Analysis of Dioxins Using Automated Sample Preparation System

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1 Introduction

In this study, we examined rapid and accurate analysis of dioxins using the automated pretreatment system (GO-EHT, MIURA CO.,LTD., Japan), as shown in Figure 1.

In dioxin analysis, purification of extracts from environmental samples, food samples and blood samples, which contain many matrix components, is very complicated. Furthermore, polychlorinated diphenyl ethers (PCDEs), which are often found in soil and fish samples, have similar physical and chemical properties to dioxins, and if PCDEs are not separated from dioxins in the pretreatment process, quantification becomes difficult even with a high-resolution mass spectrometer (HRMS). In particular, peaks of PCDEs appear in the chromatogram of PCDFs, causing a positive error in the quantitative value of PCDFs.

In addition, samples with dioxin contamination from incineration often contain higher concentrations of PCDDs/DFs than DL-PCBs. In this case, if the two are not separated in the pretreatment process, fragment peaks of PCDDs appear in the chromatogram of DL-PCBs, causing a positive error in the quantitative value of DL-PCBs.

To solve these problems, it is necessary to fractionate PCDDs/DFs/non-ortho DL-PCBs (DXN, N-PCB fraction) and mono-ortho DL-PCBs (M-PCB fraction) in the purification process. Conventional methods include purification and fractionation using multilayer silica gel columns, activated carbon dispersed silica gel columns and alumina columns, but these methods are complicated and require advanced technology to analyze dioxins with high accuracy. We have developed the pretreatment system that automatically performs this fractionation. We have evaluated the applicability of this system to various samples and report the results.

2 Materials and Methods

2.1 Procedure of automated purification

The schematic of the purification process in this system is shown in Figure 1. After loading a part of extract onto the purification column, the purification column is connected to the concentration columns（Upper and lower). The following three steps are performed automatically.

(1) While heating the purification column to 60 °C, 104 mL of hexane is passed through from the upstream side. Then, air is passed through the column to dry the hexane.

(2) While heating the column to 90 °C, pass 2.2 mL of toluene through the concentration column lower from the downstream. Approximately 1.0 mL of the toluene eluate is collected (M-PCB fraction).

(3) While heating the column to 90 °C, pass 2.2 mL of toluene through the concentration columns (Upper and lower) from the downstream side. Approximately 1.3 mL of the toluene eluate is collected (DXN and N-PCB fractions). After reducing the volume of each eluate by nitrogen flow or decompression concentration, the syringe spike is added to complete the measurement solution.

Dioxins were measured by GC-HRMS [JMS-800D UltraFOCUS (JEOL Ltd., Japan) and DFS DualData (Thermo Fisher Scientific, USA)]. The GC column was BPX-DXN (TRAJAN, Australia), which is commonly used in Japan.

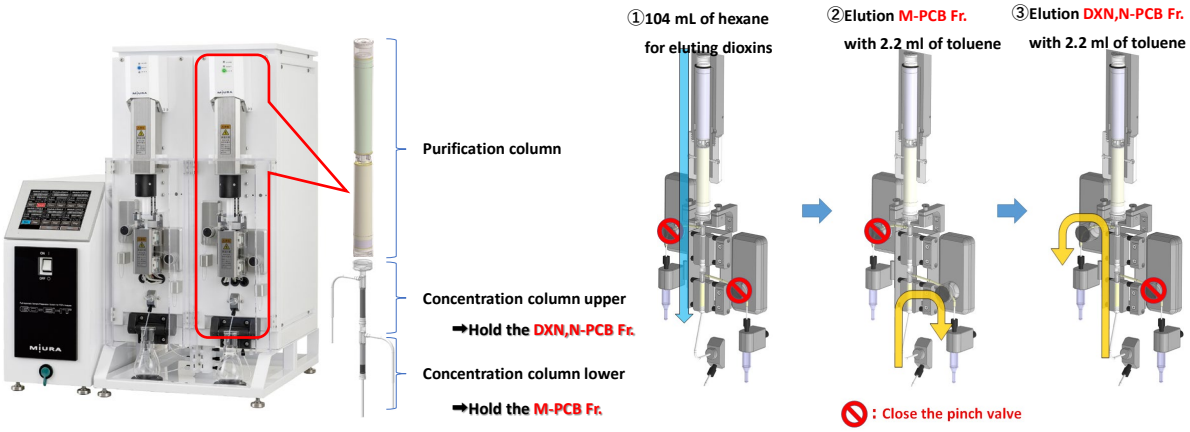

Figure 1: Automated pretreatment system (GO-EHT)

