

Loss of Polychlorinated Dibenzo-*p*-dioxins, Polychlorinated Dibenzofurans and Coplanar Polychlorinated Biphenyls during Vacuum Concentration for Ultra-trace Analysis

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We investigated the effect of kinds of organic solvent on the losses of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/DFs), coplanar polychlorinated biphenyls (Co-PCBs), and their ¹³C₁₂-labeled compounds during vacuum concentration. *n*-Hexane and toluene solutions containing the standards of those compounds were tested for the purpose. The kind of organic solvent has remarkable effect on the loss of ¹³C₁₂-Co-PCBs from the standard solutions, while it has little or no effect on the losses of Co-PCBs, PCDD/DFs, and ¹³C₁₂-PCDD/DFs from the standard solutions. The losses of lower chlorinated ¹³C₁₂-Co-PCBs with *n*-hexane solution were greater than the loss of the compounds with toluene solution. The relative amounts of ¹³C₁₂-Co-PCBs in the condensate solvent produced during the vacuum concentration show that those of the compounds with toluene solution were less than that of *n*-hexane solution. These results demonstrate that the minimization of loss of the compounds with *n*-hexane solution may be achieved by adding toluene before the vacuum concentration. We also examined the relationship between the relative amounts of Co-PCBs and ¹³C₁₂-Co-PCBs in the condensate solvents and vapor pressures of those compounds. There was the difference in the regression line slopes of the relative amounts vs. in the vapor pressures between the *n*-hexane solution and toluene solution. This fact could be useful for the optimization of the vacuum concentration conditions in the convenient analytical methods.

Key Words : Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/DFs); Coplanar polychlorinated biphenyls (Co-PCBs); Analyte loss; Vacuum concentration; Ultra-trace analysis

1 Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are found in the diverse environment throughout the world. Those compounds have garnered much attention because of their high toxicity and potential risk to human health^{1,2)}. Chemical analyses for these compounds are ultra-trace-level analyses that require the minimization of interferences from organic solvents, reagents, glassware, and other sample-processing hardware. In addition, they demand the minimization of analyte losses from sample solutions during the multi-step sample cleanup processes that precede GC/MS analysis. Particularly, macro-concentration during the processing, in which the sample solutions are concentrated from a few hundred milliliters to a few milliliters, requires special attention to minimize analyte losses because sample extracts in those processes must be macro-concentrated repeatedly for cleanup and for injection into the HPLC or GC/MS³⁻⁵⁾.

A rotary evaporator and Kuderna-Danish (K-D) concentrator

are often used for macro-concentration in PCDDs and PCDFs (PCDD/DFs), and PCBs analyses³⁻⁵⁾. A particular advantage of the rotary evaporator is the high-speed vaporization that is achieved by vacuum concentration, semi-automatic or automatic vacuum regulation, and user-friendly operation in comparison with that of a K-D concentrator. For that reason, it is often used in trace-analyses for an organic contaminant. However, the vacuum concentration technique using a rotary evaporator requires optimized conditions to minimize analyte losses because the concentration resulting from high-speed vaporization during the process can cause a remarkable loss of the solute's low boiling-point fraction⁶⁾. Moreover, the high-speed vaporization may engender bumping from the sample solution. Such losses degrade the quality of analytical data. Furthermore, such losses contribute to cross-contamination during PCDD/DFs and PCBs analysis. Few studies have been made to minimize losses of PCDD/DFs or PCBs from sample solutions during the vacuum concentration. O'Keefe *et al.*⁷⁾ investigated various concentration procedures that are used to concentrate standard solutions: 2,3,7,8-TetraCDD losses were

not observed during vacuum evaporation-to-dryness in a benzene solution. Duinker *et al.*⁶⁾ reported that the initial solvent temperature should be lower than room temperature to avoid bumping during the vacuum concentration, particularly when using pentane and dichloromethane as solvents. Such high-speed vaporization can cause remarkable loss of low boiling-point fractions of solutes. In addition, official standard test methods for PCDD/DFs, and PCBs or Coplanar PCBs (Co-PCBs) have been proposed by some organizations, such as the Japanese Industrial Standards (JIS) Committee³⁾ and the U.S. Environmental Protection Agency (U.S. EPA)^{4,5)}. The extraction procedures and liquid chromatography during the cleanup processes for the standard test methods can produce large volumes of dilute solutions containing those compounds dissolved in diverse organic solvents such as *n*-hexane, toluene, acetone, and dichloromethane. The vacuum concentration technique using a rotary evaporator was performed to recover those compounds from the dilute solutions. The standard test methods for PCDD/DFs and Co-PCBs have explained that analyte loss may occur when the vacuum concentration is performed too rapidly. However, optimized conditions of the vacuum concentration on those solvents used for the cleanup processes have never been described in detail in the standard test methods. Furthermore, studies have never assessed the effect of kinds of organic solvent on the losses of those compounds during the vacuum concentration processing. Moreover, no studies addressing the effect of kinds of organic solvent on the loss of toxic PCDD/DFs, aside from 2, 3, 7, 8-TetraCDD, have been performed because those other toxic PCDD/DFs standards were not commercially available.

