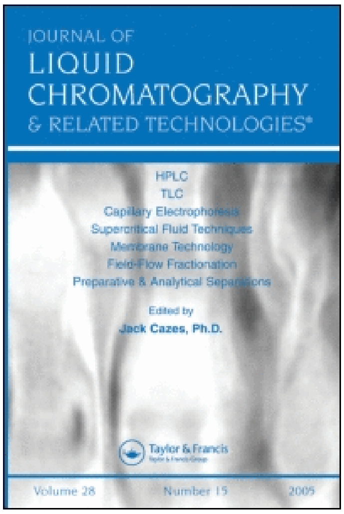

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SAMPLE PREPARATION FOR EFFECTIVE DETERMINATION OF POLYCHLORINATED DIBENZODIOXINS FROM LEACHATES

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ABSTRACT

Four pretreatment methods, including LLE-HPLC, LLE-DMSO, SPE-HPLC and SPE-DMSO are compared for the effective determination of polychlorinated dibenzodioxins (PCDDs) from a leachate sample. For the LLE-HPLC method, liquid–liquid extraction (LLE) with dichloromethane is used for the initial extraction of the analytes from leachate. For the elimination of interferences coextracted from leachate, acid treatment, followed by high performance liquid chromatographic (HPLC) column clean-up, is performed. For the LLE-DMSO method, LLE, acid treatment and extraction with dimethyl sulfoxide (DMSO) are accomplished, consecutively. For the SPE-HPLC method, solid-phase extraction of disk-type with toluene–ethanol mixture (3:7, v/v), acid treatment, and HPLC column clean-up are performed, consecutively.

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For the SPE-DMSO method, SPE with DMSO and acid treatment are performed, consecutively. After each of these methods, column clean-ups including multilayer silica gel and alumina are performed, sequentially. The final extract is analyzed by HPLC-UV. For SPE, a toluene–ethanol mixture, conventional solvent is used for the extraction of PCDDs from SPE of disk-type. However, the use of DMSO with a selective interaction with aromatic compounds shows higher efficiencies when considering time, solvent usage, and removal of interferences.

INTRODUCTION

Polychlorinated dibenzodioxins (PCDDs) have been the subject of much concern because of their toxicity (1,2). Reported procedures for the sample preparation of PCDDs require complicated experimental set-ups and are time-consuming (3–8). Solvent extraction, ether solid-phase extraction (SPE) or liquid–liquid extraction (LLE), is a common initial step of sample preparation for the analysis of PCDDs (5,7).

Recently, interest has been shown in replacing conventional LLE with SPE techniques for isolating environmental pollutants (9). LLE is less applicable to routine analysis of a large number of samples. SPE is an attractive technique that reduces the usage, exposure, and disposal costs of solvent and extraction time for sample preparation (10).

A combination of several different clean-ups is used to remove potential interferences and to obtain a clear sample. HPLC clean-up (11) and column chromatography, using a variety of adsorbents such as acid and base modified silica gel (12), alumina (13,14), florisil (15), and carbon (16) are applied.

Generally, toluene–ethanol mixture (3:7, v/v) is used as an extraction solvent for SPE of disk-type because of the higher solubility and desorption of PCDDs in toluene (17). This combination allows for the use of a strong solvent such as toluene to elute PCDDs and ethanol to address the residual water left on the SPE disk (17).

Orazio et al. (18) used DMSO to remove the large aliphatic constituents from waste oil. DMSO dissolves aromatics selectively through an association between the relatively electron-rich aromatic ring and DMSO (19).

The purpose of this study is to investigate and compare the pretreatment methods for the effective determination of PCDDs from leachate samples. LLE and SPE were performed and compared. The selective extraction method with DMSO was also evaluated in LLE and SPE. DMSO extraction solvent was compared with toluene–ethanol mixture in SPE of disk-type, because an effect of DMSO on the SPE of disk-type of PCDDs from leachate has rarely been studied.

EXPERIMENTAL

Standards and Chemicals

Reagent-grade standards of 2,2′,4,5′-tetrachlorinated biphenyl (4B), 2,3,4,5,6-pentachlorinated biphenyl (5B), 1,2,3,4-tetrachlorinated dibenzodioxin (4D), 1,2,3,4,7-pentachlorinated dibenzodioxin (5D), 1,2,3,4,7,8-hexachlorinated dibenzodioxin (6D), 1,2,3,4,6,7,8-heptachlorinated dibenzodioxin (7D) and octachlorinated dibenzodioxin (8D) were obtained from Ultra Scientific (250 Smith Street, North Kingstown). A stock solution containing a mixture of PCBs at 0.5 μg/mL and PCDDs at 0.045 was prepared in acetonitrile for each compound.

