Evaluation of automatic sample purification system for dioxins and PCBs analysis by the use of GC-HRMS and GC-MS/MS

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Introduction

In Europe, The regulations were added in 2012. COMMISSION REGULATION EU No 225/2012 is that, to enhance feed hygiene, it is necessary to provide for an obligation for feed busies operators to test fats, oils and products derived thereof for dioxins and PCBs in order to reduce risk that contaminated products enter the food chain[1]. Furthermore COMMISSION REGULATION EU No 252/2012 is the regulation that the methods for non-dioxin-like PCBs (PCB # 28, 52, 101, 138 153, 180) was added into Regulation (EC) No 1883/2006 [2,3].

Demands of PCBs analysis and number of sample for dioxins analysis has been increasing in Europe since the regulations were laid down. Therefore European dioxins testing laboratories requires the new automatic sample preparation system could save money and time for dioxins and PCBs analysis. So we have been developing the full automatic sample purification system for dioxins analysis since 2011. This system has effective features that could increase purification effect, reduce solvent dramatically and improve throughput for purification process for dioxins and PCBs analysis.

In this study, degree of purification sample solutions obtained by the use of our system was examined. This test was implemented to confirm if the purified solutions could measure with no hitch by the use of GC-MS/MS and GC-HRMS.

Materials and methods

1. *Samples and standards*

   Hen egg, Salmon and Pork meat were purchased from a supermarket in Matsuyama, Fish oil obtained from Sigma-Aldrich, Palm Fatty Acid Distillate (PFAD) were provided from a European dioxins testing laboratory in Netherlands. 50 of hen egg removed shell was mixed and homogenized, then kept at -25 ˚C in the freezer for 2days, after that freeze-dried for 1 days. 2 kilograms of salmon meat and Pork meat were directly freeze-dried, then homogenized.

   Standard solutions for dioxins, PCBs and PBDEs were purchased from Wellington laboratories or Cambridge isotope laboratories (CIL).

2. *Extraction*

   Each fat solution of Freeze-dried hen egg, salmon and pork meat were prepared by the use of Soxhlet extraction or Speed extractor E-916 (BUCHI) with mixed solvent of toluene and acetone (7:3). And then the extracts were evaporated the solvent completely.

3. *Purification*

   Automatic sample purification system and the consumable columns were developed by MIURA Co., Ltd. The flow of analysis shows in Figure 1. Aliquot of hen egg fat, salmon fat, pork fat, fish oil and PFAD, spiked with 13C12-dioxins, PCBs and PBDEs surrogate mixture, were respectively 3.5 g, 3 g, 3 g, 2 g and 3 g, these fat samples dissolved in about 5 mL of n-hexane were directly applied on the purification column by using a disposable syringe. And then columns set up to the system. Then press the start button. In about 94 min, two fractions, dioxins and PCBs fraction, could obtain automatically. Dioxins fraction in about 1.0 mL of toluene includes PCDD/DFs and non-ortho PCBs. Mono-ortho PCBs, 6-isomer PCBs and PBDEs exist in PCBs fraction of about 1.0 mL of toluene. Solvents used in all processes are only hexane and toluene, and amount of the solvents is about 100 mL of hexane 5 mL of toluene.

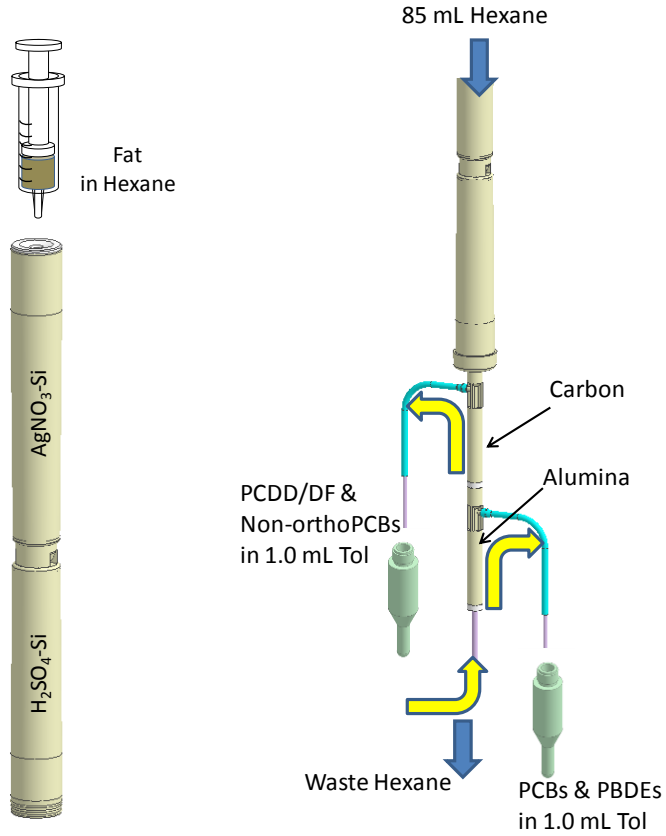

Figure 1 Consumable columns and flow of the system

4. *GC-MS measurement*

PCDD/DFs, DL-PCBs, 6-isomers PCBs and PBDEs were determined by GC-HRMS (JMS-800D-JEOL) and GC-MS/MS (Thermofisher-TSQ8000 and Agilent7000C). All of sample solutions were injected 2 µL in the splitless mode. The high resolution mass spectrometry was operated in the EI mode, using selected ion monitoring. The resolution of the MS was routinely more than 10,000 (10 % valley). The triple quadruple mass spectrometry was operated with multiple reactions monitoring (MRM) mode.

