Evaluation of Automated Sample Preparation System for Immunoassay in Dioxins Analysis

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Introduction

Environmental monitoring of Dioxins emission source and screening to grasp pollution conditions are essential. However, the analytical method of Dioxins still requires a lot of complicated processes, educated technical experts and large cost. Consequently development of the simplified instrumental and analytical method has been advanced. Recently, immunoassay as biological methods for dioxins analysis has been developed[1], and also these assays have an advantage, the lower cost and suitable for large and rapid screening. However, Dioxins in environmental samples are very lower concentration, therefore it is needed to remove matrices influenced assay as much as possible and must be concentrated sample solutions regardless of sensitivity of measurement instrument. And also it is required complicated clean-up process using various chromatographic adsorbents and concentration process. Although Power-Prep System (FMS Inc.) [2] was developed as an automated clean-up system, further function that dioxins are sequentially concentrated and dissolved in suitable solvent such as Dimethyl sulfoxide (DMSO) are desired for immunoassay.

We have just developed automated Sample Preparation System for immunoassay that has three special functions[3]: purification, concentration and solvent substitution. In this report, it was examined the validation of this system. Repeatability, accuracy and recovery were studied with flue gas sample and fly ash sample using GC-MS. And also we investigated evaluation of this system for application to immunoassay.

Materials and Methods

Samples

The reference flue gas sample (1.4ng-TEQ /about $2m^3N$) was a mixture of approx. 30 kinds of crude extracts prepared according to JIS K 0311: 1999. The reference fly ash sample (3.8ng-TEQ/about 0.04g) was prepared by soxhlet extraction after HCl treatment. The reference contaminated soil sample (16ng-TEQ/5g-dry) was prepared by soxhlet extraction after air-drying.

Procedure of Sample Preparation System

The diagram of Sample Preparation System is shown in Fig. 1□Hexane solution of crude extract was applied to the top of the disposable multilayer column (12.5×200mm) (1). Multilayer column was composed of sequentially of silica gel (0.5g), 10% $AgNO_3$ silica gel (3.0g), 44% H_2SO_4 silica gel (10g), silica gel (0.5g). This multilayer column and other disposable parts (joint (2), alumina column packed in alumina (0.8g) (3), sample bottle (4), PTFE tube (15, 16)) were set to the system. Multilayer column was preheated at 60°C by heating jacket(12) for 10 minute, and then 90ml hexane was loaded from top of the multilayer column (at a flow-late of 2.5ml/min□load volume 105ml). Dioxins eluted from the multilayer column were adsorbed on alumina column. After alumina column kept at 60°C by heating jacket (13) was dried with nitrogen (6) through a sample bottle (4), followed by eluting dioxins from alumina column with 1ml DMSO kept at 60°C (by reverse-flow at a flow-late of 1.25ml/min, total volume 3 ml). Thereafter, 1ml DMSO solution was corrected in the sample bottle.

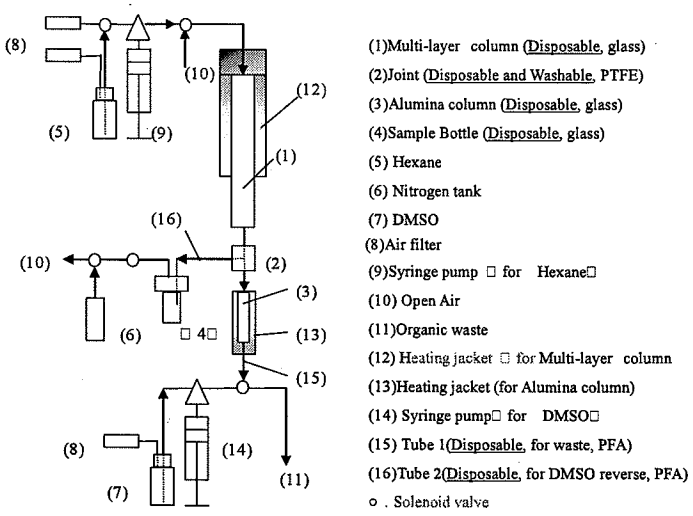

Fig.1 Diagram of Sample Preparation System

Evaluation Method of Sample Preparation System

Following by three items for evaluation were studied using this system.

1. Comparison of recoveries of dioxins in eluates from manual multilayer column and heated multilayer column.
2. Repeatability, accuracy and recovery of dioxins in DMSO solution prepared by Sample Preparation System.
3. Recorded blank level after higher contaminated soil sample loading.

Reference samples spiked with internal standard were purified and substituted to DMSO solutions by Sample Preparation System. Again, the DMSO solutions were substituted to hexane by liquid-liquid extraction. Followed by the hexane extracts were fractionated using Active Carbon-dispersed Silica gel chromatography. And then dioxins fraction was determined by HRGC-HRMS (JMS-700D and JMS-700S, JEOL).

