APPLICATION OF A SOLVENT-CUT LARGE-VOLUME INJECTION SYSTEM USING DEANS SWITCH-TYPE SILFLOW IN A DIOXIN ANALYSIS OF HUMAN BLOOD

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Introduction

The 'Yusho incident', which occurred in western Japan in 1968, was a mass food poisoning by polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDFs). PCDFs are highly toxic and are major causative agent of yusho disease. In 2004, the 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) concentration in the blood was added to the diagnostic criteria of yusho disease, and the concentrations of dioxins in the blood were measured at the health checkups of yusho. The blood concentrations of dioxins can be measured by high-resolution gas chromatography/mass spectrometry (HRGC/HRMS). Todaka et al. developed a highly sensitive method for dioxins measurement using HRGC/HRMS equipped with a solvent-cut large-volume (SCLV) injection system (SGE Analytical Science, Victoria, Australia) and a large-volume injection
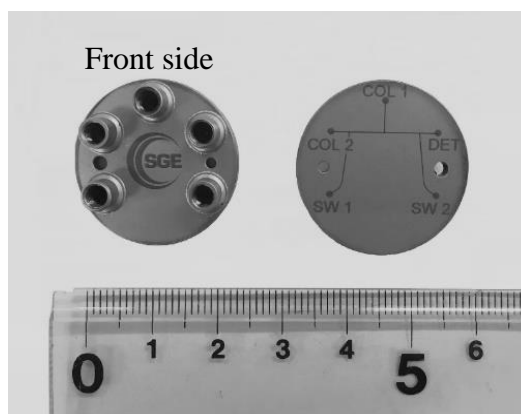

Figure 1 Deans Switch type SilFlow

(LVI) system of stomach type insert (Aisti Science, Wakayama, Japan)[1]. This LVI-SCLV method made it possible to measure dioxin concentrations in approximately 5 g blood samples. The SCLV injection system is one of the key devices for measuring blood dioxins in health checkup of yusho. However, since the LVI-SCLV system has many connection points with the capillary column, the risk of leakage is high. When leakage has occurs in the SCLV, much effort is needed to eliminate the cause of the leak. In addition, the SCLV system has been discontinued, and a system to replace it is necessary to measure dioxins in blood with high sensitivity.

The Deans Switch is a valveless switch based on pressure balance. It can be used for analyses using multiple columns, heart-cutting of specific components, and switching of the flow path to two columns[2,3]. In recent years, microfluid devices usable for gas chromatography have been developed based on the application of semiconductor technology. A Deans switch-type microflow channel device is now available, and its application examples are reported[4]. In the present investigation, we used the SilFlow™ Deans switch kit (hereafter SilFlow) manufactured by SGE Analytical Science (Victoria, Australia) (Figure 1) as a Deans switch-type microflow channel device, and we observed that the SilFlow can substitute for a SCLV injection system for the measurement of dioxins in human blood.

Materials and methods
Chemicals and materials

Native and labeled dioxin standards of polychlorinated dibenzo-p-dioxins (PCDDs), PCDFs, and non-ortho-dioxins like polychlorinated biphenyls (PCBs) were purchased from Wellington Laboratories (Ontario, Canada). The silver nitrate silica gel was purchased from Kanto Chemical Industries (Tokyo). Activated carbon was purchased from Nacalai Tesque, (Kyoto, Japan). All reagents and solvents of dioxin analysis grade were supplied by Kanto Chemical Industries. A control sample of frozen pool serum was used for quality control (Nissui Pharmaceuticals, Tokyo).

Procedure for determining dioxins in human blood

The extraction and purification of dioxins from human blood was performed according to the method of Todaka et al[1]. Blood samples (5 g) were spiked with ^{13}C labeled dioxin standards. The samples were extracted using n-hexane by accelerated solvent extraction. The extract was concentrated and purified by sulfuric acid treatment and a silver nitrate silica gel column. Next, impurities were removed with an activated carbon column, and dioxins excluding mono-ortho PCBs were fractionated with toluene. Eluted toluene was concentrated, a syringe spike was added. The determination of dioxins was performed on an Autospec Premier mass spectrometer (Waters, Manchester, UK) equipped with a 6890N Gas Chromatograph (Agilent, Santa Clara, CA), LVI system, and SilFlow; this is the LVI-SilFlow syetem. A BPX-5 capillary column (7 m × 0.25 mm i.d., 0.25 μm film) and a BPX-DIOXIN-I capillary column (30 m × 0.15 mm i.d., both columns from SGE Analytical Science) were used for pre-column and analytical column, respectively. The following parameters were used: initial injector temperature, 120°C; injection volume, 100 μL; source temperature, 280°C; filament current, 750 μA, and resolution, 10000 (10% valley definition).

