A Simplified Dioxin Analysis System - Automatic Sample Preparation Devise and Dioxin Biosensor -

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Introduction

In Japan, monitoring of dioxin emission sources is essential to quantifying their role in pollution. However, available analytical methods are complicated, require trained personnel and often involve high costs. Because of these factors the development of simplified instrumental analytical methods has attracted wide attention.

On 27 December 2004, the Law Concerning Special Measures against Dioxins (Dioxins Law) in Japan was partially revised, including specified added application of the simplified dioxins analysis methods based on biological methods, such as binding method via Ah-receptor or using specific antibodies for dioxins[1,2].

In order to improve accuracy and reproducibility of biological methods for dioxin analysis we first attempted to improve sample preparation. We described the performance of an automated sample preparation device (SPD-600) in a previous report [3, 4]. Now we have completed development an automated dioxin biosensor (DXS-600).

In this work, we describe the rapidand simple dioxin analysis system constituted of SPD-600 and DXS-600, and report the capability, availability and application for various environmental samples.

Materials and Methods

Samples

The reference sample of flue gas was a mixture of crude extracts of 100 samples. Twenty-seven samples of flue gas extracts, eighteen samples of fly ash extracts, twenty-two samples of bottom ash extracts and twenty-eight samples of soil extractswere used for the evaluation of this system. These samples were analyzed in parallel by the standard GC/MS method.

Results and Discussions

The Dioxin Biosensor DXS-600

The DXS-600 is an automated flow immunoassay analyzer based on the kinetic exclusion assay (KinExA) [5,6,7], KinExA is a fluorescence based flow through immunoassay system designed to measure the amount of unbound antibody after an antibody and analyte are allowed to react in solution. This system has achieved the antibody affinity based theoretical detection limit for analyte[5]. The schematic diagram of KinExA is shown in **Fig. 1**.The remaining fluorescent signal measured with a photodiode is the response signal indicating the dioxin concentration.

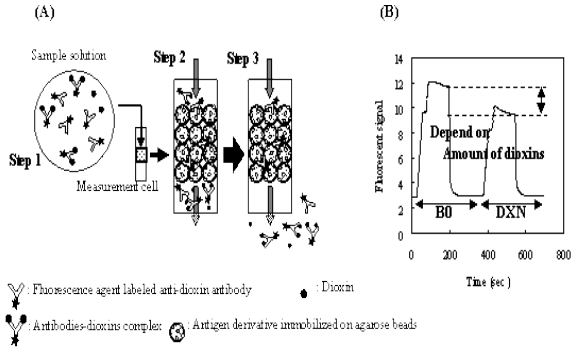

Fig. 1 (A) Schematic Diagram Principle of KinExA with DXS-600. **Step1**: the environmental sample prepared by SPD-600 was mixed with anti-dioxin antibody labeled fluorescent agent. An equilibrated mixture of antigen-antibody complex was formed in the measurement sample solution. **Step2**. The sample solution was run into the measurement cell without competitive reaction. **Step3**. Unbound antibody was caught by measurement cell packed with the dioxin derivative immobilized on agarose beads, and excess anti-dioxin antibody was washed out by buffer. **(B)** Typical Sensorgram of DXS-600. Measuring dioxins concentration is performed on a sample containing dioxins (**DXN** measurement), after a sample without dioxins (**B0** measurement).

Calibration curves of 2,3,4,7,8-Pentachlorodibenzofuran

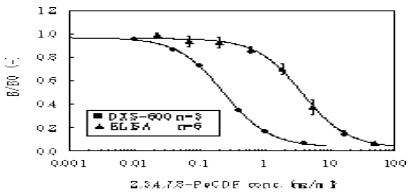

Fig. 2 Comparison of calibration curves between DXS-600 and ELISA.

Fig.2 showed the calibration curves for 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8- PeCDF) with DXS-600 and ELISA. In the case of DXS-600, 0.04 ng/ml was obtained as the lower detection limit and Relative Standard Deviation (RSD) value was less than 3% based on triplicate measurements each using a different measurement cell. In the comparison of the calibration curves between DXS-600 and ELISA using the same anti-dioxin antibody, the DXS-600 analyzer showed 25 times or more higher sensitivity than the ELISA method along with superior reproducibility.