The Gas chromatograph temperature programs for each compound were followed,

Gas chromatograph program for PCDD/DFs and dl-PCBs was applied:

In GC equipped with DB-5ms (J&W Scientific, 60m x 0.25mm, 0.25µm Film thickness, Agilent Technologies), Initial Temprature is at 150 ºC (Hold time 1min), increasing to 180 ºC at rate of 20 ºC/ min, no hold, then increasing to 280 ºC at rate of 2 ºC/ min, no hold, and then increasing to 310 ºC at rate of 20 ºC/ min, final hold time 7 min. Total run time is 42 minutes. The inlet temp. was maintained at 250 ºC.

Gas chromatograph program for 6-isomers PCBs was applied:

In GC equipped with HT8-PCB (60m x 0.25mm, 0.2µm Film thickness, SGE USA), Initial Temprature is at 120 ºC (Hold time 1min), increasing to 180 ºC at rate of 20 ºC/ min, no hold, then increasing to 280 ºC at rate of 2 ºC/ min, final hold time 3 min. Total run time is 42 minutes. The inlet temp. was maintained at 250 ºC.

Gas chromatograph program for PBDEs was applied:

In GC equipped with DB-5HT (J&W Scientific, 15m x 0.25mm, 0.10µm Film thickness, Agilent Technologies), Initial Temprature is at 135 ºC (Hold time 3min), increasing to 215 ºC at rate of 10 ºC/ min, no hold, then increasing to 280 ºC at rate of 5 ºC/ min, no hold, and then increasing to 325 ºC at rate of 20 ºC/ min, final hold time 3.75 min. Total run time is 30 minutes. The inlet temp. was maintained at 260 ºC.

Results and discussion

1. Comparison of soxhlet extruction with BUCHI extructer

   For evaluation of extruction efficiency of dioxins and PCBs with Speed extractor E-916, freeze-dried salmon and egg were extracted with Speed extractor E-916 and conventional soxhlet extructer. The extructs obtained from each extructers were cleanuped by the automatic system. These results, concentrations of PCBs in extracts were quantified with GC-HRMS. Table 1 shows the concentrations in each extructs. Although some of 2,3,7,8-substituted PCDD/DFs isomers were background levels, no significant differences could be observed for each extraction.

Table 1 Comparison of soxhlet extruction with BUCHI speed extructer (E-916)

	Soxhlet	E-916			Soxhlet	E-916	
Sample / unit	Salmon pg/g-fat				Hen egg pg/g-fat		
TrCB (#28)	261.8	224.6	234.3		250.8	291.1	287.5
TeCB (#52)	517.3	471.1	504.1		73.2	95.6	92.4
PeCB (#105)	992.8	1030.9	948.0		17.9	22.2	28.9
PeCB (#118)	757.9	682.2	727.6		232.5	240.1	256.8
HxCB (#153)	2338.5	2133.0	2255.3		489.3	516.5	561.0
HxCB (#138)	1563.7	1324.9	1627.3		351.3	313.5	426.8
HpCB (#180)	917.2	835.7	918.6		140.4	148.5	148.5

2. Recoveries

   Table 2 shows the recoveries of Dioxins and PCBs fractions. These results are shown by avarage of repeatedly 3 times operations by use of the system. PCDD/DFs and non-ortho PCBs including the dioxins fraction shows good recoveries. And mono-ortho PCBs could be well separated from dioxins faraction with the specialized carbon column as a concentration column. Furthermore one more concentration column for adsorption of mono-ortho PCBs and 6-isomers PCBs also get good results in the recoveries. This separation technique is charactrized by just using hexane (without use of dichlorometane) and a reduction in the solvent.