Results and discussion

The comparison of LVI-SilFlow and LVI-SCLV (the conventional method)

Figure 2 shows the schematic diagram of LVI-SilFlow system. In the conventional method, i.e., LVI-SCLV, the sample is introduced to the mass spectrometer (MS) in the following process. A sample introduced from the LVI system is maintained in the pre-column. By raising the temperature of the gas chromatograph (GC) oven, dioxins are maintained in the pre-column, and a large volume of solvent and low-boiling-point compounds are discharged from the purge line. The purge line is closed and dioxins are introduced into the cold trap. After all dioxins are cold-trapped, the purge line is opened, and impurities remaining in the pre-column

Figure 2 The schematic diagram of the LVI-SilFlow system

are discharged. The temperature of the GC oven is lowered, the cold trap is released, and the temperature of the oven is raised again so that the dioxins are introduced into the MS.
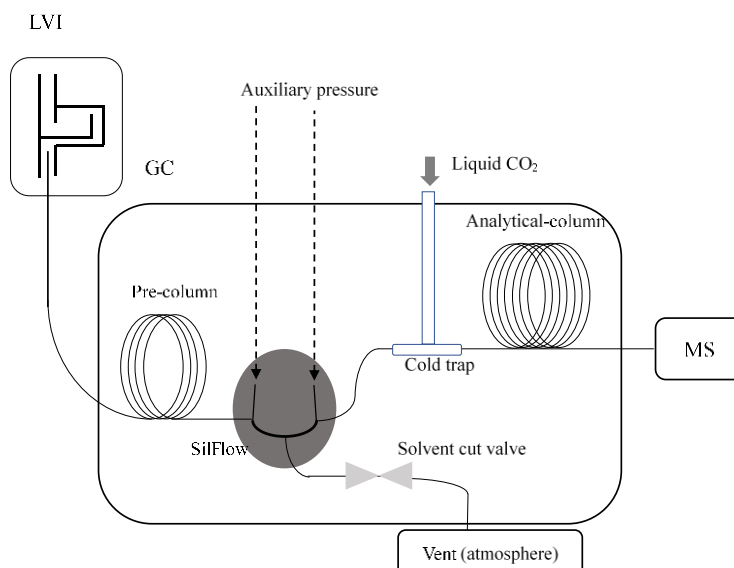
In the measurement of the standard solution of dioxins in the LVI-SCLV system, tetrachlorobiphenyls (TeCBs) are detected first and octachlorodibenzofuran (OCDF) is detected last. We injected 100 μL of the standard solution (0.0025 ng/mL) of dioxins into LVI-SilFlow and LVI-SCLV systems, respectively. Figure 3 shows mass chromatograms of TeCBs, PeCDFs and OCDF. Since the same chromatogram was obtained using the LVI-SCLV and LVI-SilFlow systems, we concluded that two systems were operating in the same mechanism.

Evaluation of reproducibility for dioxin measurements by the LVI-SilFlow system

In our measurement of dioxins in the blood per one batch, we have extracted and purified 24 samples containing control samples. Every time, dioxin standards and control samples were measured with HRGC/HRMS, and the validity of each measurement was evaluated using those measurments. In this study, we used the LVI-SilFlow system to measure the dioxins in the blood of 8 batches. Table 1 shows the relative response factor (RRF) of the dioxin standards in each batch. The concentration of the control sample was

calculated per wet weight to eliminate errors in the measurement of fat mass. In Japan's manual for measuring dioxins of blood[5], it is stipulated that the variation of RRF should be within ±20% compared with the previous measurement. As shown in Table 1, the RRF at each measurement was -10.5% to 12.5% compared to the average value of RRF of 8 batches in all congeners and was thus within the range of ±20%. Therefore, it was possible to measure dioxins with good reproducibility by using the LVI-SilFlow system.

Table 2 shows the measurement results of the control samples in each batch and the mean value, standard deviation (SD) and relative standard deviation (RSD) of each congener. According to Japan's manual for measuring dioxins in blood[5], cross-check results require that the difference between the mean of each measured concentration

Figure 3 Mass chromatograph of TeCBs, PeCDFs, and OCDF

and the measured concentration is within 50%. As shown in Table 2, the RSD of each congener was 4.1% at the minimum and 12.1% at the maximum, and thus SilFlow made it possible to measure blood dioxins with good reproducibility. The RSD of the toxicity equivalency quantity (TEQ) of dioxins with a TEF excluding mono-ortho PCBs was 3.2%, and the LVI results showed high reproducibility. In addition, this control sample result is similar to that reported by Todaka et al. using the LVI-SCLV[1]. Their measurement result was 0.063 pg-TEQ/g, which was equivalent to our measurement result (0.067 pg-TEQ/g). Since the SilFlow has a very small dead volume compared to the SCLV, the influence of contamination is small and handling is easy. Therefore, the SilFlow method can be substituted for the conventional method SCLV, and the LVI-SilFlow system can be applied to measurement of dioxins in the blood.