The effects of rotary evaporation on the losses of PCDD/DFs and PCBs, as described above, are correctable because those compounds are quantified using labeled internal standards and isotope dilution. However, unacceptable recovery losses of those compounds during the analyses decrease the method performance³⁻⁵⁾. For that reason, the minimization of recovery losses of those compounds caused by rotary evaporation will contribute to maintaining the analytical method performance and the system quality.

Previous studies have specifically pursued trace level analysis for organic contaminants in various environmental media. In addition, we have reviewed studies that have attempted to optimize analytical conditions of contaminants⁸⁻¹⁰⁾. However, the trials are time consuming because those analytical procedures are labor intensive. Furthermore, studies of aspects of physical chemistry for organic contaminants have never been performed sufficiently to obtain optimized conditions. Therefore, we undertake the present investigation to obtain criteria for optimal design of the vacuum concentration conditions for PCDD/DFs and PCBs analysis. The present study examines the effect of kinds of organic solvent on the losses of PCDD/DFs and Co-PCBs designated as toxic by the World Health Organization (WHO). In addition, we examine their ¹³C₁₂-labeled compounds during the vacuum concentration process. Thereafter, we analyze the relationship between the results and vapor pressures of those compounds.

2 Experimental

2.1 Apparatus and conditions

The experiments were performed with a rotary evaporator (BÜCHI Rotavapor Model R-205V; Sibata Scientific Technology Ltd., Tokyo, Japan) equipped with a vacuum system (BÜCHI

Vacuum System Model V-503N; Sibata Scientific Technology Ltd.) and a vacuum controller (BÜCHI Vacuum Controller Model V-805; Sibata Scientific Technology Ltd.). A circulator (CCA1100; Tokyo Rikakikai Co., Ltd., Tokyo, Japan) was used to cool a condenser. Vacuum concentration conditions were referred from information on choosing optimum distillation conditions for organic solvents¹¹⁾. They were as follows: working pressure for the boiling point of solvent at 40°C, *n*-hexane, 335 ± 30 hPa, toluene, 77 ± 10 hPa; heating bath temperature, 60 ± 1°C; cooling condenser temperature, 10 ± 1°C; flask rotation speed, 90 rpm.

2.2 Materials

Erlenmeyer flask (500 ml, clear joint, size 29/32), pear-shaped flask (10 ml, clear joint, joint size 15/25), and an oval solvent-splash guard adaptor as a bumping trap (200 ml, clear joint, top outer joint size 29/42, lower inner joint size 29/32) were purchased from Kimura Rikagaku Seisakusho Co., Ltd. (Tokyo, Japan). A round solvent-splash guard adaptor (400 ml, vertical type, clear joint, top outer joint size 29/42, lower inner joint size 15/25), and a Kjeldahl flask (200 ml, SPC joint, joint size 29/32) were purchased from Sibata Scientific Technology Ltd. All glassware used for this study was baked at 450°C for 4 h and washed with acetone, then *n*-hexane prior to use. Cartridge filters (CCF-005-D1B and CCF-050-C1H; Advantec Toyo Kaisha Ltd., Tokyo, Japan) were used for ambient indoor-air purification.

2.3 Standards and reagents

The following PCDD/DFs, Co-PCBs, and their ¹³C₁₂-labeled internal standards in nonane solution were purchased from Wellington Laboratories, Inc. (Ontario, Canada): PCDD/DFs (DF-ST-A, 1,000 pg/μl), Co-PCBs (PCB-ST-A, 2,000 pg/μl), ¹³C₁₂-labeled cleanup internal standard (DF-LCS-A, 1,000 pg/μl; PCB-LCS-A, 2,000 pg/μl), ¹³C₁₂-labeled injection internal standard (DF-IS-I, PCB-IS-B, 1,000 pg/μl). The PCDD/DFs, Co-PCBs, their ¹³C₁₂-labeled cleanup internal compounds (¹³C₁₂-PCDD/DFs and ¹³C₁₂-Co-PCBs), and ¹³C₁₂-labeled injection internal compounds were diluted using nonane.

Acetone, *n*-hexane, toluene, and nonane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All solvents used for this study were of dioxin-analysis grade.

2.4 Procedure

Figure 1 shows the experimental procedure schematically. *n*-Hexane and toluene were used for this study as organic solvents because they are often used during the cleanup process for PCDD/DFs and PCBs analysis³⁻⁵⁾. Each PCDD/DF, Co-PCB, ¹³C₁₂-PCDD/DF, and ¹³C₁₂-Co-PCB isomer (Table 1) was put separately into 300 ml of either *n*-hexane or toluene in an Erlenmeyer flask. Each flask was connected to an oval solvent-splash guard adaptor. In addition, the flask was connected to a rotary evaporator. Vacuum was promptly applied to the system; we began rotating the Erlenmeyer flask. Thereafter, the flask was lowered into the preheated heating bath, then removed from the heating bath. Its rotation stopped when the sample solutions in the flask reached an apparent volume of *ca.* 1–2 ml. In addition, ambient indoor-air was slowly and carefully admitted into the system. The air was purified by passage through two different cartridge filters that were connected in series to a manual-leak vent. After vacuum concentration, two samples were obtained: a concentrated sample in the Erlenmeyer flask, and a condensate

sample, which was caught in the solvent-splash guard adaptor. The concentrated sample in the flask was transferred into a pear-shaped flask. A round solvent-splash guard adaptor was connected to the rotary evaporator. In addition, the flask was connected to the adaptor. The sample was evaporated to ca. 0.5 ml in the manner explained above. Thereafter, the sample was slowly and carefully evaporated to near dryness using a nitrogen-gas blowdown apparatus. The nitrogen-gas blowdown condition was conducted according to a reported condition¹⁰. Addition of 15 μ l of nonane and 10 μ l of ¹³C₁₂-labeled injection internal compounds into the flasks reconstituted the concentrated sample.

