EVALUATION OF THE RAPID CLEANUP METHOD FOR THE ANALYSIS OF DIOXINS IN FOODSTUFF

Ueda Y*, Watanabe I, Honda K

Center of Advanced Technology for the Environment, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime, 790-8566, Japan

Introduction

Dioxins (PCDDs: polychlorinated dibenzo-*p*-dioxins, PCDFs: polychlorinated dibenzo furans and DL-PCBs: dioxin like polychlorinated biphenyls) are a group of toxic and persistent organic pollutants that affects human health and the environment. The uptake of dioxins in human is known to be mainly *via* food intake. Therefore, continuous monitoring of dioxins levels in foodstuffs is needed. However, as foodstuffs have relatively low amounts of dioxins and include relatively large amount of lipids, the analysis of dioxins requires a skilled technique and also spends a lot of time and cost. Our group has reported a rapid cleanup method for analysis of dioxins in fly ash using semi-automated cleanup device that allowed rapid, easy and accurate analysis, and less consumption of organic solvents[1].

In this study, we used the semi-automated cleanup device (SZ-DX-PT050, SEEDS TEC, Japan.,as shown in Fig. 1) for the purification of the dioxin extract in nine samples of foodstuffs, and evaluated the recovery rates and cleanup efficiency of dioxins analysis.

Materials and methods

Internal standard solution

$^{13}C_{12}$-labeled internal standard mixtures of dioxins ($^{13}C_{12}$-labelled dioxins) were purchased from Wellington Laboratories (Canada). The internal standard solution was diluted with decane to 10pg/μL.

Foodstuff sample

The food samples (potato, soybean, spinach, apple, mushroom, seaweed, yellowtail fish, beef and egg) were purchased from a supermarket in Ehime, Japan. Each sample was homogenized and stored at -20℃ until analysis.

(A)

(B)

Fig. 1. (A) Semi-automated cleanup device (SZ-DX-PT050), (B) Multilayer silica gel column(left) and activated alumina column(right).