Immunoassay

Two methods, ELISA (enzyme-linked immunosorbent assay) and KinExA (kinetic exclusion assay), were used to measure the DMSO solution of flue gas sample prepared by this system.
Measurement by KinExA was performed using EndoBioSensor™ (Sapidyne Instruments, Inc.) and anti-dioxin monoclonal antibody as described in Glass et al.[4] The ELISA was carried out using anti-dioxin monoclonal antibody as described in Takagi et al.[5]

Results and Discussion

Evaluation of heated multilayer column

Recoveries of $^{13}C_{12}$-labeled internal standard in hexane solution that was eluted from multilayer column are shown in Table 1 (flue gas sample (n=3) and fly ash sample (n=3)). There were not a remarkable difference and lower recovery of internal standards by heating treatment in multilayer column on instrument.

The pattern of TCDF chromatograms of a fly ash sample cleaned up by heated multilayer column and the manual multilayer column respectively is shown in Fig.2. There were not a variation of pattern and profile of two chromatograms in each isomer of each sample. Moreover, it indicated dioxins composition of sample was not changed in both cases.

Evaluation of Recovery and Repeatability

Table 1. **Recoveries of internal**

	preparation system recovery(%)	RSD(%)
2,3,7,8-TeCDD	90	2
1,2,3,7,8-PeCDD	101	2
1,2,3,4,7,8-HxCDD	104	3
1,2,3,6,7,8-HxCDD	105	1
1,2,3,7,8,9-HxCDD	103	2
1,2,3,4,6,7,8-HpCDD	101	1
OCDD	104	2
2,3,7,8-TeCDF	91	2
1,2,3,7,8-PeCDF	99	2
2,3,4,7,8-PeCDF	90	1
1,2,3,4,7,8-HxCDF	103	3
1,2,3,6,7,8-HxCDF	95	3
1,2,3,7,8,9-HxCDF	102	4
2,3,4,6,7,8-HxCDF	97	3
1,2,3,4,6,7,8-HpCDF	99	3
1,2,3,4,7,8,9-HpCDF	98	1
OCDF	102	2
3,4,4',5-TeCB #81	83	3
3,3',4,4'-TeCB #77	82	2
3,3',4,4',5-PeCB #126	89	3
3,3',4,4',5,5'-HxCB #169	93	2

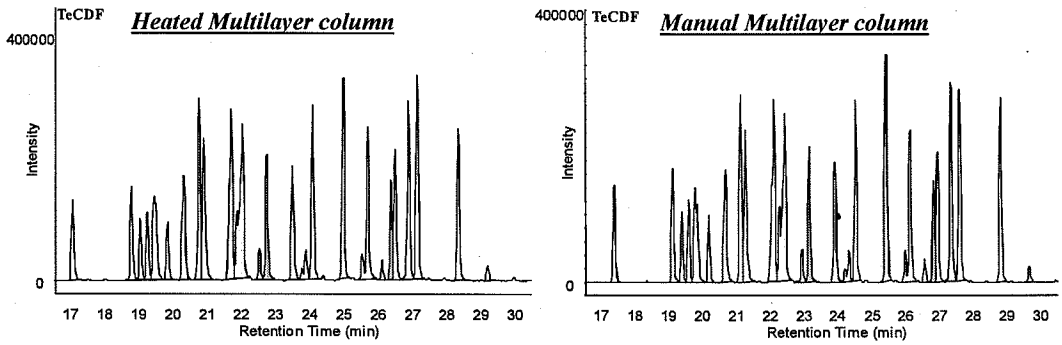

Fig.2 Comparison of GCMS-SIM chromatograms of TeCDFs of a fly ash sample purified by the heated multilayer column and non-heated multilayer column.

The level of dioxins in flue gas sample and fly ash sample cleaned up by this system are shown in Table 2. The recoveries of PCDD/Fs and non-ortho-PCB in flue gas sample were 82-101% (RSD Max. 6%) and 71-73% (RSD Max. 3%), respectively. The recovery rates of PCDD/Fs and non-ortho-PCB in fly ash sample were 83-100% (RSD Max. 6%) and 69-76% (RSD Max. 3%), respectively. Repeatability and accuracy are most important matter for immunoassay that does not spike a ^{13}C to obtain accurate concentration. Consequently, these results were consistently below RSD 6%. We confirmed recovery and repeatability are very high. In Compared to results of evaluation of heated multi-layer column, these values were slightly lower recoveries of PCDD/Fs and non-ortho-PCBs. This difference could be attributed to an efficiency of liquid-liquid extraction method in DMSO Solution.

