DEVELOPMENT OF A SAMPLE PREPARATION SYSTEM FOR DIOXINS: APPLICATION TO IMMUNOASSAY

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Introduction

Dioxins are highly toxic and bioaccumulative compounds among persistent organic pollutants, and their environmental monitoring and screening are essential. In Japan, conventional testing for dioxins uses the Japan Industrial Standard method (JIS K 0311). This method is expensive and has a relatively slow analytical cycle time. This constrains the number of samples that can be economically tested in projects, leading to uncertainties in the application and interpretation of results for management of site remediation. Immunoassay and bioassay are one way of overcoming these problems[1)]. However, there still remain several problems in clean-up for dioxins contaminated environmental matrices and in analytical complications such as solvent concentration and substitution. For a wide recognition of these assays as monitoring and screening, a suitable pre-treatment method for the assay systems should be developed for all sorts of measurements of analyte.

This study was carried out to develop the automated sample preparation system of dioxins for immunoassay, and the following items were examined; (1) the efficiency of purification by heating multi-layer silica gel column, (2) solvent substitution method using solid-phase adsorbents, and (3) application to immunoassay.

Methods and Materials

The following study was performed by the experimental instrument, as shown in Fig.1.

Samples

Two reference samples of flue gas and fly ash were used for this experiment. Flue gas sample was a mixture of n-Hexane solutions extracted from approx. 30 kinds of flue gas collecting by DioANA® filter or an impinjer, according to JIS K 0311: 1999. Another fly ash sample was obtained from one type of incinerator. After HCl treatment, dioxins were extracted by soxhlet extraction method and then followed by n-Hexane substitution.

Purification column (Multi-layer silica gel column) chromatography

For a validation of the purification column, multi-layer silica gel column was prepared as follows: The packing materials; a few Na_2SO_4(anhydride), 2% KOH silica(0.8g), silica gel(0.05g), 44% H_2SO_4 silica(7.3g), silica gel(0.05g), 10% $AgNO_3$ silica(3.5g), and a few Na_2SO_4(anhydride), were laminated in sequence into the LC glass column with closed ends (10mm×150mm). Each five ml of one blank and two reference samples was applied on this column, and then eluted with n-Hexane at 60ºC or a room temperature.

Each of the eluted solutions was concentrated to 4ml and 100µl using a nitrogen evaporative concentrator and then measured by ultraviolet-visible (V-550 type, Nippon Bunko) and gas chromatography-mass spectrometry (JMS-AUMS200 type, JEOL) respectively.

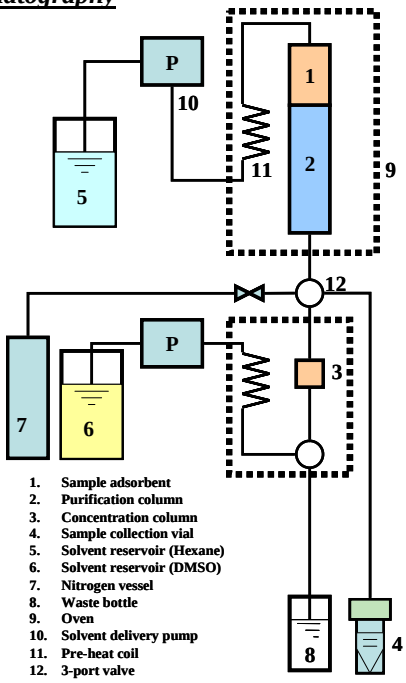

Fig.1: Diagram of experimental purification and solvent substitution system.

Concentration, Separation and Solvent Substitution / Application to Immunoassay

For investigations of concentration, separation and substitution, an alumina column was set under multi-layer silica gel column (Fig.1). Each reference sample of flue gas and fly ash was applied on a top of multi-layer silica gel column. After heating the column at 60ºC, dioxins were eluted with 90ml of n-Hexane kept at 60ºC, and then followed by drying the alumina column with N_2 gas. Thereafter, dioxins in alumina column were reversibly eluted with 1ml of DMSO solution kept at 60ºC, and determined by HRGC-HRMS (JMS-700D, JEOL) after n-Hexane substitution. These values of dioxins were compared with the results obtained in conventional treatment, i.e., manual multi-layer silica gel column according to the JIS.