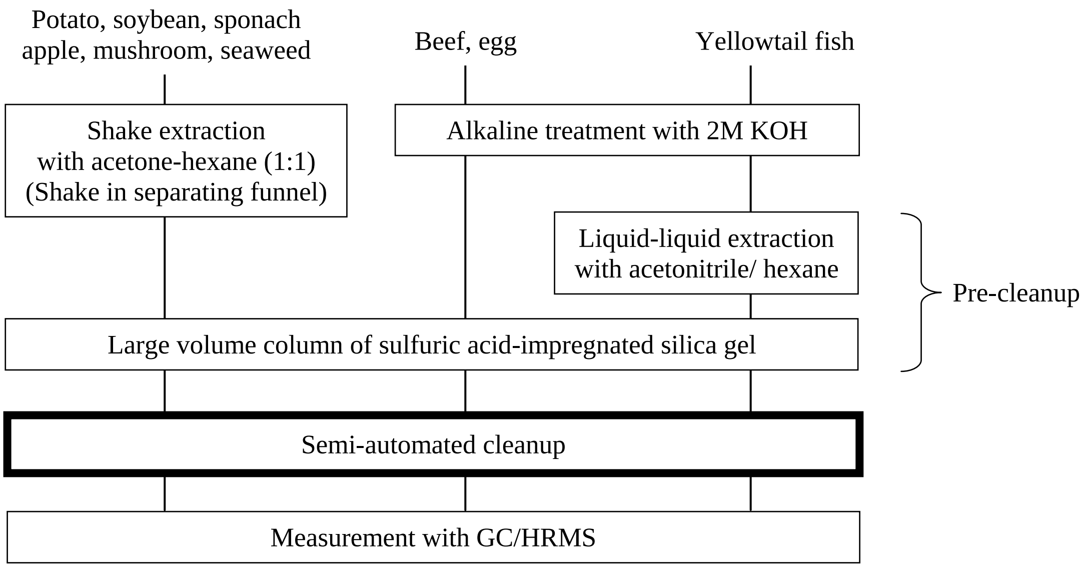

Fig. 2. Flow diagram of dioxins analysis in food samples

Extraction and pre-cleanup

The sample extraction followed the guideline for the analysis of dioxins in foods in Japan[2], as shown in Fig. 2. About 100g for each sample (potato, soybean, spinach, apple, mushroom and seaweed) was twice extracted with acetone-hexane (1:1) by shaking at room temperature. After the filtrate was washed with water, the hexane layer was dehydrated with sodium sulfate and concentrated to 10mL.

For yellowtail, beef and egg, about 50g for each sample was treated with 2M KOH for 12 hours at room temperature, and then the dioxins were extracted with methanol and hexane. The hexane layer was washed with water,dehydrated with sodium sulfate, the hexane solution was concentrated to 10mL.

The liquid-liquid partition by acetonitrile and hexane was used for removing the large amounts of lipids in the crude extract of yellowtail. After the crude extract was treated with acetonitrile and hexane, the extracted hexane layer was dehydrated with sodium sulfate and concentrated to 10mL.

Furthermore, for all other samples, pre-treatment using sulfuric acid silica gel column was performed for removing relatively large amounts of lipids in the extracted solutions. This column, being 3cm i.d., consists of 7.5g of sodium sulfate, 10g of silica gel impregnated with 22% sulfuric acid and 25g of silica gel impregnated with 44% sulfuric acid. 10mL of the sample extract was directly applied to this column, which was previously conditioned with 50mL of hexane, and dioxins were eluted with 200mL of hexane.

Purification using the semi-automated cleanup device

The multilayer silica gel column/ activated alumina column cartridge was used for purification of the pre-cleanup solutions using the semi-automated cleanup device. The pre-cleanup solution was spiked with $^{13}C_{12}$-labelled dioxins and concentrated to 200μL. The sample was applied onto the multilayer silica gel column cartridgefollowed by setting on the PTC (Positive Temperature Coefficient) heater, which was preheated to 60℃. Then, the multilayer silica gel column was heated on the heater for 30 minutes. After the multilayer silica gel column cooled to approximately 40℃, an activated alumina column cartridge was connected to the multilayer silica gel column cartridge. Dioxins were eluted with 40mL of hexane at a flow rate of 2mL min^{-1}. The activated alumina column cartridgewith the dioxins trapped in it was separated from the multilayer silica gel column, and reversibly set on the PTC heater, which was maintained at 85℃. The activated alumina cartridge was then dried on the heater, with a rate of flow of fresh air of 1.0mL min^{-1}. Dioxins were eluted by the addition of 900μL of toluene onto the activated alumina which was kept at 85℃ on the heater. Dioxins were collected in a vial. $^{13}C_{12}$-labelled dioxin as a syringe spike was added and finally concentrated to 50μL by nitrogen flow. The entire cleanup procedure was completed within 2 hours.

Measurement

The purified extracts were analyzed by GC-HRMS (SIM) (JMS-800D, JEOL) using DB-5MS (60m × 0.25mm i.d., 0.25μm film, J&W) capillary column. The resolution of the instrument was more than 10,000 and the verification of the resolution in the working range was obtained reference peaks by measuring PFK.

Results and discussion

Recovery rate of $^{13}C_{12}$-labelled dioxins

The sample solutions after the pre-cleanup treatment were purified by the multilayer silica gel column set on the semi-automated cleanup device, and the concentrations of the spiked dioxins were measured by GC-HRMS. The recoveries of $^{13}C_{12}$-labelled dioxins added as internal standard are summarized in Table 1.

The recoveries of $^{13}C_{12}$-labelled dioxins in all the sample of foodstuff were in the range of 73% and 110% (potato: 76-100%, soybean: 80-110%, spinach: 75-98%, apple: 75-100%, mushroom: 81-98%, seaweed: 79-110%, yellowtail: 73-110%, beef: 89-110%, egg: 75-110%). These results agreed well with the guideline values suggestedfor the analysis of dioxins in food in Japan (permitted limit of recovery rate: 40-120%), indicating that the purification method using the semi-automated cleanup device affords a high recovery of dioxins.

