APPLICATION OF SOLVENT CUT LARGE VOLUME (SCLV) INJECTION SYSTEM USING NARROW-BORE COLUMN TO DIOXINS ANALYSIS IN HUMAN BLOOD

KAZUHIRO TOBIISHI[1], Takashi Todaka[2], Hironori Hirakawa[1], Tsuguhide Hori[1], Takao Iida[1]

[1]Fukuoka Institute of Health and Environmental Sciences, Fukuoka
[2]Japan Food Hygiene Association, Tokyo

Introduction

An SCLV injection system has been developed as a quantitative technique on the scale of a few femtograms per microlitre.[1,2] It is advantageous in that it allows a large volume injection without GC injector modification, and it can be carried out under high vacuum because of its narrow-bore column. This system should therefore contribute to improving column noise level and resolution. There has been a few information regarding different types of capillary columns and analytical conditions.

We presented comparison of the SCLV injection system with the conventional technique for analyzing dioxins by HRGC-HRMS using cyanopropyl phase capillary columns.[3,4] We reported on the new protocol of dioxins analysis in human blood using SCLV injection system.[5] We also reported on improvement of the SCLV injection system using narrower-bore column.[6] In this paper, we apply an SCLV injection system using narrow-bore column to dioxins analysis in human blood.

Methods and Materials

HRGC-HRMS Measurement: All HRGC-HRMS analysis was conducted on a 6890 series GC (Agilent Technology, USA) equipped with Autospec-Ultima NT (Micromass, UK). The SCLV injection system (SGE, Australia) was equipped with a BPX-Dioxin-II (3m×0.15mm) capillary column (SGE, Australia) as the pre-column and a BPX-5 (10m×0.1mm×0.1μm) narrow-bore capillary column (SGE, Australia) as the analytical column. For the conventional SCLV injection system, a BPX-5 (6m×0.25mm×0.25μm) capillary column (SGE, Australia) as the pre-column and a BPX-Dioxin-I (30m×0.15mm) capillary column (SGE, Australia) as the analytical column were used. The analytical conditions for the SCLV injection system are shown in Table 1. Perfluorotributylamine (PFTBA) was used for lock-mass system under the measurement of narrow-bore capillary column system.

Sample: In order to confirm the conditions of the equipment, five microlitre of standard (0.2ng/mL, 2,3,7,8-substituted PCDDs and PCDFs mixture) in nonane was injected. And then, five grams of control serum sample was used for the analysis of human blood. The details of the clean-up method for human blood were described in another paper.[5]

Table 1 Analytical conditions for the SCLV injection system

	(A)	(B)
Pre-column	BPX-Dioxin-II (3m×0.15mm)	BPX-5 (6m×0.25mm×0.25μm)
Analytical column	BPX-5 (10m×0.1mm×0.1μm)	BPX-Dioxin-I (30m×0.15mm)
Purge on time	5 min	
Injector temperature	280ºC	
Oven temperature	160ºC(4min)→20ºC/min→ 280ºC(8.5min)→70ºC/min→ 210ºC (0.5min)→10ºC/min→ 280ºC (1.5min)	160ºC(3min)→20ºC/min→ 300ºC(8min)→60ºC/min→ 210ºC (0.5min)→3ºC/min→ 300ºC (1min)
Injector pressure	688kPa (4min)→2kPa/min→ 689kPa (14min)→249kPa/min→ 440kPa (0.5min)→8kPa/min→ 496kPa (1.5min)	469kPa (3min)→418kPa/min→ 678kPa (14.5min)→338kPa/min→ 340kPa (1min)→3.4kPa/min→ 442kPa (1min)
Cold trap	3.5-20 min cooling	2.5-20 min cooling
Solvent cut	4-18.5 min solvent cut: off	3-18 min solvent cut: off
MS Resolution	>10000	
Channels	5-11	
Cycle time	188-374 ms	310-610 ms
Analysis time	28.5 min	57.0 min

Results and Discussion

HRGC-HRMS Measurement: The peak width of 2,3,7,8-TeCDD in the chromatogram for the narrow-bore capillary column system was about 3 seconds, while that for the conventional column system was about 10 seconds. It is considered that the cycle time per scan must be set shorter in the narrow-bore capillary column system than in the conventional column system. So, the cycle time was set 188-374ms by decreasing the monitor and delay time. All PCDD/F analytes were eluted within about 28.5 minutes with the narrow-bore capillary column system, whereas elution took about 57 minutes with the conventional column system. We expect that the measurement using PFTBA contribute for decreasing the noise level but the differences could not be observed.

Comparison of Chromatograms: Chromatograms of the dioxin standard were compared between the narrow-bore capillary column system and the conventional column system. The chromatograms of TeCDD are presented in Figure 1; (A) stands for the use of the 10m×0.1mm column, and (B) for

the use of the 30m×0.15mm column. From this comparison, the resolution of the chromatograms obtained using the narrow-bore capillary column system was slightly lower than that of the conventional column system. The signal level from two systems is equal but the noise level from the narrow-bore capillary column system is higher than that from the conventional column system. We have a plan to compare with the column of different length and type.

Measurement of dioxins standard: Five microlitre of dioxins standard (0.2ng/mL) was injected seven times. Table 2 summarizes the retention time, average of amount, standard deviation and relative standard deviation (rsd%). Average of amount agreed on 1.0pg very well. The data of rsd% among the injections agreed by 1.60-6.77%. From these results, it is concluded that the narrow-bore capillary column system is effective for determining low-level dioxins.

Measurement of human blood: Concentrations of dioxins in control serum sample were measured using the narrow-bore capillary column system and the conventional column system. Table 3 summarizes the comparison of dioxins concentrations using the two systems. This comparison demonstrates agreement between the two systems.