Simplified Dioxin Analysis System

The simplified dioxin analysis system is composed of the sample preparation device SPD-600 and the dioxin biosensor DXS-600, as shown in Fig.3. In only 4 hours this analysis system is able to clean up crude extracts of environmental samples and analyze TEQ-value of dioxins included in each samples. The SPD-600 has multi-layer silica gel columns with automated heating units andcan simultaneously prepare three samples suited to bioassay and immunoassay within 3 hours[3, 4]. The DXS-600 can analyze a prepared sample in as little as 16 min.Satisfactory results of accuracy and reproducibility were achieved when used on the simplified dioxin analysis system.

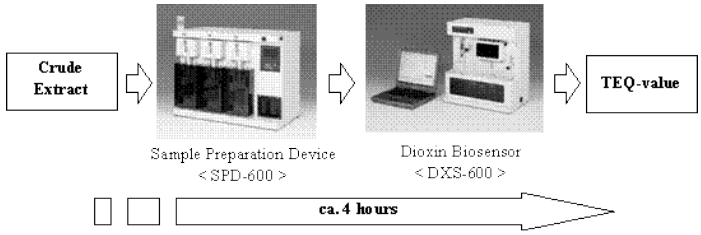

Fig. 3 Images of the Simplified Dioxin Analysis System.

Validation of the Simplified Dioxin Analysis System

Preparation of the reference sample (5.14ng-TEQ/m^3N) was performed three times respectively using SPD-600, and each sample was measured by DXS-600 in triplicate.

Table 1 shows the results of the simplified dioxin analysis system with SPD-600 and DXS-600.Within-assay of DXS-600 was good result (RSD: < 3%) and also this system showed high reproducibility (RSD: 3.26%) based on triplicate experiments. The TEQ-value (5.15ng-TEQ/m^3N) obtained by this system was similar to GC/MS data. These results proved high precision of the simplified dioxin analysis system.

Table 1. Results of the simplified dioxin analysis system with SPD-600 and DXS-600.

	SPD-600	DXS-600				Equivalent value	TEQ-value	
N	Preparation vol. ml	Assay vol. µl	n	Signal	Average RSD,%	pg/ml	ng-TEQ/m^3N	Average RSD,%
1	0.7847	5	1	6.3036				
			2	6.2784	6.2844	0.094	4.97	
			3	6.0711	2.81%			
2	0.7998	5	1	6.4807				
			2	6.2824	6.2666	0.094	5.10	5.15
			3	6.0957	2.56%			3.26%
3	0.8830	5	1	6.5692				
			2	6.3205	6.3966	0.090	5.38	
			3	6.3002	1.91%			
Bo		0	1	9.7444				
			2	9.4794	9.6388			
			3	9.6926	1.19%			

Notes N: Sample preparation number, **Preparation vol.**: Volume of prepared sample solution (ml), **Assay vol.**: Usage of sample for immunosensing (µl), **n**: Number of within-assay using the same cell, **Signal**: Detected fluorescent intensity, **Equivalent value**: Fluorescent signal converted to 2,3,4,7,8-PeCDF concentration based on calibration curve,
TEQ-value: TEQ-value = Equivalent value × Preparation vol. ÷ Assay vol. × 339.01

Evaluation of Correlation between GC/MS and the Simplified Dioxin Analysis System

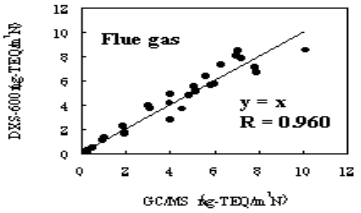

Fig. 4 Correlation between GC/MS values and DXS-600 values of flue gas samples.

Fig. 4 shows the good correlation between GC/MS values and DXS-600 values of flue gas samples (R=0.960). Similarly, good correlation was observed in fly ash samples (R=0.945), bottom ash samples (R=0.975) and soil (estimated dioxins pollution emitted from incinerators) samples (R=0.960).This system is thus proven as an analysis system with high-precision for dioxin pollution samples emitted from incinerators.

Conclusions

Development of the simplified dioxin analysis system allows dioxin analysis in environmental samples within 4 hours. Moreover the system excludes human error, improves accuracy and reproducibility of dioxin analysis and reduces the risk of human exposure to dioxins.

Now we are investigating application of this system to soil samples including more complicated contaminants. Furthermore we anticipate wide applications of this system to foods, animals and other things.

Acknowledgements

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