Furthermore, application to immunoassay using flow immunosensor (EndoBioSensor™, Sapidyne Instruments Inc.) and monoclonal anti-dioxin antibody[2,3] was examined on recovery tests of 2,3,4,7,8-PeCDF added in DMSO solution obtained from the preparation system mentioned above.

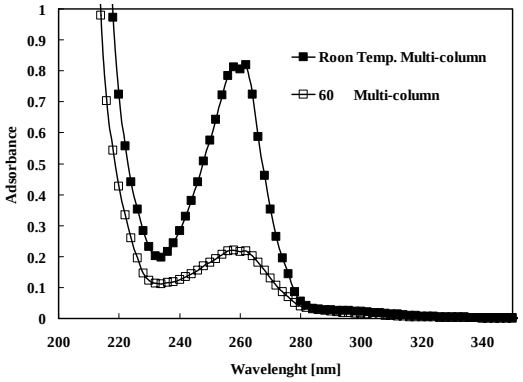

Fig.2: UV spectra of flue gas samples purified by multi-layer silica gel column at a room temperature and 60ºC.

Results and Discussion

Purification column (Multi-layer silica gel column) chromatography

In multi-layer silica gel column (Fig.1), dioxins were completely eluted with 130ml of n-hexane at a room temperature and with 90ml of n-hexane at 60ºC. This indicates that it is possibility to make a short time of analytical cycle and reduce a volume of organic solvent.

UV spectra of flue gas samples purified by multi-layer silicagel column at a room temperature (Fig.2) showed relatively high absorbance of 260nm (230-300nm) and it means presences of aromatic and unsaturated compounds and sources of electron transition. While UV spectra at 60ºC showed relatively low absorbance, indicating that interferences of immunoassay such as poly aromatic hydrocarbon (PAH) were removed from the flue gas samples. More detailed information was found in the result of GC-MS spectrum (Fig.3). The peaks of PAH, alkyl benzene (ABz) and alkyl biphenyl markedly disappeared. Similar results were also found in fly ash sample.

These observations indicate that progressing in chemical reaction by means of heating multi-layer silica gel column is useful for purification of dioxins analysis.

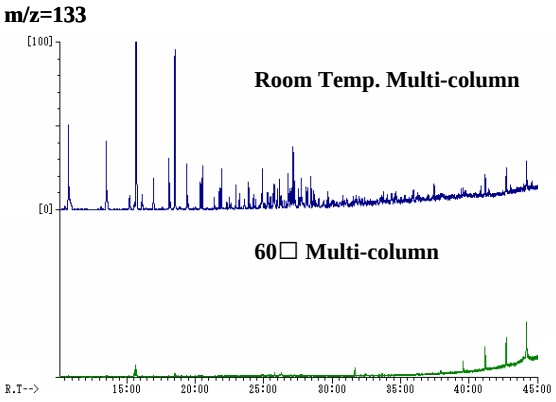

Fig.3: GC-LRMS chromatograms of flue gas samples purified by multi-layer silica gel column.

Concentration, Separation and Solvent Substitution Method

Recovery tests of dioxins were examined on the automated pre-treatment system (Fig.1) as shown in Fig.4, and their results were compared with those of manual method (multi-layer silica gel column chromatography) according to the JIS(Table 1). High recoveries of dioxins were obtained in this system, as same as those of the JIS method, and their RSD% exhibited smaller values compared to those of the JIS. This indicates that the automated pre-treatment system has a high confidence for dioxins analysis, and also that a process of concentration, separation and solvent substitution using alumina column gives improvement in efficiency.