Table 2. Repeatability and Recovery of Sample flue gas sample and Fly ash sample

	flue gas sample(n=3) Conc.□pg/ml□	RSD(%)	Recovery internal-reference	Fly ash sample(n=3) Conc.□pg/ml□	RSD(%)	Recovery internal-reference
2,3,7,8-TeCDD	80	2	92	88	2	90
1,2,3,7,8-PeCDD	390	4	95	1100	0.5	94
1,2,3,4,7,8-HxCDD	290	5	94	1600	2	100
1,2,3,6,7,8-HxCDD	420	6	96	3400	2	99
1,2,3,7,8,9-HxCDD	340	6	92	2900	0.4	100
1,2,3,4,6,7,8-HpCDD	1700	3	88	26000	0.3	92
OCDD	1700	2	84	37000	1	95
2,3,7,8-TeCDF	570	5	91	360	3	90
1,2,3,7,8-PeCDF	910	0.8	92	940	0.4	91
2,3,4,7,8-PeCDF	890	3	91	1300	0.4	91
1,2,3,4,7,8-HxCDF	810	0.7	93	2000	3	93
1,2,3,6,7,8-HxCDF	720	1	85	2000	0.9	85
1,2,3,7,8,9-HxCDF	49	6	101	190	3	98
2,3,4,6,7,8-HxCDF	700	0.6	89	3000	2	91
1,2,3,4,6,7,8-HpCDF	1700	3	82	9700	0.5	83
1,2,3,4,7,8,9-HpCDF	190	3	86	1300	3	86
OCDF	530	3	82	5400	2	86
3,4,4',5-TeCB #81	440	0.6	72	220	0.9	71
3,3',4,4'-TeCB #77	1300	0.7	73	660	3	76
3,3',4,4',5-PeCB #126	710	1	71	760	4	69
3,3',4,4',5,5'-HxCB #169	167	3	72	360	3	71

Evaluation of Blank Levels

Result of blank level is shown in Table 3. The blank level was very low and all of congeners were not detected in this system. We confirmed free of contamination. Especially, these results are responsible for using disposable parts and non-valve on sample flow line.

Table 3. Blank levels in case of using a contaminated soil

	Soil Sample pg□volume of addition)	Blank pg/ g-dry
2,3,7,8-TeCDD	440	<0.63
1,2,3,7,8-PeCDD	2000	<0.95
1,2,3,4,7,8-HxCDD	2100	<0.76
1,2,3,6,7,8-HxCDD	3800	<0.76
1,2,3,7,8,9-HxCDD	2900	<0.85
1,2,3,4,6,7,8-HpCDD	24000	<1.1
OCDD	53000	<0.92
2,3,7,8-TeCDF	4300	<0.37
1,2,3,7,8-PeCDF	11000	<0.37
2,3,4,7,8-PeCDF	11000	<0.44
1,2,3,4,7,8-HxCDF	13000	<0.94
1,2,3,6,7,8-HxCDF	12000	<0.81
1,2,3,7,8,9-HxCDF	600	<0.8
2,3,4,6,7,8-HxCDF	15000	<0.71
1,2,3,4,6,7,8-HpCDF	61000	<1.34
1,2,3,4,7,8,9-HpCDF	4400	<1.3
OCDF	21000	<2.8
3,4,4',5-TeCB #81	3500	<0.79
3,3',4,4'-TeCB #77	28000	<0.67
3,3',4,4',5-PeCB #126	12000	<1.1
3,3',4,4',5,5'-HxCB #169	4100	<0.39

Immunoassay

Seven flue gas samples prepared by Sample Preparation System were measured by ELISA and EndoBioSensor™. The correlations between TEQ values of HRGC-HRMS and 2,3,4,7,8-PeCDF (F114) value of each immunoassay are shown in Fig.3 and 4. Good correlations were observed in each immunoassay. These results showed that flue gas samples were cleaned up and both

interferences of ELISA and KinExA were removed by Sample Preparation System. Therefore this system was useful for both methods in immunoassay.

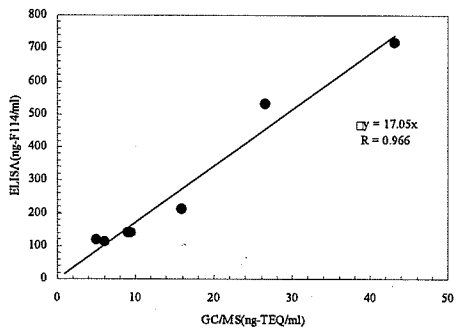

Fig.3 Correlation between GC/MS values and ELISA values of flue gas samples

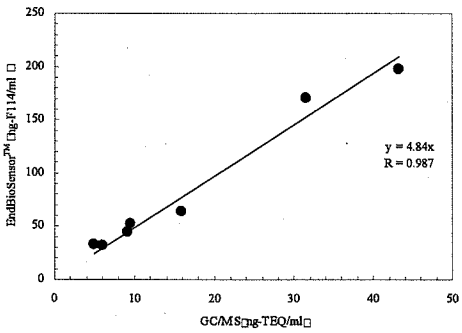

Fig.4 Correlation between GC/MS values and EndoBioSensor™ values of flue gas samples

Conclusions

The automated Sample Preparation System has demonstrated that Dioxins sample for immunoassay can be prepared from crude extracts without any contamination. In addition, dioxins could be measured by EndoBioSensor™ within about 2 hours including sample preparation process. Moreover it avoids personal errors of sample preparation and reduces the risks of human exposure and improves the accuracy.

Acknowledgements

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