2.2 Environmental samples (Soil)

Soil samples containing high levels of PCDEs were used. Dioxins in soil were extracted using the Soxhlet extraction method (toluene) of the "Manual for Soil Survey and Measurement Related to Dioxins[1]" (Official method of Soil in Japan, Ministry of the Environment). The clean-up spike was added when the extract was loaded to the purification column to evaluate the recovery in the purification process (the same procedure were applied to food and blood samples below). A quantity of the extract equivalent to 2.5 g of soil was used for purification. Since toluene in the extract solution causes fractionation errors in the purification process, toluene was sufficiently removed by decompression and concentration, replaced with hexane, and used in the purification process. Figure 2 shows procedure for making the extract and measurement solution.

2.3 Food samples

The following commercial foods were used: Fish (sea bass, mackerel), meat (chicken breast, chicken tender) and oyster. Dioxins in each food were extracted using the Soxhlet fat extraction method (acetone/toluene) of the "Tentative Guidelines for Determination of Dioxins in Foods[2]" (Official method of Foods in Japan, Ministry of Health, Labor and Welfare)". A quantity of the extract equivalent to 20 g of food was used for purification. Figure 2 shows procedure for making the extract and measurement solution.

2.4 Blood samples

The following commercial bloods were used: Blood A, B, O and AB types, Each CPDA-1, Human (COSMO BIO CO., LTD.). Dioxins in blood were extracted using the saturated ammonium sulfate addition-ethanol/hexane solvent extraction method of the "Tentative Manual for Determination of Dioxins in Blood[3]" (Official method of Blood in Japan, Ministry of Health, Labor and Welfare). A quantity of the extract equivalent to 20 g of blood was used for purification. Figure 2 shows procedure for making the extract and measurement solution.

2.5 Multi-sample processing apparatus

The following multi-sample processing apparatuses were used for the operations indicated by * in Figure 2. These apparatuses are compact and useful, as shown in Figure 3. Soxhlet extraction: UniversalExtractor E-800 (BUCHI, Switzerland). Evaporation (1st): SyncorePlus Analyst (BUCHI, Switzerland). Evaporation (2nd): SyncorePlus Polyvap (BUCHI, Switzerland). Concentration of eluate: Centrifugal Evaporator EC300 (SAKUMA Inc., Japan).

2.6 Standards of PCDDs/DFs and DL-PCBs

As mass-labelled dioxins standard solutions, DFP-LCS-B was used for the clean-up spike and DF-IS-J for the syringe spike, both diluted. DFP-CVS-B1 was used for calibration curves (both from WELLINGTON LABORATORIES JAPAN INC., Japan).