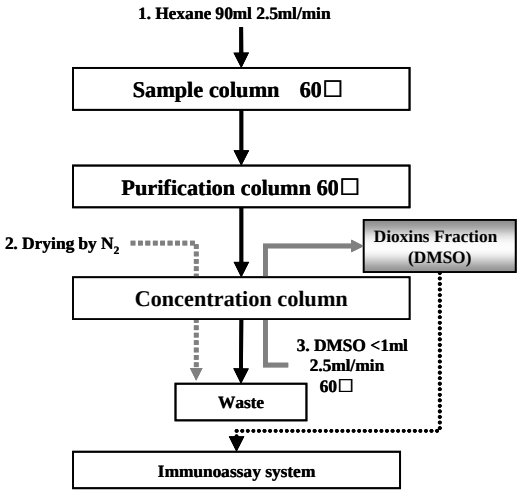

Fig. 4: Flow chart of the purification and solvent substitution for immunoassay

Application to Immunoassay

Immunoassay using EndoBioSensor™ was tested on DMSO solutions obtained by the automated system, and their results are shown in Fig.5. The values of 2,3,4,7,8-PeCDF added in DMSO solution linearly increased with an increase in the concentration of 2,3,4,7,8-PeCDF, and recoveries of 2,3,4,7,8-PeCDF were quantitative. The results indicate that the interferences of this immunoassay were removed, further this pre-treatment system is useful for immunoassy.

Table 1: Recoveries and RSD (%) of dioxins for reference flue gas samples. These values are representative of a whole purification and substitution process.

	Manual (n=3)		Alumina (n=3)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
2,3,7,8-TeCDD	79	12	93	2
1,2,3,7,8-PeCDD	91	12	100	4
1,2,3,4,7,8-HxCDD	99	14	99	4
1,2,3,6,7,8-HxCDD	104	13	92	3
1,2,3,7,8,9-HxCDD	97	17	106	5
1,2,3,4,6,7,8-HpCDD	122	5	105	4
OCDD	122	2	102	8
2,3,7,8-TeCDF	78	15	92	2
1,2,3,7,8-PeCDF	90	13	103	2
2,3,4,7,8-PeCDF	88	13	90	2
1,2,3,4,7,8-HxCDF	93	15	104	2
1,2,3,6,7,8-HxCDF	100	15	97	3
1,2,3,7,8,9-HxCDF	91	19	87	4
2,3,4,6,7,8-HxCDF	99	13	98	3
1,2,3,4,6,7,8-HpCDF	121	6	102	2
1,2,3,4,7,8,9-HpCDF	113	7	107	3
OCDF	118	1	101	5

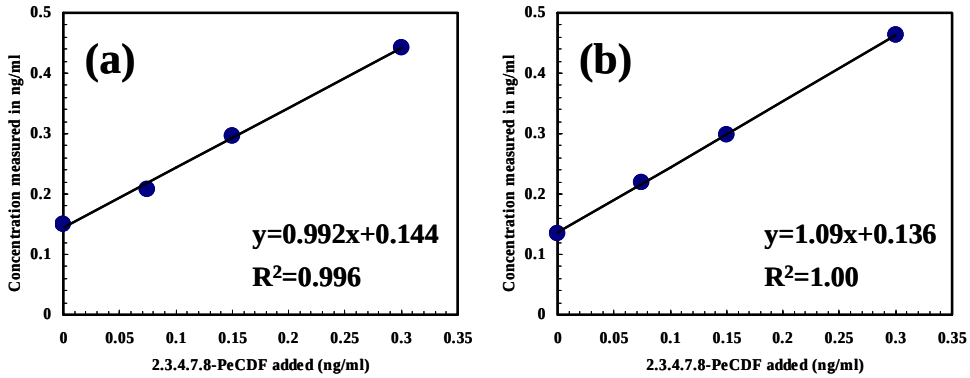

Fig.5: Results of recoveries of 2,3,4,7,8-PeCDF from flue gas (a) and fly ash (b) sample. Three different concentrations of 2,3,4,7,8-PeCDF were spiked to 1/10 diluted DMSO solutions prepared on the automated preparation system, and each value of dioxins was measured by EndoBioSensor™.

Conclusions

An automated pre-treatment system, which is composed of two columns, a multi-layer silica gel column and alumina, is useful for immunoassay of dioxins using EndoBioSensor™. Especially, two special techniques, i.e. heating multi-layer silica gel column and solvent substitution by alumina column, could remove interferences of immunoassay such as aromatic hydrocarbons, and concentrate directly to a small volume of DMSO solution.

Acknowledgements

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References

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