A leachate sample was obtained from a waste landfill (Seoul, Korea). Silica gel (230–400 mesh, Merck, Darmstadt, Germany) and alumina (70–230 mesh, Merck) were first rinsed with methanol twice, and then with dichloromethane twice. Next, it was activated at 180°C for at least 12 h. Anhydrous sodium sulfate (Merck) was used to protect the packing materials in the multilayer silica gel column (20) and to remove the water content of extracts.

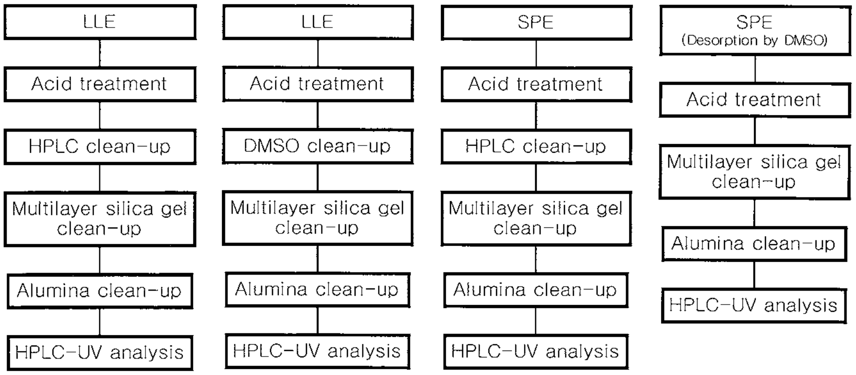
All solvents were HPLC grade from J. T. Baker (Philipsburg, USA). Dimethyl sulfoxide (DMSO) and sulfuric acid were obtained from Duksan Pharmaceutical (Korea). SPE-disk (47 mm, C18) and apparatus were obtained from 3M (St. Paul, MN, USA) and Millipore (Bedford, MA, USA), respectively.

200 mL of leachate was spiked at 100 ppt with 0.5 ppm stock solution of 4B and 5B, and at 9 ppt with 0.045 ppm stock solution of 4D, 5D, 6D, 7D, and 8D. The sample, controlled to pH 3 with sulfuric acid, was homogenized in an ultrasound (bath type, Fisher Scientific Solid State/ultrasonic FS-28, Pittsburgh, PA, USA) for 30 min and left to equilibrate overnight. These spiking levels were used because the detection limits and quantification of PCDDs by the HPLC analysis were considered.

In this study, LLE-HPLC, LLE-DMSO, SPE-HPLC, and SPE-DMSO methods are performed and then compared in order to eliminate the interferences and determine PCDDs from leachate (see Fig. 1).

LLE-HPLC Method

A LLE-HPLC method indicates that LLE acid treatment and clean-up, using HPLC multilayer silica gel and alumina column, are performed consecutively. LLE was accomplished using 50 mL of dichloromethane three times. The extract of LLE was treated more than seven times with 30 mL of concentrated sulfuric acid until the dichloromethane phase became colorless. The extract was washed with water and dried with an anhydrous sodium sulfate column. After the concentration of extract, it was applied to HPLC column clean-up.



(a) LLE-HPLC (b) LLE-DMSO (c) SPE-HPLC (d) SPE-DMSO

Figure 1. Schemes of four pretreatments for the determination of PCDDs from leachate.

The clean-up was performed on a Shodex C18-5B (250 × 4.6 mm, 5 μL; Shoko, Kyoto, Japan) column. The HPLC system was a Shimadzu Liquid Chromatograph equipped with an SPD-10A UV-visible detector and C-R6A integrator. Chromatograms were recorded at 250 nm (A.U.F.S = 0.005). The flow rate of the mobile phase was 1.0 mL/min and the temperature was 40°C. An acetonitrile–water (9:1, v/v) solution was used to separate PCDDs, with retention time of 11–30 min, from PCBs and large amounts of bulk interferences. Recovered eluate, including PCDDs, was dried by anhydrous sodium sulfate column. The acetonitrile solution was transferred to *n*-hexane for the next clean-up step of multilayer silica gel column.