The condensate sample in the oval solvent-splash guard adaptor was transferred into a Kjeldahl flask, which was evaporated to ca. 1–2 ml using the rotary evaporator. The concentrated sample was transferred into a pear-shaped flask. The sample was prepared as explained above from the vacuum concentration step (II) to the reconstitution step. Reconstituted samples were collected in a sample vial for HRGC/HRMS analysis. Control standard solutions were prepared as described above from the reconstitution step. They were used for calculating percent recovery and percent volatility. Control standard solutions were collected in a sample vial for HRGC/HRMS analysis. The test samples and control test samples were stored in the dark at 4 °C until HRGC/HRMS analysis. Percent recoveries were calculated as follows: the ratio of the integrated areas of respective compounds to the ¹³C₁₂-labeled injection internal compounds in the standard solutions)/(the ratio of the integrated area of respective compounds to the ¹³C₁₂-labeled injection internal compounds in the control standard solution) ×

Table 1 CAS numbers and added amounts of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/DFs), coplanar polychlorinated biphenyls (Co-PCBs), and their ¹³C₁₂-labeled compounds.

Compound	Unlabeled analyte		Labeled analyte ^a	
	CAS No. ^b	Added amount / μ g	CAS No. ^b	Added amount / μ g
PCDD/DFs				
2,3,7,8-TetraCDD	1746-01-6	500	76523-40-5	250
1,2,3,7,8-PentaCDD	40321-76-4	500	109719-79-1	250
1,2,3,4,7,8-HexaCDD	39227-28-6	500	109719-80-4	250
1,2,3,6,7,8-HexaCDD	57653-85-7	500	109719-81-5	250
1,2,3,7,8,9-HexaCDD	19408-74-3	500	109719-82-6	250
1,2,3,4,6,7,8-HeptaCDD	35822-46-9	500	109719-83-7	250
OctaCDD	3268-87-9	1000	114423-97-1	500
2,3,7,8-TetraCDF	51207-31-9	500	89059-46-1	250
1,2,3,7,8-PentaCDF	57117-41-6	500	109719-77-9	250
2,3,4,7,8-PentaCDF	57117-31-4	500	116843-02-8	250
1,2,3,4,7,8-HexaCDF	70648-26-9	500	114423-98-2	250
1,2,3,6,7,8-HexaCDF	57117-44-9	500	116843-03-9	250
1,2,3,7,8,9-HexaCDF	72918-21-9	500	116843-04-0	250
2,3,4,6,7,8-HexaCDF	60851-34-5	500	116843-05-1	250
1,2,3,4,6,7,8-HeptaCDF	67562-39-4	500	109719-84-8	250
1,2,3,4,7,8,9-HeptaCDF	55673-89-7	500	109719-94-0	250
OctaCDF	1010-77-1	1000	- ^c	500
¹³C₁₂-labeled injection internal standard				
1,2,7,8-TetraCDF	-	-	-	250
1,2,3,4,6-PentaCDF	-	-	-	250
1,2,3,4,6,9-HexaCDF	-	-	-	250
1,2,3,4,6,8,9-HeptaCDF	-	-	-	250
Co-PCBs				
3,3',4,4'-TetraCB (#77) ^d	32598-13-3	500	105600-23-5	250
3,4,4',5'-TetraCB (#81)	70362-50-4	500	208461-24-9	250
2,3,3',4,4'-PentaCB (#105)	32598-14-4	500	208263-62-1	250
2,3,4,4',5'-PentaCB (#114)	74472-37-0	500	208263-63-2	250
2,3,4,4',5'-PentaCB (#118)	31508-00-6	500	104130-40-7	250
2,3,4,4',5'-PentaCB (#123)	65510-44-3	500	208263-64-3	250
3,3',4,4',5'-PentaCB (#126)	57465-28-8	500	208263-65-4	250
2,3,3',4,4',5'-HexaCB (#156)	38380-08-4	500	208263-68-7	250
2,3,3',4,4',5'-HexaCB (#157)	69782-90-7	500	235416-30-5	250
2,3,3',4,4',5'-HexaCB (#167)	52663-72-6	500	208263-69-8	250
3,3',4,4',5,5'-HexaCB (#169)	32774-16-6	500	208263-70-1	250
2,2',3,3',4,4',5'-HeptaCB (#170)	35065-30-6	500	-	250
2,2',3,3',4,4',5,5'-HeptaCB (#180)	35065-29-3	500	-	250
2,3,3',4,4',5,5'-HeptaCB (#189)	39635-31-9	500	208263-73-4	250
¹³C₁₂-labeled injection internal standard				
2,3',4',5'-TetraCB (#70)	-	-	-	250
2,3,3',5,5'-PentaCB (#111)	-	-	235416-29-2	250

a. ¹³C₁₂-Labeled Cleanup Internal Standard.

b. Registry numbers of the Chemical Abstracts Service.

c. Not registered.

d. International Union of Pure and Applied Chemistry number.