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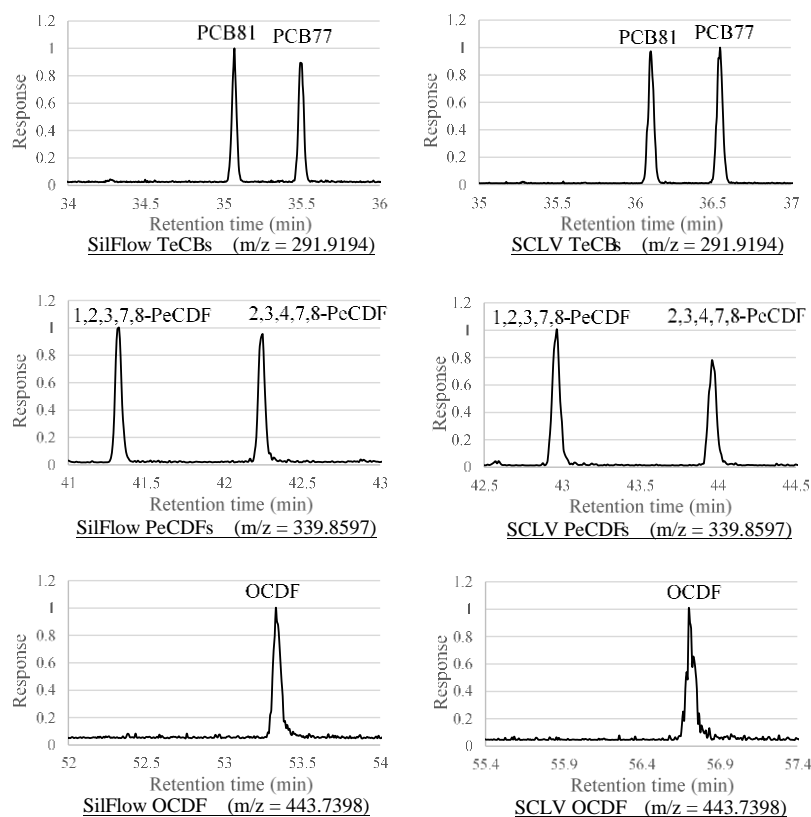
Table 1 The reproducibility of the relative response factor (RRF) of dioxin standards in LVI-SilFlow

| Congener | Relative Response Factor (RRF) | | | | | | | | | Min. RRF / | Max. RRF/ |
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Mean	Mean (%)	Mean (%)
2,3,7,8-TCDD	1.013	0.995	1.039	1.006	0.884	0.986	1.001	0.976	0.988	89.5	105.2
1,2,3,7,8-PeCDD	0.923	0.888	0.907	0.821	0.952	0.903	0.882	0.890	0.896	91.6	106.3
1,2,3,4,7,8-HxCDD	0.908	0.987	0.937	0.948	0.969	0.904	0.885	1.005	0.943	93.9	106.6
1,2,3,6,7,8-HxCDD	0.786	0.819	0.857	0.920	0.880	0.845	0.805	0.905	0.852	92.2	107.9
1,2,3,7,8,9-HxCDD	0.794	0.903	0.899	0.895	0.877	0.908	0.920	1.008	0.901	88.1	112.0
1,2,3,4,6,7,8-HpCDD	1.160	1.040	1.094	1.102	1.170	1.187	1.081	1.087	1.115	93.2	106.5
OCDD	0.990	1.033	1.046	1.088	0.971	1.011	1.053	1.197	1.049	92.6	114.2
2,3,7,8-TCDF	0.920	1.118	0.925	0.999	0.976	1.003	0.964	0.971	0.984	93.4	113.6
1,2,3,7,8-PeCDF	0.934	0.912	0.932	1.022	0.911	1.012	0.921	0.898	0.943	95.3	108.4
2,3,4,7,8-PeCDF	1.068	1.008	0.995	0.955	1.058	1.104	0.981	1.043	1.026	93.1	107.5
1,2,3,4,7,8-HxCDF	0.956	1.063	1.007	1.000	0.939	0.968	0.945	0.992	0.984	95.4	108.0
1,2,3,6,7,8-HxCDF	0.912	0.935	1.008	0.932	0.988	0.924	0.974	0.909	0.948	95.9	106.4
2,3,4,6,7,8-HxCDF	1.038	1.041	1.006	1.032	1.014	1.121	1.044	1.180	1.060	94.9	111.4
1,2,3,7,8,9-HxCDF	1.049	1.065	1.086	0.999	1.097	1.028	1.046	1.070	1.055	94.7	104.0
1,2,3,4,6,7,8-HpCDF	0.927	1.003	0.944	1.013	0.917	1.046	0.896	0.939	0.961	93.3	108.9
1,2,3,4,7,8,9-HpCDF	1.049	1.014	1.094	1.066	0.974	0.989	0.984	0.949	1.015	93.5	107.8
OCDF	0.952	0.978	1.061	0.902	0.901	0.985	0.896	0.870	0.943	92.2	112.5
344'5-TCB(PCB81)	0.936	0.950	0.916	0.947	0.916	0.950	0.932	0.966	0.939	97.5	102.9
33'4'4'-TCB(PCB77)	1.025	1.025	1.021	1.019	1.046	1.006	1.027	1.062	1.029	97.8	103.2
33'44'5-PenCB(PCB126)	1.132	1.037	1.110	1.102	1.063	1.084	0.979	1.066	1.072	91.3	105.6
33'44'55'-HxCB(PCB169)	0.926	0.845	0.954	0.927	0.888	0.995	0.897	0.973	0.926	91.3	107.5