The column for the clean-up was packed in the order of neutral (1 g), acidic (7 g) and neutral (1 g) silica gel. The column was eluted with 50 mL of *n*-hexane. Afterwards, clean-up of the alumina column packed with 10 g was performed. Two fractionations were performed: elution with 70 mL of *n*-hexane and then with 50 mL of hexane-dichloromethane (8:2, v/v). The first fraction was discarded.

The second fraction was concentrated to 2–3 mL and transferred into a 7-mL vial. Nitrogen evaporation was performed to remove *n*-hexane, and then 100 μL of acetonitrile was added for the quantification by HPLC-UV.

LLE-DMSO Method

LLE, acid treatment, washing with water and drying with anhydrous sodium sulfate column of leachate sample, were the same as those in LLE-HPLC method. After the concentration of the extract (dichloromethane solution), *n*-hexane was added. The *n*-hexane solution was extracted three times with 50 mL of DMSO. After the addition of water, the DMSO–water mixture was homogenized using ultrasound energy. This solution was back-extracted with 50 mL of *n*-hexane, three times, and the collection of *n*-hexane was washed with water for the elimination of DMSO residue and dried with anhydrous sodium sulfate column. Column clean-up steps including multilayer silica gel and alumina were also the same as those in the LLE-HPLC method.

SPE-HPLC Method

The leachate sample was controlled to pH 2 with hydrochloric acid prior to SPE in order to lessen the plugging of core of disk membrane. The disk membrane was placed in the filtration apparatus attached to a vacuum source. In order to wash the disk, 20 mL of the final elution solvent was added to the filtration reservoir, and then drawn slowly through the disk by applying slight vacuum. After drawing air through the disk for several minutes, 20 mL of methanol was added and drawn slowly through the disk. Before drying, the disk was washed with 30 mL of water. Afterwards, the prepared sample was added to the reservoir.

After the entire sample was drawn through, the disk was air-dried. To desorb the analytes from the disk, 120 mL of toluene–ethanol mixture (3:7, v/v) was used. Again, 40 mL of solvent mixture was added in the reservoir, three times. Acid treatment, washing with water, drying with anhydrous sodium sulfate column, and column clean-up including HPLC, multilayer silica gel, and alumina were the same as those in LLE-HPLC method.

SPE-DMSO Method

This method was accomplished similar to the way SPE-HPLC method was. Instead of a toluene–ethanol mixture, DMSO was added to the disk where the analytes were adsorded. 150 mL of water was added to DMSO extracts, followed by back-extraction with 50 mL of *n*-hexane, three times. The collection of *n*-hexane solution was treated with sulfuric acid. After drying and concentration, the column clean-ups including multilayer silica gel and alumina were performed, the same as that in the LLE-HPLC method.

HPLC-UV Analysis

The system and conditions were the same as those used for HPLC clean-up, as mentioned above. 90-μL aliquots of the concentrated solution (100 μL of acetonitrile) were injected onto the column.

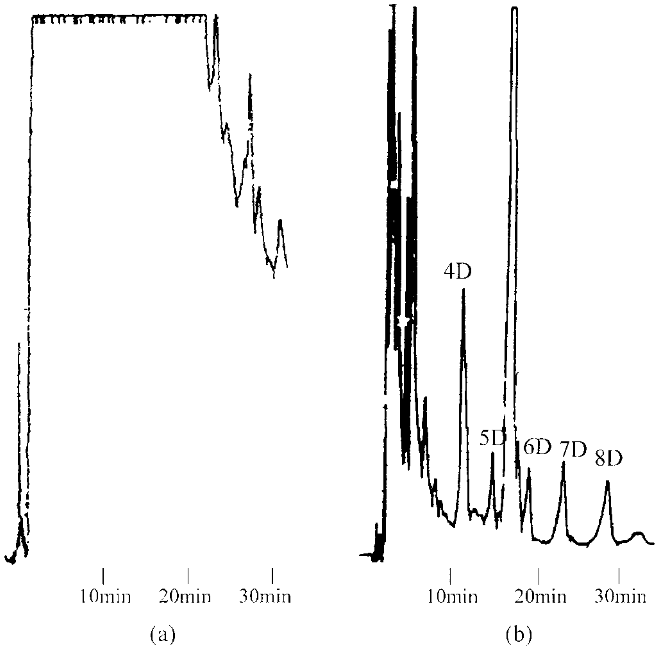


Figure 2. Chromatograms corresponding to PCDDs from leachate— a) without the HPLC or DMSO clean-up. b) without alumina clean-up, last step. 4D, 5D, 6D, 7D, and 8D are abbreviated in the text.