Table 2 Recoveries of isomers in dioxins and PCBs fraction. (PFAD 2 to3 g, n=3)

Dioxins fraction				PCBs fraction	
2,3,7,8-TeCDD	94%	TeCB (#81)	98%	TrCB (#28)	91%
1,2,3,7,8-PeCDD	97%	TeCB (#77)	96%	TeCB (#52)	96%
1,2,3,4,7,8-HxCDD	96%	PeCB (#126)	99%	HxCB (#153)	94%
1,2,3,6,7,8-HxCDD	96%	HxCB (#169)	99%	HpCB (#180)	96%
1,2,3,7,8,9-HxCDD	93%	PeCB (#123)	3%	OcCB (#194)	101%
1,2,3,4,6,7,8-HpCDD	89%	PeCB (#118)	8%	PeCB (#123)	100%
OCDD	88%	PeCB (#105)	4%	PeCB (#118)	99%
2,3,7,8-TeCDF	95%	PeCB (#114)	1%	PeCB (#105)	99%
1,2,3,7,8-PeCDF	92%	HxCB (#167)	4%	PeCB (#114)	100%
2,3,4,7,8-PeCDF	93%	HxCB (#156)	3%	HxCB (#167)	100%
1,2,3,4,7,8-HxCDF	96%	HxCB (#157)	5%	HxCB (#156)	101%
1,2,3,6,7,8-HxCDF	92%	HpCB (#189)	4%	HxCB (#157)	102%
1,2,3,7,8,9-HxCDF	91%			HpCB (#189)	105%
2,3,4,6,7,8-HxCDF	95%				
1,2,3,4,6,7,8-HpCDF	88%				
1,2,3,4,7,8,9-HpCDF	90%				
OCDF	84%				

3. Comparison of GC-MS/MS with GC-HRMS

   Table 3 shows the concentration quantified with GC-HRMS and GC-MS/MS. The fat samples (PFAD 2 ~ 3 g fat) were prepared by the automatic system. It was evaluated by coefficient of variance (CV) caluclated

the data that 3 times injected PCBs fraction each. CV was below 20 % for both GC-MS. And the concentration of GC-MS/MS is roughly in agreement with the concentration of GC-HRMS. It indicates that 6-isomers PCBs in the PCBs fraction obtained from the system could have no issue about GC-MS/MS measurement.

Table 3 Comparison of quantitive concentrations of 6-isomers PCBs

	GC-HRMS		GC-MS/MS	
	pg/g-fat	CV%	pg/g-fat	CV%
TrCB (#28)	62.5	4%	63.7	2%
TeCB (#52)	26.9	2%	28.4	4%
PeCB (#101)	6.2	9%	6.8	17%
PeCB (#118)	6.1	6%	6.4	7%
HxCB (#153)	6.3	14%	6.1	4%
HxCB (#138)	5.7	4%	6.8	10%
HpCB (#180)	5.9	2%	5.6	5%

Repeatability and reproducibility of two makers GC-MS/MS measurement with 5 kind of sample matrixes purified by the system illustrates in Table 4. Each solutions were injected 3 times. Samples except for the PFAD and pork fat with a RSD value of 20 % observed for PCBs, which can be explained by the low concentration value. However, it was noticed that no significant differences were observed from concentrations in each fat sample.

Table 4 Coefficient of variance of quantified concentrations with GC-MS/MS of two makers

GC-MS/MS (A)	FishOil	HenEgg	PFAD	Pork	Salmon		GC-MS/MS (B)	FishOil	HenEgg	PFAD	Pork	Salmon
TrCB (#28)	1%	2%	2%	3%	2%		TrCB (#28)	3%	3%	2%	4%	2%
TeCB (#52)	1%	4%	4%	7%	4%		TeCB (#52)	5%	3%	4%	5%	6%
PeCB (#101)	1%	6%	17%	4%	4%		PeCB (#101)	1%	4%	58%	9%	3%
PeCB (#123)	3%	6%	-	-	9%		PeCB (#123)	9%	10%	-	-	4%
PeCB (#118)	1%	5%	7%	4%	4%		PeCB (#118)	1%	5%	12%	7%	1%
PeCB (#114)	2%	17%	-	-	11%		PeCB (#114)	1%	5%	-	-	6%
HxCB (#153)	1%	3%	4%	3%	1%		HxCB (#153)	2%	3%	-	10%	9%
PeCB (#105)	1%	4%	10%	2%	5%		PeCB (#105)	2%	5%	13%	11%	2%
HxCB (#138)	1%	2%	10%	3%	2%		HxCB (#138)	2%	2%	54%	6%	8%
HxCB (#167)	1%	3%	-	16%	2%		HxCB (#167)	3%	5%	-	15%	5%
HxCB (#156)	1%	4%	9%	10%	1%		HxCB (#156)	2%	2%	20%	39%	7%
HxCB (#157)	1%	3%	-	14%	2%		HxCB (#157)	2%	17%	87%	27%	11%
HpCB (#180)	2%	2%	5%	1%	1%		HpCB (#180)	3%	2%	66%	11%	1%

-: Not found. (Not detected)

References:

1. COMMISSION REGULATION (EU) No 225/2012 of 15 March 2012 amending Annex II to Regulation (EC) No 183/2005 of the European Parliament and of the Council as regards the approval of establishments placing on the market, for feed use, products derived from vegetable oils and blended fats and as regards the specific requirements for production, storage, transport and dioxin testing of oils, fats and products derived thereof

2. COMMISSION REGULATION (EU) No 278/2012 of 28 March 2012 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels and action thresholds for dioxins and polychlorinated biphenyls

3. COMMISSION REGULATION (EU) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin- like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006