71.1% with a static extraction mode at 2,000 psi (40 °C). Overall, the trend suggests the addition of a dynamic mode significantly improves the recovery efficiency of each analyte. However, the result indicates that the head-space SFE methodology by means of a combination of static with dynamic mode at 1,200 psi (40 °C) is not still enough to recover efficiently

Table 2. The % mean recoveries of PCBs and OCPs in water obtained using the head-space SFE methodology with a static SFE mode

Organochlorine compounds ^a	Head-space SFE methodology		
	% mean recovery (40 °C, 1,200 psi) static SFE mode <i>Equi. time</i> ; 10 min X ± SD ^b	% mean recovery (40 °C, 1,500 psi) static SFE mode <i>Equi. time</i> ; 10 min X ± SD	% mean recovery (40 °C, 2,000 psi) static SFE mode <i>Equi. time</i> ; 10 min X ± SD
Lindane	32.9 ± 9.1	47.1 ± 1.8	57.8 ± 4.4
Heptachlor	47.3 ± 9.6	63.2 ± 4.6	86.5 ± 3.0
Aldrin	49.7 ± 2.3	59.3 ± 4.1	79.9 ± 3.7
PCB (# 96)	46.3 ± 3.1	52.0 ± 7.5	77.4 ± 1.1
Dieldrin	41.9 ± 2.4	58.4 ± 6.3	75.1 ± 3.3
PCB (# 77)	29.9 ± 3.9	48.6 ± 3.3	68.1 ± 1.6
Endrin	34.8 ± 3.9	52.7 ± 4.7	70.3 ± 1.8
PCB (# 176)	37.6 ± 1.4	55.6 ± 4.4	69.4 ± 4.6
4,4'-DDT	33.0 ± 1.0	52.0 ± 3.1	68.7 ± 4.3
PCB (# 126)	34.2 ± 10.8	49.3 ± 5.0	63.3 ± 3.6
PCB (# 114)	33.5 ± 0.6	48.6 ± 5.1	65.1 ± 3.4
Average for all compounds	38.3 ± 4.4	53.3 ± 4.5	71.1 ± 3.2

^aEach individual organochlorine compounds is identified by its retention time of GC/ECD. ^bStandard deviations (SD) were based on results of the analysis averaged from five trials performed at each condition.