Table 2 The reproducibility of concentrations of control sample in LVI-SilFlow

| Congener | Concentrations (pg/g) | | | | | | | | Mean | SD | RSD |
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8			
2,3,7,8-TCDD	0.0042	0.0043	0.0046	0.0040	0.0046	0.0042	0.0045	0.0040	0.0043	0.00022	5.0%
1,2,3,7,8-PeCDD	0.016	0.019	0.017	0.020	0.018	0.016	0.019	0.018	0.018	0.0012	6.7%
1,2,3,4,7,8-HxCDD	0.021	0.017	0.022	0.020	0.018	0.019	0.017	0.022	0.020	0.0018	9.3%
1,2,3,6,7,8-HxCDD	0.15	0.18	0.17	0.14	0.15	0.15	0.16	0.14	0.16	0.013	8.5%
1,2,3,7,8,9-HxCDD	0.026	0.027	0.024	0.024	0.029	0.027	0.032	0.025	0.027	0.0025	9.2%
1,2,3,4,6,7,8-HpCDD	0.37	0.39	0.37	0.33	0.40	0.35	0.47	0.47	0.40	0.048	12.1%
OCDD	2.4	2.4	2.5	2.3	2.5	2.4	2.3	2.2	2.4	0.099	4.1%
2,3,7,8-TCDF	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-
1,2,3,7,8-PeCDF	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-
2,3,4,7,8-PeCDF	0.015	0.016	0.020	0.018	0.019	0.020	0.015	0.018	0.018	0.0018	10.4%
1,2,3,4,7,8-HxCDF	0.020	0.023	0.027	0.021	0.022	0.026	0.023	0.021	0.023	0.0023	10.3%
1,2,3,6,7,8-HxCDF	0.020	0.022	0.023	0.018	0.021	0.021	0.019	0.018	0.020	0.0016	7.8%
2,3,4,6,7,8-HxCDF	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-
1,2,3,7,8,9-HxCDF	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-
1,2,3,4,6,7,8-HpCDF	0.042	0.049	0.054	0.044	0.048	0.045	0.048	0.052	0.048	0.0039	8.1%
1,2,3,4,7,8,9-HpCDF	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-
OCDF	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-
344'5-TCB(PCB81)	ND	ND	ND	ND	ND	ND	ND	ND	-	-	-
33'4'4'-TCB(PCB77)	0.097	0.091	0.10	0.083	0.098	0.094	0.100	0.083	0.093	0.0066	7.0%
33'44'5-PenCB(PCB126)	0.062	0.063	0.073	0.059	0.060	0.066	0.070	0.057	0.064	0.0052	8.2%
33'44'55'-HxCB(PCB169)	0.050	0.058	0.062	0.058	0.061	0.055	0.057	0.054	0.057	0.0036	6.4%
TEQ from PCDDs	0.045	0.050	0.048	0.046	0.047	0.045	0.050	0.047	0.047	0.0017	3.6%
TEQ from PCDFs	0.010	0.011	0.012	0.011	0.012	0.012	0.011	0.011	0.011	0.00072	6.4%
TEQ from PCDDs/PCDFs	0.056	0.061	0.060	0.057	0.059	0.057	0.060	0.058	0.059	0.0017	3.0%
TEQ from Non-ortho-PCBs	0.0077	0.0081	0.0091	0.0077	0.0078	0.0083	0.0088	0.0073	0.0081	0.00058	7.1%
Total-TEQ	0.063	0.069	0.069	0.065	0.067	0.065	0.069	0.065	0.067	0.0021	3.2%