Cleanup efficiency

The GC/HRMS (SIM) chromatograms of dioxins purified by using the semi-automated device showed very little influence imposed by the impurities in all of the foodstuff samples in this study.

Furthermore, as the purification procedure was controlled by semi-automated cleanup device and completed within 2 hours, this rapid cleanup minimizes personal error, and needs less consumption of organic solvent and time, and thus improves the accuracy of the experiment.

Acknowledgements

This work was financially supported by JST-JICA through SATREPS program titled Multi-beneficial measure for the mitigation of climate change in Vietnam and Indochina countries by development of biomass energy.

References

1. Hong J, Miki Y, Honda K, Toida H. (2012), *Chemosphere*. 88: 1287-1291
2. Ministry of Health and Welfare of Japan. (2008), *The guideline for the analysis of dioxins in foods in Japan*

Table 1.Recoveries of $^{13}C_{12}$-labelled dioxins in food samples with semi-automated cleanup device[%]

	Potato	Soybean	Spinach	Apple	Mushroom	Seaweed	Beef	Egg	Yellowtail
2,3,7,8-TeCDD	76	80	75	75	84	79	90	75	70
1,2,3,7,8-PeCDD	91	94	87	87	90	90	97	92	89
1,2,3,4,7,8-HxCDD	95	97	93	100	94	96	100	96	94
1,2,3,6,7,8-HxCDD	96	100	91	100	94	100	110	100	92
1,2,3,7,8,9-HxCDD	92	92	84	86	91	85	89	86	85
1,2,3,4,6,7,8-HpCDD	87	88	85	96	85	88	98	97	99
OCDD	88	86	82	100	90	91	100	100	96
2,3,7,8-TeCDF	88	90	83	84	86	88	93	91	87
1,2,3,7,8-PeCDF	89	92	83	87	84	91	93	86	95
2,3,4,7,8-PeCDF	89	97	90	92	89	93	98	96	100
1,2,3,4,7,8-HxCDF	96	100	93	98	93	99	96	95	92
1,2,3,6,7,8-HxCDF	97	98	91	100	93	93	92	95	88
1,2,3,7,8,9-HxCDF	88	89	82	88	89	85	100	89	83
2,3,4,6,7,8-HxCDF	97	97	97	100	92	97	98	98	92
1,2,3,4,6,7,8-HpCDF	98	99	91	100	98	100	96	92	92
1,2,3,4,7,8,9-HpCDF	86	83	79	84	82	84	93	87	92
OCDF	86	98	84	100	88	90	90	98	97
3,4,4',5-TeCB (#81)	95	100	97	96	90	110	100	100	94
3,3',4,4'-TeCB (#77)	94	100	97	93	90	100	98	100	94
3,3',4,4',5-PeCB (#126)	100	110	96	99	98	110	110	100	94
3,3',4,4',5,5'-HxCB (#169)	88	96	86	85	81	95	100	100	100
2',3,4,4',5-PeCB (#123)	94	100	89	92	91	110	99	97	93
2,3',4,4',5-PeCB (#118)	96	110	95	95	92	110	94	92	96
2,3,3',4,4'-PeCB (#105)	98	110	94	99	95	110	100	98	91
2,3,4,4',5-PeCB (#114)	93	110	91	93	93	110	100	97	91
2,3',4,4',5,5'-HxCB (#167)	93	100	95	95	91	100	110	100	88
2,3,3',4,4',5-HxCB (#156)	94	100	98	96	91	100	110	110	110
2,3,3',4,4',5'-HxCB (#157)	93	100	91	94	87	100	100	100	98
2,3,3',4,4',5,5'-HpCB (#189)	98	110	94	99	94	110	100	98	90