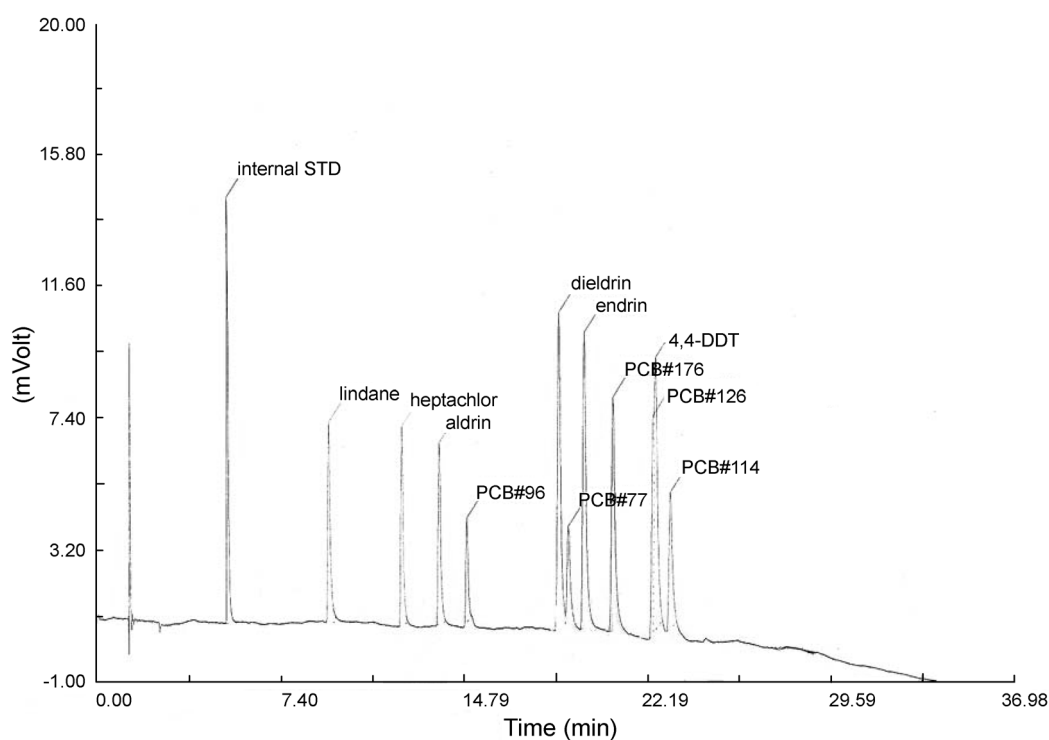


Figure 5. GC chromatogram of organochlorine compounds after the head-space SFE methodology with a static mode at 40 °C and 1,200 psi.

Table 3. The % mean recoveries of PCBs and OCPs in water obtained using the head-space SFE methodology with a combination of static with dynamic SFE mode

Organochlorine compounds ^a	Head-space SFE methodology		
	% mean recovery (40 °C, 1,200 psi) static/dynamic SFE mode <i>Equi. time</i> ; 10 min	% mean recovery (40 °C, 1,500 psi) static/dynamic SFE mode <i>Equi. time</i> ; 10 min	% mean recovery (40 °C, 2000 psi) static/dynamic SFE mode <i>Equi. time</i> ; 10 min
	$X \pm SD^b$	$X \pm SD$	$X \pm SD$
Lindane	60.3 ± 5.6	65.2 ± 4.5	94.3 ± 1.7
Heptachlor	82.4 ± 6.5	84.6 ± 8.2	94.5 ± 0.8
Aldrin	80.3 ± 3.3	84.1 ± 3.3	96.0 ± 2.5
PCB (# 96)	73.1 ± 1.9	74.9 ± 5.8	96.1 ± 3.2
Dieldrin	68.6 ± 4.4	79.4 ± 7.2	98.5 ± 1.2
PCB (# 77)	71.4 ± 3.7	76.3 ± 1.8	97.9 ± 1.3
Endrin	72.1 ± 1.8	80.5 ± 2.9	98.6 ± 0.5
PCB (# 176)	70.3 ± 4.8	77.2 ± 3.3	93.1 ± 1.7
4,4'-DDT	70.3 ± 5.2	71.7 ± 7.2	90.6 ± 0.5
PCB (# 126)	68.8 ± 3.6	75.6 ± 4.5	94.6 ± 5.4
PCB (# 114)	63.5 ± 4.9	66.7 ± 3.1	94.4 ± 3.4
Average for all compounds	71.0 ± 4.2	76.0 ± 4.7	95.3 ± 2.0

^aEach individual organochlorine compounds is identified by its retention time of GC/ECD. ^bStandard deviations (SD) were based on results of the analysis averaged from five trials performed at each condition.

organochlorine compounds in water.

It was observed that, when increasing the pressure from 1,200 to 2,000 *via* 1,500 psi with a combined static/dynamic SFE mode mentioned earlier, there was a marked difference in the recovery efficiency. The mean recoveries were over 90% for all analytes, as shown in Figure 6, under study at 40 °C and 2,000 psi, and the SDs were less than 5%. An

increase of recovery efficiency with increasing the pressure is probably due to the higher solubility of analytes in supercritical CO₂ fluid.