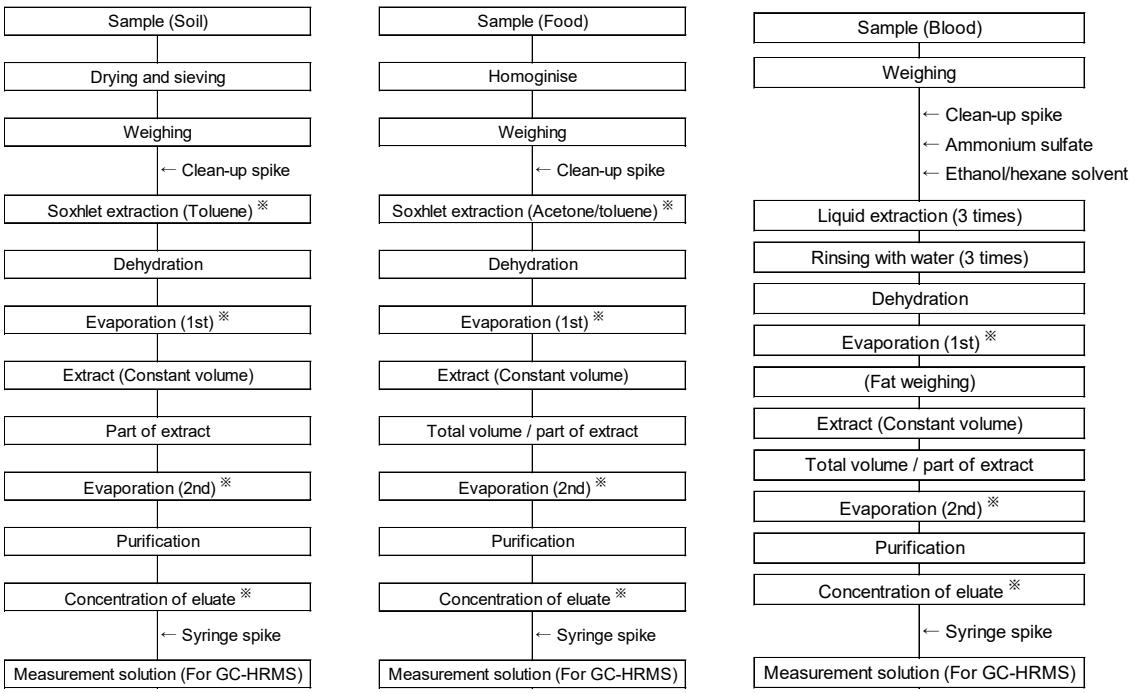

Figure 2: Procedure for making each extract and measurement solution (Official method in Japan)

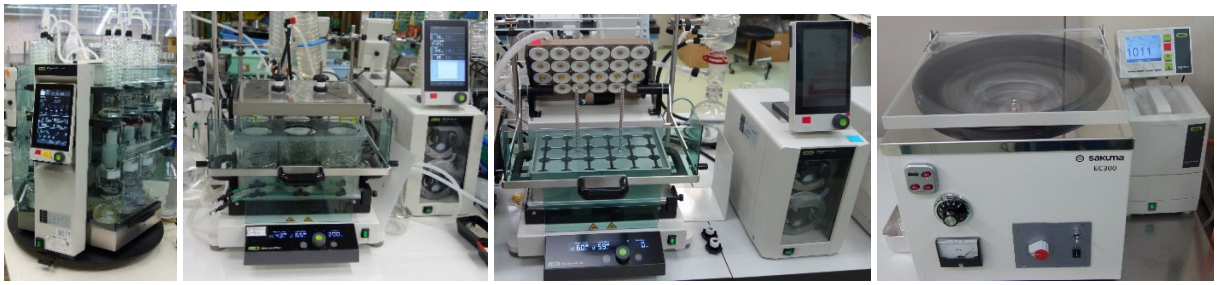

Figure 3: Multi-sample processing apparatus in our laboratory

3 Results and Discussion

3.1 Removal performance of PCDEs (Soil)

The removal performance of PCDEs in soil samples was evaluated. Chromatograms of DXN, N-PCB and M-PCB fractions were compared without and with fractionation. The chromatogram of PeCDFs and HxCDFs are shown in Figure 4 and 5, respectively.

In the case without fractionation, large peaks of PCDEs were identified on the chromatogram, and some isomers were difficult to quantify. On the other hand, when fractionated, PCDEs were separated into M-PCB fractions, and PCDEs peaks disappeared in DXN and N-PCB fractions. This facilitates quantification and improves the reliability of the quantitative values. These results indicate that this system has high removal performance for PCDEs.