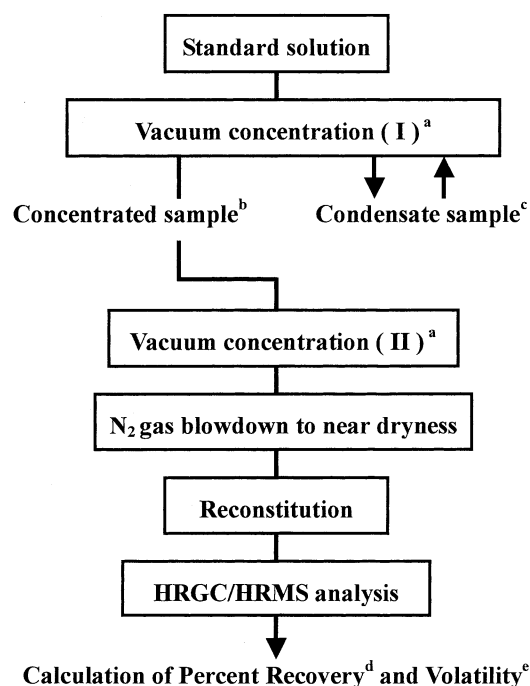


Figure 1 Schematic illustration of the experimental procedure. (a) A rotary evaporator was used. (b) A concentrated sample in an Erlenmeyer flask. (c) A condensate sample that was caught in a solvent-splash guard adaptor with a condensed solvent. (d) Relative amounts of analytes in the concentrated sample produced during vacuum concentration (I), %. (e) Relative amounts of analytes in the condensate sample produced during vacuum concentration (I), %.

100 (%). Percent volatilities were calculated as follows: the ratio of the integrated areas of respective compounds to the ¹³C₁₂-labeled injection internal compounds in the condensate solutions)/(the ratio of the integrated area of respective compounds to the ¹³C₁₂-labeled injection internal compounds in the control standard solution) × 100 (%). Experiments were carried out in a class 10,000 clean room.

2.5 HRGC/HRMS analysis

We performed HRGC/HRMS analysis using a gas chromatograph (Agilent 6890; Agilent Technologies Inc., Palo Alto, CA, USA) with an injector (Agilent 7683; Agilent Technologies Inc.) that was connected directly to a mass spectrometer (JMS-700D; JEOL, Tokyo, Japan). The GC operating conditions were: TetraCDD/DFs to HexaCDD/DFs separation column (SP2331, 60 m × 0.32 mm i.d.; Supelco, Bellefonte, PA, USA), stationary phase film thickness of 0.2 μ m; non-activated guard column (0.25 mm i.d.; GL Sciences Co. Ltd., Tokyo, Japan); 1-min splitless time; column temperature of 100 °C (1-min hold) → 20 °C/min → 200 °C → 2 °C/min → 265 °C (21.5-min hold); helium carrier gas of 1.5 ml/min (constant flow); HeptaCDD/DFs to OctaCDD/DF separation column (DB17, 30 m × 0.25 mm i.d.; J&W Scientific Inc., Folsom, CA, USA), stationary phase film thickness of 0.15 μ m; non-activated guard column (0.25 mm i.d.; GL Sciences Co. Ltd.); 1-min splitless time; column temperature of 120 °C (1-min hold) → 20 °C/min → 280 °C (15-min hold); helium carrier gas of 1.0 ml/min (constant flow); TetraCBs to HeptaCBs (Co-PCBs) separation column (HT8, 50 m × 0.22 mm i.d.; SGE Japan, Inc.), stationary phase film thickness of 0.25 μ m; non-activated guard

column (0.25 mm i.d.; GL Sciences Co. Ltd.); 1-min splitless time; column temperature of 100°C (1-min hold) → 20°C/min → 180°C → 3°C/min → 280°C (7-min hold), helium carrier gas of 1.0 ml/min (constant flow). MS conditions were: ion source temperatures of 280°C for TetraCDD/DFs to HexaCDD/DFs and Co-PCBs and 300°C for HeptaCDD/DFs to OctaCDD/DF; electron impact ionization mode; 600 μA filament current; 38 eV ionization voltage; 10 kV accelerating voltage; selected ion monitoring detection method; resolution >10,000 (10% valley definition); monitoring ion (*m/z*), quantitative ions, (M+2)⁺ for TetraCDD/DFs to HeptaCDD/DFs and TetraCBs to HeptaCBs, (M+4)⁺ for OctaCDD/DF, reference ions, M⁺ for TetraCDD/DFs and TetraCBs, (M+4)⁺ for PentaCDD/DFs to HeptaCDD/DFs and PentaCBs to HeptaCBs, and (M+2)⁺ for OctaCDD/DF. Diok software (JEOL) was used to analyze chromatographic data.

3 Results and Discussion

3.1 Preliminary experiments for ascertainment of actual distillation conditions.

Before starting any work on actual vacuum concentration, preliminary experiments in actual distillation conditions were performed to ascertain whether optimum distillation conditions for solvents¹¹ used in the present study might engender bumping. We also ascertained the concentration time of standard solutions under the above conditions.