Both the recovery and the standard deviation obtained appeared to be equivalent or better than those reported for the U.S. EPA method 8080. Furthermore, the comparison of total analysis time showed that the head-space SFE method-

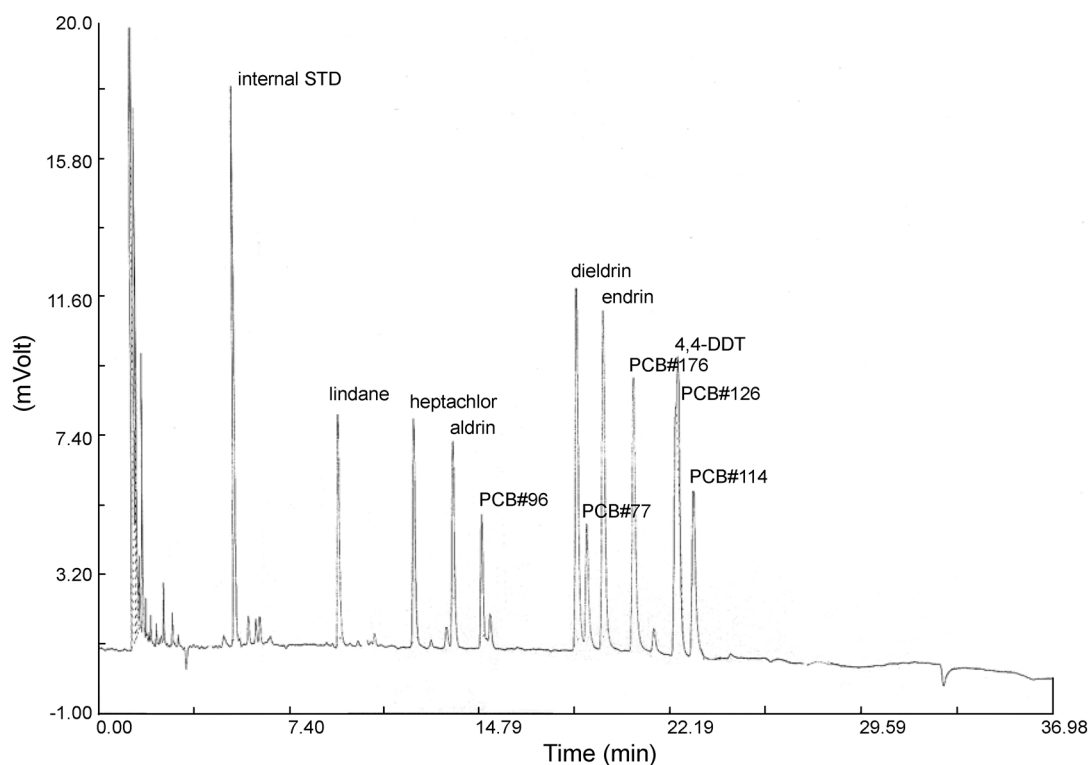


Figure 6. GC chromatogram of organochlorine compounds after the head-space SFE methodology with the combined static/dynamic mode at 40 °C and 2,000 psi.

ology was considerably faster than the U.S. EPA method 8080, because the former did not require multiple sample treatments. Consequently, it is demonstrated that the head-space SFE methodology, at the studied SFE conditions (a combined static/dynamic SFE mode at 2,000 psi (40 °C)), can be potentially applied on water containing organochlorine compounds. The major limitation of the proposed head-space SFE methodology was the maintenance of the pressure throughout the extraction. For a dynamic mode rather than a static mode, it was difficult to preserve the exact extraction pressure since the pressure during the SFE extraction was not kept constant. Hence, it is thought that the solubility of the supercritical CO₂ fluid might be fluctuated slightly.

Conclusions

The goal of this study was to develop the alternative methodology through the head-space SFE, and its application on the determination of organochlorine compounds including PCBs and OCPs in aqueous matrix. It has been shown that analytical procedures based on the proposed head-space SFE methodology are simple to undertake and faster than those of the conventional method (the U.S. EPA method 8080), which is commonly used for the analysis of organochlorine compounds in water. For the analysis of a sample, the total analysis time of the head-space SFE methodology took about 30 minutes to complete except for the instrumental analysis, as compared to 4 hours in the conventional method. In addition, less additional sample

preparations were also required. At the selected conditions, over 90% of all investigated analytes was recovered from water with the SDs < 5%, indicating these values are equivalent or better to the conventional method. However, the proposed head-space SFE methodology requires the optimization in depth since the recovery efficiency is strongly dependent on the SFE conditions. Therefore, further investigation and optimization of this type of methodology is continuing.

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