RESULTS AND DISCUSSION

The objective of this study was to investigate a simple, fast, and reliable extraction and clean-up procedure for the determination of PCDDs in contaminated water, such as leachate. A variety of bulk interferences in leachate made HPLC analysis of PCDDs difficult, although acid treatment and column clean-up with the use of multilayer silica gel and alumina were performed (see Fig. 2a).

This phenomenon needed an additional clean-up step using HPLC or DMSO. When the clean-up step using the alumina column, the last step, was applied, interference that had a similar retention time to 4D, resulted in always limiting the accurate determination of PCDDs (see Fig. 2b).

LLE-HPLC Method

Compared with *n*-hexane and toluene, dichloromethane was chosen as the LLE solvent because of the higher recovery of PCDDs. HPLC column clean-up made the efficient elimination of PCBs and bulk interferences, having shorter

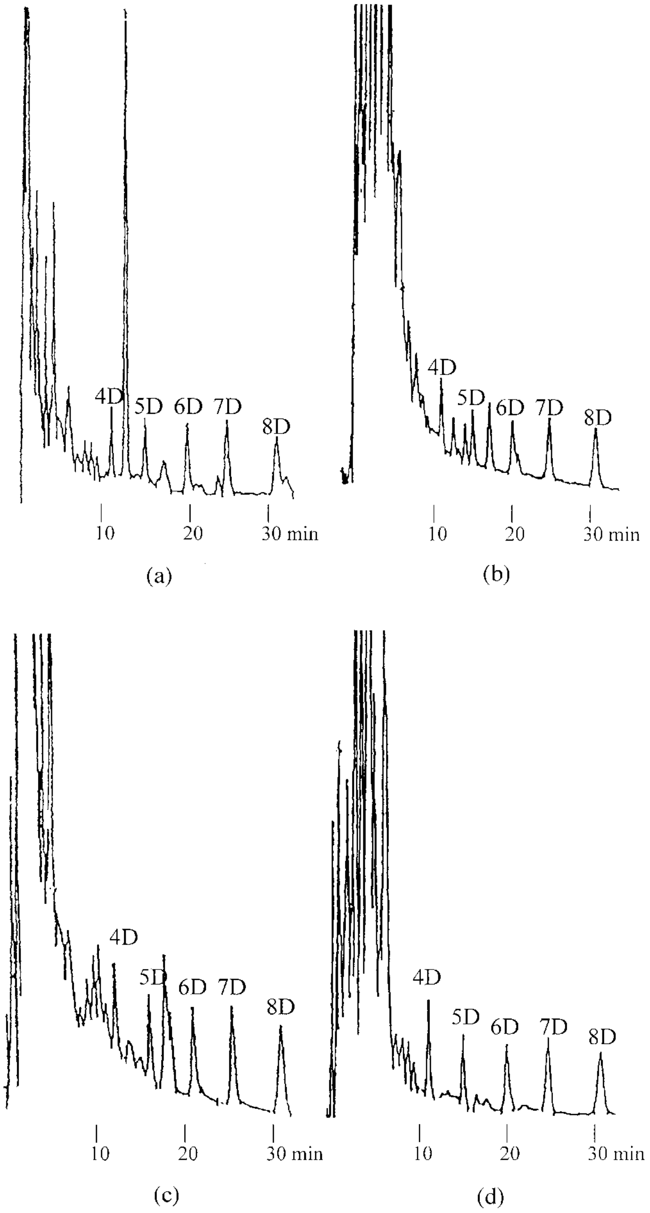


Figure 3. Chromatograms of leachate extract in HPLC-UV. a) LLE-HPLC; b) LLE-DMSO; c) SPE-HPLC; d) SPE-DMSO method. 4D, 5D, 6D, 7D, and 8D are abbreviated in the text.