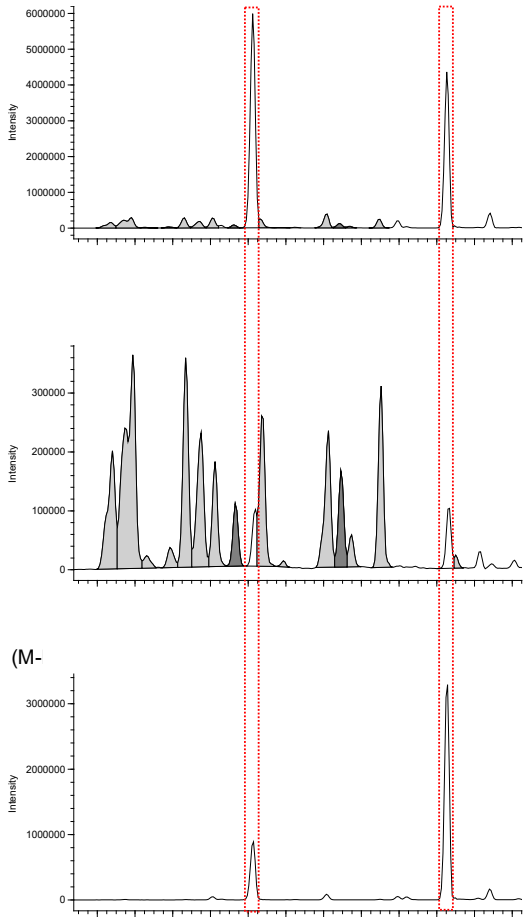

Figure 4: Chromatogram of PeCDFs (BPX-DXN)

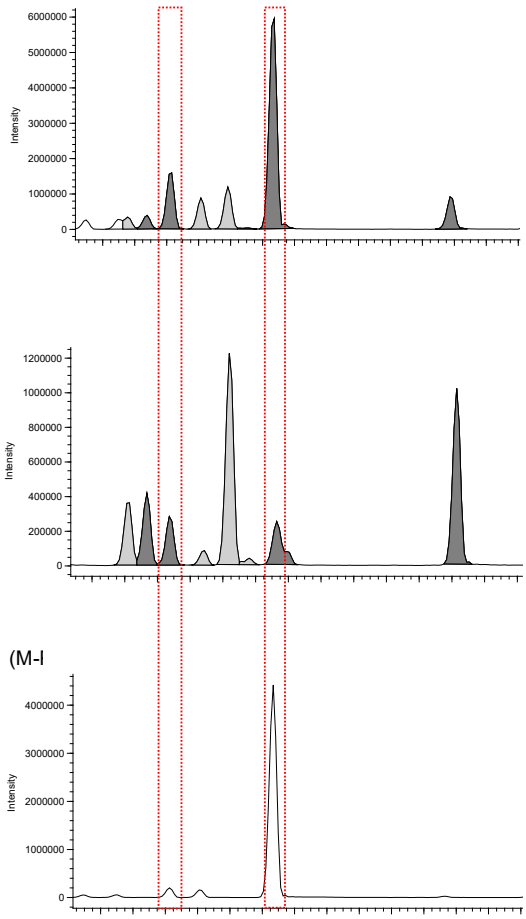

Figure 5: Chromatogram of HxCDFs (BPX-DXN)

3.2 Evaluation of purification effects by TIC and Lock mass monitor (Food)

The purification effect by fractionation in various foods was evaluated. TIC and Lock mass monitor are shown Figure 6 and 7, respectively. In the TIC, several peaks derived from the food matrix were detected, although their intensities were low. However, no Lock mass variation was observed and there were no problems with quantitation. These results indicate that this system is highly effective in purifying the matrix of food samples.

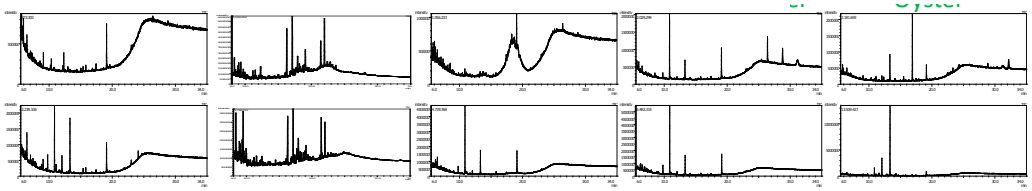

Figure 6: TIC in food samples by GC-LRMS (GCMS-TQ8050NX, SHIMADZU, Japan)