Those examinations showed no bumping or visible boiling of sample solutions in these conditions. In addition, concentration times of about 16 min for *n*-hexane and 26 min for toluene were required for each solvent to reach complete concentration. According to the U.S. EPA method^{4,5}, the distillation condition for sample solutions is required to cause no bumping or visible boiling of the sample solutions during the process. In addition, the proper concentration time required to complete concentration is recommended as 15–20 min. Concentration times used in the present study were between 16 min and 26 min, as described above, which were equal to or longer than that of the U.S. EPA method. Therefore, it is likely that the difference in concentration time between *n*-hexane and toluene solutions do not cause the losses of PCDD/DFs and Co-PCBs including their ¹³C₁₂-labeled compounds.

3.2 Effect of kinds of organic solvent on percent recovery.

The effect of kinds of organic solvent on the percent recoveries of PCDD/DFs, Co-PCBs, and their ¹³C₁₂-labelled compounds from standard solutions was studied. The percent recoveries represent relative amounts of those compounds in the standard solutions during the vacuum concentration as described in section 2.4.

Those results are tabulated in Table 2. Most of the mean percent recoveries of PCDD/DFs and their ¹³C₁₂-labeled compounds in the *n*-hexane solutions were 100 ± 10%. Most of those in the toluene solutions showed similar results. These results suggest that kinds of organic solvent have little or no effect on the percent recoveries of PCDD/DFs and ¹³C₁₂-PCDD/DFs. Furthermore, not only the mean percent recoveries of Co-PCBs and their ¹³C₁₂-labeled compounds in the toluene solutions, but also those of Co-PCBs in the *n*-hexane solution were 100 ± 10%. However, only the mean percent recoveries of ¹³C₁₂-Co-PCBs in the *n*-hexane solution range from 81.3–97.7%. Particularly, mean percent recoveries of ¹³C₁₂-3,3',4,4'-TetraCB (#77) and ¹³C₁₂-3,4,4',5-TetraCB (#81) in the *n*-

Table 2 Effect of kinds of organic solvent on percent recoveries of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/DFs), coplanar polychlorinated biphenyls (Co-PCBs), and their ¹³C₁₂-labeled compounds.

Compound (IUPAC Number)	Recovery ± S.D. / %			
	<i>n</i> -Hexane		Toluene	
	Unlabeled analyte	Labeled analyte	Unlabeled analyte	Labeled analyte
PCDD/DFs				
2,3,7,8-TetraCDD	97.9 ± 2.0	98.2 ± 4.4	94.1 ± 1.4	99.6 ± 2.1
1,2,3,7,8-PentaCDD	102.1 ± 1.6	113.7 ± 5.0	101.7 ± 1.6	117.1 ± 2.2
1,2,3,4,7,8-HexaCDD	106.2 ± 1.9	99.5 ± 2.8	105.6 ± 3.9	95.5 ± 0.7
1,2,3,6,7,8-HexaCDD	97.2 ± 5.4	100.1 ± 4.7	97.1 ± 0.8	99.8 ± 2.7
1,2,3,7,8,9-HexaCDD	107.1 ± 3.6	95.9 ± 2.8	106.3 ± 5.5	96.1 ± 1.5
1,2,3,4,6,7,8-HeptaCDD	99.2 ± 2.3	105.3 ± 3.9	98.7 ± 1.8	111.6 ± 5.5
1,2,3,4,6,7,8,9-OctaCDD	100.3 ± 5.2	98.3 ± 2.9	102.3 ± 6.1	95.6 ± 1.2
1,3,6,8-TetraCDF	96.0 ± 3.0	90.2 ± 2.1	93.8 ± 5.3	91.7 ± 5.5
2,3,7,8-TetraCDF	97.6 ± 0.3	93.8 ± 2.5	103.7 ± 2.2	95.8 ± 3.7
1,2,3,7,8-PentaCDF	99.4 ± 1.7	106.2 ± 6.6	98.9 ± 1.2	110.8 ± 2.7
2,3,4,7,8-PentaCDF	98.9 ± 0.8	111.0 ± 8.8	97.6 ± 1.3	106.0 ± 2.2
1,2,3,4,7,8-HexaCDF	102.9 ± 1.6	93.1 ± 4.3	104.4 ± 2.6	89.6 ± 4.0
1,2,3,6,7,8-HexaCDF	105.9 ± 5.4	99.1 ± 4.1	106.4 ± 1.6	98.9 ± 6.3
1,2,3,7,8,9-HexaCDF	98.6 ± 4.7	94.2 ± 6.8	98.1 ± 2.6	92.3 ± 2.5
2,3,4,6,7,8-HexaCDF	102.5 ± 2.2	91.0 ± 1.1	100.6 ± 1.9	89.6 ± 2.4
1,2,3,4,6,7,8-HeptaCDF	106.0 ± 1.8	103.8 ± 7.6	115.8 ± 2.3	104.9 ± 4.7
1,2,3,4,7,8,9-HeptaCDF	106.3 ± 3.0	100.1 ± 6.5	98.8 ± 3.4	99.5 ± 4.9
1,2,3,4,6,7,8,9-OctaCDF	100.9 ± 1.8	102.0 ± 1.7	95.1 ± 3.3	104.5 ± 6.8
Co-PCBs				
3,3',4,4'-TetraCB (#77)	97.8 ± 0.6	84.8 ± 1.2	98.2 ± 0.3	92.1 ± 1.8
3,4,4',5-TetraCB (#81)	95.5 ± 1.4	81.3 ± 1.8	96.7 ± 1.6	91.0 ± 4.0
2,3,9,4',5-PentaCB (#105)	99.4 ± 1.9	93.3 ± 3.6	100.2 ± 1.3	94.4 ± 4.3
2,3,4,4',5-PentaCB (#114)	99.4 ± 1.1	89.0 ± 2.9	99.5 ± 1.3	92.6 ± 2.4
2,3,4,4',5-PentaCB (#118)	97.2 ± 1.4	90.2 ± 2.7	98.6 ± 0.7	92.1 ± 3.6
2',3,4,4',5-PentaCB (#123)	101.1 ± 2.5	89.5 ± 1.6	101.3 ± 1.1	93.3 ± 3.6
3,3',4,4',5-PentaCB (#126)	99.2 ± 2.4	92.9 ± 2.4	101.3 ± 1.4	95.3 ± 3.4
2,3,3',4,4',5-HexaCB (#156)	96.2 ± 2.5	93.1 ± 3.4	98.5 ± 4.8	97.9 ± 3.9
2,3,3',4,4',5-HexaCB (#157)	97.2 ± 2.1	97.7 ± 3.1	100.0 ± 2.6	102.9 ± 4.0
2,3',4,4',5,5'-HexaCB (#167)	97.0 ± 0.8	91.5 ± 3.7	98.8 ± 2.1	99.1 ± 4.8
3,3',4,4',5,5'-HexaCB (#169)	96.8 ± 2.4	95.4 ± 2.3	98.8 ± 2.4	101.6 ± 2.3
2,2',3,3',4,4',5'-HeptaCB (#170)	93.8 ± 3.1	92.7 ± 0.3	99.8 ± 4.1	98.4 ± 6.1
2,2',3,3',4,4',5'-HeptaCB (#180)	95.3 ± 4.1	91.7 ± 2.6	98.0 ± 3.1	95.2 ± 5.2
2,3,3',4,4',5,5'-HeptaCB (#189)	93.8 ± 4.3	91.8 ± 2.5	98.0 ± 4.6	95.9 ± 1.5