Table 1. Recoveries (%) and Standard Deviations (%) of PCDDs from Leachate, and the Efficiencies of Performance by Four Different Methods

		LLE-HPLC ($n = 4$)	LLE-DMSO ($n = 3$)	SPE-HPLC ($n = 3$)	SPE-DMSO ($n = 3$)
% Recovery (Standard deviation)	4D	94.7(2.1)	88.9(0.7)	95.0(1.9)	107(7.8)
	5D	90.4(6.5)	68.3(5.4)	88.9(4.4)	87.6(12)
	6D	91.5(9.9)	55.7(2.7)	81.0(1.0)	93.9(13)
	7D	89.9(8.1)	61.4(0.2)	87.7(4.5)	88.8(5.6)
	8D	91.4(10)	81.5(2.2)	99.6(0.2)	80.8(9.0)
Time (h)		92	13	18	12
Solvent usage (mL)		900	1000	800	700
Removal of interference[a]		++	++	++	+++
Convenience		+	++	+	+++
Exchange of solvent[b]		1	1	1	0
Acid treatment		7	7	7	3
Clean-up step		4	4	4	3
Drying step		2	1	2	1
Concentration		4	4	4	3

[a] The symbol of +, ++ and +++ means good, better, and best, respectively.

[b] Exchange of solvent represents that a solvent is concentrated and then diluted by another solvent.

retention time than 4D, possible, and facilitated the determination of PCDDs (see Fig. 3a). The recoveries and standard deviations of PCDDs were 90–95% and 2.1–10%, respectively (see Tab. 1).

LLE-DMSO Method

The comparison between chromatograms of Figures 3a and b indicated that the removal of HPLC chromatographic interferences with PCDDs was efficiently done through the LLE-DMSO method, rather than the LLE-HPLC method. The recoveries and standard deviations of PCDDs were 56–89% and 0.2–5.4%, respectively. Although the recoveries of PCDDs were relatively lower, the LLE-DMSO method was the more effective method, when extraction time and convenience were considered (see Tab. 1).

SPE-HPLC Method

For the SPE method, toluene–ethanol mixture is the conventional solvent. In this study, various compositions of ethanol in toluene were tested and compared (ethanol:toluene, 0:10, 3:7, 5:5, 7:3, v/v). Only 100% of toluene showed recovery less than 50%, whereas other compositions had little differences (results not shown). Like the LLE-HPLC method, interferences having shorter retention time than 4D were removed by an HPLC column clean-up (see Fig. 3c).

The recoveries and standard deviations of PCDDs were 81–99.6% and 0.2–4.5%, respectively. This method had a disadvantage of being time-consuming and was laborious because of the drying and concentration steps (see Tab. 1).

SPE-DMSO Method

Throughout SPE, generally, the simultaneous performance of extraction and concentration step can be obtained. Because DMSO extracted aromatic compounds selectively, the desorption by DMSO in SPE made the more selective separation of PCDDs from bulk interference in leachate possible, resulting in accomplishing the extraction and clean-up step simultaneously. However, the LLE-DMSO method required a series of LLE with dichloromethane, concentration, dilution with *n*-hexane, and clean-up with DMSO. DMSO clean-up of PCDDs from *n*-hexane solution gave lower values of 50–60%, as represented in Table 1.

The SPE-DMSO method had the advantage that the DMSO clean-up step could be carried out directly after desorption of PCDDs from disk without intermediate steps, such as concentration and dilution. In addition, unlike the other methods above, this method required at least two acid treatments.

When a toluene–ethanol mixture—the conventional extraction solvent—was used in an SPE-HPLC method, HPLC clean-up was needed for the separation of bulk interferences from PCDDs. However, the use of DMSO did not require HPLC clean-up and showed a cleaner chromatogram (see Fig. 3d). The reason may also be why DMSO is a selective extraction solvent for the extraction of aromatic compounds from a matrix.

The recoveries and standard deviations of PCDDs ranged between 81–107% and 5.6–13%, respectively (see Tab. 1). Considering pretreatment time, solvent consumption, interference removal, and procedure convenience, this method was the most efficient (see Tab. 1).

CONCLUSION

The comparison of SPE and LLE showed that SPE was more effective than LLE. The use of DMSO eliminated the necessity of HPLC clean-up for the removal of interferences. Although a toluene–ethanol mixture was a conventional solvent in SPE, the extraction using DMSO was more efficient because it was time-effective and showed the cleanest HPLC chromatograms.

The SPE-DMSO method comprised a single step, including clean-up together with extraction and concentration, and was superior to other methods. These results will provide the guidelines for the analysis of aromatic compounds from various aqueous matrices.

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