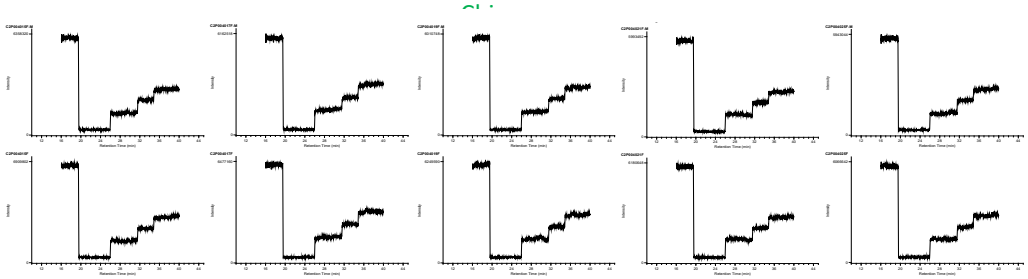

Figure 7: Lock mass monitor in food samples

3.3 Evaluation of recovery rates (Blood)

Recovery rates in blood samples were evaluated. Recovery rates for clean-up spikes are shown in Figure 8 (n=5 for each sample). The recovery rates were generally better than 80%.

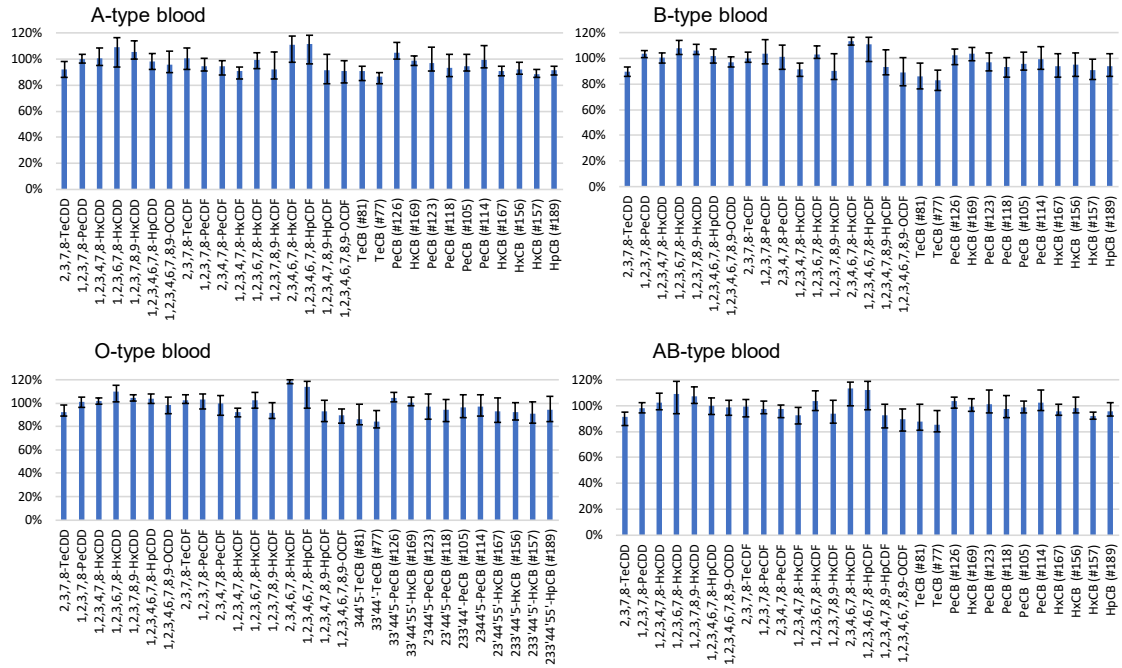

Figure 8: Recovery rates of PCDDs/DFs/PCBs in blood samples

4 Conclusions

This automated sample preparation system enables rapid and accurate analysis of dioxins in various samples. This system shows high removal performance of PCDEs by fractionation into DXN, N-PCB and M-PCB fractions.

5 References

1. https://www.env.go.jp/air/dioxin/post_60.html
2. https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/kenkou_iryou/shokuhin/kagaku/dioxin/index.html
3. http://www.nihs.go.jp/mhlw/chemical/dioxin/index.html