IUPAC Number = International Union of Pure and Applied Chemistry Number.

S.D. = Standard deviation. Experiments were made in triplicate.

hexane solution were low, and their percent recoveries were 84.8% and 81.3%, even though the recommended distillation conditions^{4,5} were used in the present study. These results suggest that kinds of organic solvent have a remarkable effect on the percent recoveries of ¹³C₁₂-TetraCBs. Therefore, we conclude that optimal vacuum concentration conditions must be determined for each kind of solvent to minimize the loss of volatile isomers from the standard solution.

3.3 Effect of kinds of organic solvent on percent volatility.

The relative amounts of PCDD/DFs, Co-PCBs, and their ¹³C₁₂-labelled compounds in the condensate solvent produced during the vacuum concentration was also studied to elucidate the effect of kinds of organic solvent on the percent recoveries of those compounds in greater detail. The relative amounts of those compounds in the condensate solvent represent percent volatilities of those compounds as described in section 2.4.

Those results are tabulated in Table 3. The mean percent volatilities of PCDD/DFs and ¹³C₁₂-PCDD/DFs were almost under 0.1% or were 0.1–0.9%. These results suggest that kinds of organic solvent have little or no effect on losses of PCDD/DFs and ¹³C₁₂-PCDD/DFs from the standard solutions. The results are consistent with the percent recovery results for PCDD/DFs and ¹³C₁₂-PCDD/DFs described in the previous section. In contrast, all mean percent volatilities of the Co-PCBs and ¹³C₁₂-Co-PCBs range from 0.1–5.7%. The result indicates that kinds of organic solvent have a great effect on the loss of Co-PCBs and ¹³C₁₂-Co-PCBs compared with those of PCDD/DFs and ¹³C₁₂-PCDD/DFs from the standard solutions. The results are also consistent with the percent recovery results for Co-PCBs and ¹³C₁₂-Co-PCBs described in the previous section. In addition, the mean percent volatilities of lower

Table 3 Effect of kinds of organic solvent on percent volatilities of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/DFs), coplanar polychlorinated biphenyls (Co-PCBs), and their $^{13}\text{C}_{12}$ -labeled compounds.