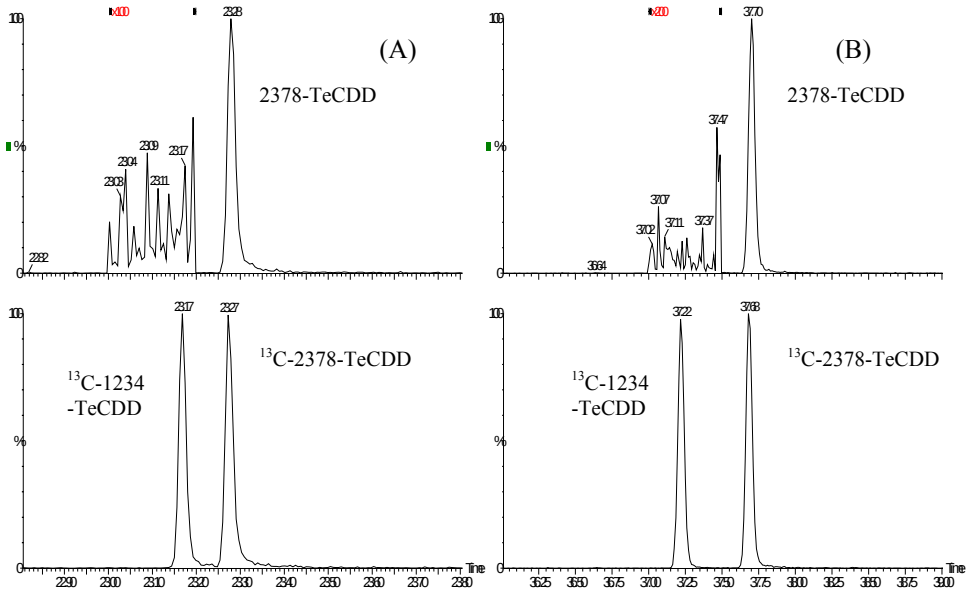

Figure 1 Chromatograms of TeCDD
(A) Narrow-bore capillary column system, (B) Conventional column system

Table 2 Measurement of dioxins standard using narrow-bore capillary column system

	Retention time	Average (pg)	Std	Rsd%
344'5-TeCB(#81)	22.42	0.998	0.0217	2.17
33'44'-TeCB(#77)	22.52	1.00	0.0361	3.61
33'44'5-PeCB(#126)	23.35	0.998	0.0494	4.95
33'44'55'-HxCB(#169)	24.28	1.00	0.0455	4.55
2378-TeCDD	23.28	1.00	0.0542	5.42
12378-PeCDD	24.32	1.00	0.0626	6.25
123478-HxCDD	25.37	1.00	0.0677	6.77
123678-HxCDD	25.42	1.00	0.0568	5.67
123789-HxCDD	25.51	1.00	0.0751	7.50
1234678-HpCDD	26.56	1.00	0.0588	5.88
OCDD	27.75	1.00	0.0676	6.76
2378-TeCDF	23.17	1.00	0.0347	3.47
12378-PeCDF	24.01	0.999	0.0160	1.60
23478-PeCDF	24.28	1.00	0.0322	3.22
123478-HxCDF	25.09	0.999	0.0384	3.85
123678-HxCDF	25.14	0.999	0.0168	1.69
234678-HxCDF	25.35	1.00	0.0439	4.39
123789-HxCDF	25.65	1.00	0.0290	2.90
1234678-HpCDF	26.13	1.00	0.0266	2.66
1234789-HpCDF	26.77	1.00	0.0171	1.71
OCDF	27.83	0.998	0.0343	3.43

Table 3 Comparison of concentrations of dioxins in control serum sample (pg/g-lipid)

	narrow-bore capillary column system	Conventional column system
344'5-TeCB(#81)	nd	nd
33'44'-TeCB(#77)	63	47
33'44'5-PeCB(#126)	25	23
33'44'55'-HxCB(#169)	22	23
2378-TeCDD	nd	1.9
12378-PeCDD	9.4	5.9
123478-HxCDD	7.1	6.6
123678-HxCDD	44	53
123789-HxCDD	9.2	10
1234678-HpCDD	130	130
OCDD	2200	1900
2378-TeCDF	nd	nd
12378-PeCDF	nd	nd
23478-PeCDF	9.4	6.4
123478-HxCDF	7.9	7.7
123678-HxCDF	10	7.7
234678-HxCDF	3.6	2.1
123789-HxCDF	nd	nd
1234678-HpCDF	17	19
1234789-HpCDF	nd	nd
OCDF	nd	nd

Acknowledgements

The authors wish to thank Mr. Tatsuya Ezaki and Mr. Hiromasa Fujii at SGE Japan for their technical information and valuable discussions in producing this paper.

References

1 Masuzaki Y., Matsumura T., Ezaki T., Ohashi M., Morita M. (2001) Organohalogen Compounds, 50, 194.
2 Ezaki T., Masuzaki Y., Matsumura T., Ohashi M., Ito H., Morita M. (2001) Organohalogen Compounds, 50, 190.
3 Tobiishi K., Hori T., Kurokawa Y., Ishiguro Y. and Iida T. (2002) Organohalogen Compounds, 55, 179.
4 Hori T., Tobiishi K., Ashizuka Y., Nakagawa R. and Iida T. (2002) Organohalogen Compounds, 55, 95.
5 Todaka T., Hirakawa H., Tobiishi K. and Iida T. (2003) Fukuoka Acta Med., 94, 148.
6 Tobiishi K., Hori T., Ishiguro Y. and Iida T. (2003) Organohalogen Compounds, 60, 383.