Compound (IUPAC Number)	Volatility / %			
	<i>n</i> -Hexane		Toluene	
	Unlabeled analyte	Labeled analyte	Unlabeled analyte	Labeled analyte
PCDD/DFs				
2,3,7,8-TetraCDD	0.4	0.8	0.6	0.6
1,2,3,7,8-PentaCDD	0.1	0.3	0.2	0.4
1,2,3,4,7,8-HexaCDD	< 0.1	< 0.1	< 0.1	< 0.1
1,2,3,6,7,8-HexaCDD	< 0.1	< 0.1	< 0.1	< 0.1
1,2,3,7,8,9-HexaCDD	< 0.1	< 0.1	< 0.1	< 0.1
1,2,3,4,6,7,8-HeptaCDD	0.4	< 0.1	< 0.1	< 0.1
1,2,3,4,6,7,8,9-OctaCDD	0.4	< 0.1	0.1	< 0.1
2,3,7,8-TetraCDF	0.4	0.9	0.6	0.6
1,2,3,7,8-PentaCDF	0.2	< 0.1	0.4	< 0.1
2,3,4,7,8-PentaCDF	0.2	< 0.1	0.3	< 0.1
1,2,3,4,7,8-HexaCDF	0.2	< 0.1	0.2	< 0.1
1,2,3,6,7,8-HexaCDF	0.1	< 0.1	0.2	< 0.1
1,2,3,7,8,9-HexaCDF	< 0.1	< 0.1	< 0.1	< 0.1
2,3,4,6,7,8-HexaCDF	< 0.1	< 0.1	< 0.1	< 0.1
1,2,3,4,6,7,8-HeptaCDF	0.9	< 0.1	0.8	< 0.1
1,2,3,4,7,8,9-HeptaCDF	0.4	< 0.1	0.2	< 0.1
1,2,3,4,6,7,8,9-OctaCDF	< 0.1	< 0.1	< 0.1	< 0.1
Co-PCBs				
3,3',4,4'-TetraCB (#77)	0.5	3.5	0.5	2.0
3,4,4',5'-TetraCB (#81)	1.0	5.7	0.6	2.3
2,3,3',4,4'-PentaCB (#105)	0.3	2.0	0.4	1.6
2,3,4,4',5'-PentaCB (#114)	0.8	3.1	0.5	1.3
2,3',4,4',5'-PentaCB (#118)	0.7	3.0	0.5	1.3
2,3,4,4',5'-PentaCB (#123)	0.7	2.7	0.5	1.3
3,3',4,4',5'-PentaCB (#126)	0.3	1.5	0.3	1.0
2,3,3',4,4',5'-HexaCB (#156)	0.2	2.5	0.2	2.0
2,3,3',4,4',5'-HexaCB (#157)	0.1	0.9	0.2	0.9
2,3',4,4',5'-HexaCB (#167)	0.4	1.7	0.3	0.9
3,3',4,4',5'-HexaCB (#169)	0.2	1.0	0.2	0.7
2,2',3,3',4,4',5'-HeptaCB (#170)	0.1	0.6	0.2	0.8
2,2',3,4,4',5,5'-HeptaCB (#180)	0.3	1.3	0.3	0.7
2,3,3',4,4',5,5'-HeptaCB (#189)	0.1	0.6	0.2	0.6

IUPAC Number = International Union of Pure and Applied Chemistry Number.

S.D. = Standard deviation. Percent volatility (%) = Relative amounts of analytes in the condensate solvent produced during vacuum concentration. Experiments were made in triplicate.

chlorinated $^{13}\text{C}_{12}$ -Co-PCBs ($^{13}\text{C}_{12}$ -Tetra through PentaCBs) in the *n*-hexane solution range from 1.5–5.7%, were higher than 1.0–2.3% for those in the toluene solution. Especially, percent volatilities of $^{13}\text{C}_{12}$ -3,3',4,4'-TetraCB (#77) and $^{13}\text{C}_{12}$ -3,4,4',5'-TetraCB (#81) in the *n*-hexane solution were 3.5 and 5.7%; those levels are exceptionally higher than those of the other isomers. The same tendency for #77 or #81 is shown in the Co-PCBs results. These results suggest that the losses of lower chlorinated Co-PCBs and $^{13}\text{C}_{12}$ -Co-PCBs tend to become greater than those of the higher chlorinated isomers. Therefore, losses of Co-PCBs and $^{13}\text{C}_{12}$ -Co-PCBs from the solutions might result from vaporization from the solutions⁶⁾ because those losses increase with decreasing chlorine number.

Consequently, the losses of PCDD/DFs and $^{13}\text{C}_{12}$ -PCDFs from the standard solutions were less than those of Co-PCBs and $^{13}\text{C}_{12}$ -Co-PCBs. This may be caused by the difference in the vapor pressures between Co-PCBs and PCDD/DFs because the vapor pressure of PCDD/DFs is 1–4 orders of magnitude lower than that of Co-PCBs^{12,13)}. Furthermore, minimization of loss of some lower chlorinated Co-PCBs with *n*-hexane solution may be achieved by adding toluene before the vacuum concentration because those losses with toluene solution were less than those losses with *n*-hexane solution.

3.4 Comparison between percent volatility of Co-PCBs and their vapor pressures.

It follows from the discussion thus far that the kinds of organic solvent used for this study have effect on loss of lower chlorinated Co-PCBs during the vacuum concentration processing. Their behavior is attributable to vapor pressure of the compounds. For

that reason, we examined the relationship between vapor pressure of Co-PCBs and their percent volatilities are considered as below.

Figures 2A and 2B show the relationship between mean percent volatilities of Co-PCBs and the vapor pressures at 25°C¹²⁾. The vapor pressures had been estimated using the retention index data that were determined using an intermediate polarity capillary column and non-polar packed column. Regression analysis revealed that, for Co-PCBs, the coefficient of determination (r^2) and line slope were 0.61 and 0.15, for the *n*-hexane solution and 0.92 and 0.10, for the toluene solution, respectively. Furthermore, Figures 2C and 2D show the relationship between mean percent volatilities of $^{13}\text{C}_{12}$ -Co-PCBs and their vapor pressures. Here we assume that vapor pressures of Co-PCBs and $^{13}\text{C}_{12}$ -Co-PCBs are almost equal. For $^{13}\text{C}_{12}$ -Co-PCBs, the coefficient of determination and line slope were 0.73 and 0.78, for the *n*-hexane solution and 0.26 and 0.44, for the toluene solution, respectively. These coefficients of determination results indicate that vapor pressures of Co-PCBs and $^{13}\text{C}_{12}$ -Co-PCBs have an effect on their losses from the standard solution. Such relationships of Co-PCBs and $^{13}\text{C}_{12}$ -Co-PCBs were not observed in the comparison between percent volatilities of PCDD/DFs and $^{13}\text{C}_{12}$ -PCDD/DFs in standard solutions and vapor pressures of those compounds. Therefore, these line slope results suggest that the difference between the

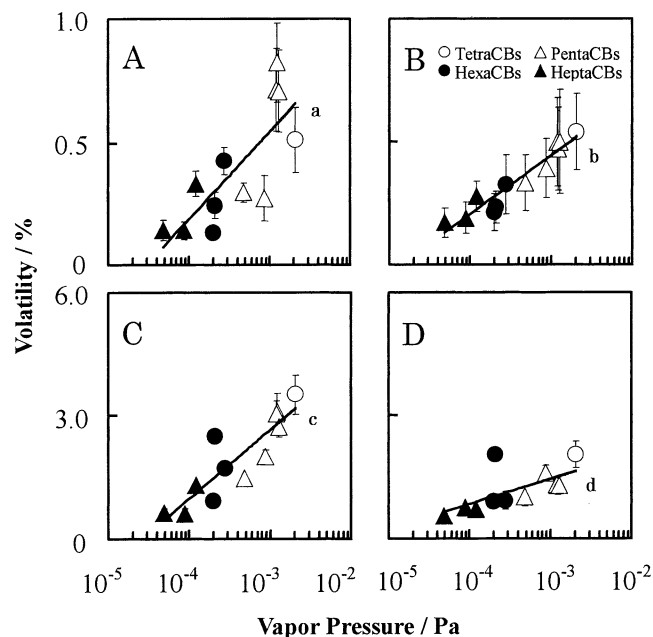


Figure 2 Plots of mean percent volatilities of Co-PCBs and $^{13}\text{C}_{12}$ -Co-PCBs versus vapor pressures of those compounds: (A) Co-PCBs, *n*-hexane solution; (B) Co-PCBs, toluene solution; (C) $^{13}\text{C}_{12}$ -Co-PCBs, *n*-hexane solution; (D) $^{13}\text{C}_{12}$ -Co-PCBs, toluene solution. Regression equation: (a) Percent volatility = 0.15 Ln (vapor pressure) + 1.6, $r^2 = 0.61$; (b) Percent volatility = 0.10 Ln (vapor pressure) + 1.2, $r^2 = 0.92$; (c) Percent volatility = 0.73 Ln (vapor pressure) + 7.7, $r^2 = 0.78$; (d) Percent volatility = 0.26 Ln (vapor pressure) + 3.3, $r^2 = 0.44$. The vapor pressures of Co-PCBs at 25°C used were obtained from Ref. 12. Plots of mean percent volatilities of $^{13}\text{C}_{12}$ -Co-PCBs and their vapor pressures assume that vapor pressures of $^{13}\text{C}_{12}$ -Co-PCBs and Co-PCBs are equal. Percent volatility (%) = Relative amounts of those compounds in the condensate solvent produced during vacuum concentration.

regression line slopes of *n*-hexane solution and that of the toluene solution is related strongly to the kinds of organic solvent. Such relationships of line slopes were not observed in the comparison between the percent recoveries in standard solutions and the vapor pressures of PCDD/DFs and Co-PCBs including their ¹³C₁₂-labeled compounds.

Further studies to elucidate the effect of various kinds of organic solvent and other vacuum concentration conditions on losses of PCDD/DFs and Co-PCBs including their ¹³C₁₂-labeled compounds during the processing may be required. Further information from real samples during the processing is required. However, the comparison of the relationship of line slope that expresses the difference in the effect of kinds of organic solvent might be a convenient method for optimizing vacuum concentration conditions in PCDD/DFs and Co-PCBs analyses because of the reasonable relationship between the loss of those compounds and their vapor pressures.

Conclusion

This study demonstrated that the kinds of organic solvent used in vacuum concentration remarkably affect the loss of Co-PCBs and ¹³C₁₂-Co-PCBs from standard solutions. The losses of some lower chlorinated ¹³C₁₂-Co-PCBs with toluene solution were less than the losses of those with *n*-hexane solution. The minimization of the loss of the compounds with *n*-hexane solution may be achieved by adding toluene before the vacuum concentration. The percent volatilities of Co-PCBs and ¹³C₁₂-Co-PCBs with *n*-hexane and toluene solutions show strong relevance to vapor pressures of those compounds. The comparison of the relationship of line slope that expresses the difference in the effect of kinds of organic solvent could facilitate optimization of the vacuum concentration conditions in PCDD/DFs and Co-PCBs analyses.

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