

## EXTRACTION METHOD ENHANCEMENT TECHNIQUES FOR THE ANALYSIS OF PCDDS AND PCDFS IN MEAT SAMPLE

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### Introduction

Dioxins are the most concerned persistent organic pollutants(POPs) to human beings and being fat soluble tend to accumulate in higher animals including humans. Dioxins has 75 congeners of PCDD and 135 congeners of PCDF, but generally only 7 congeners of PCDD and 10 congeners of PCDF are analyzed because of their toxicity, stability and so on.

Since dioxins contamination is regarded as a global issue, a large amount of samples have been analyzed, and various methods for measuring dioxins have been developed and improved. Dioxins analysis needs very complicated analytical procedure including extraction, cleanup and instrument analysis. Because analytical procedure is very complicated and needs many steps, conventional analytical procedure are very time consuming and use large volumes of solvents. So current methods are time consuming and very expensive.

In this study, speedy and cost reducing methods without reducing recovery and stability should be focused. So to present the new extraction method to simplify and stabilize of extraction method, the recovery and stability of 7 congeners of PCDD and 10 congeners of PCDF using soxhlet, forced convection dry oven and microwave oven were compared.

### Materials & Method

Sample : 5 g aliquots of corn oil(Sigma, USA) as lipid simulant were weighed and spiked with diluted PAR(Precision & Recovery Stock Solution, Wellington lab Inc.) and LCS(Labelled Compound Stock Solution, Wellington lab Inc.) on

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them. The samples were extracted in 5 replications and a solvent blank was always performed whenever a sample batch was analysed.

**Standard** : PAR standard solution was diluted with acetone(J.T.Baker, for organic residue analysis, USA) to prepare a diluted spiking solution. The concentration of spiked PAR was equal to that of the native compound of CSL(Extended calibration standard, low level, Wellington lab Inc.). LCS was diluted by a factor of 50 with acetone prior to use. Before extraction, diluted LCS spiking solution were added to the sample aliquots. CSS(Cleanup Standard Spiking Solution, Wellington lab Inc.) was  $^{37}\text{Cl}_4$ -2,3,7,8-TCDD in nonane at 0.8ng/ml. The cleanup standard was added to all extracts prior to cleanup to measure the efficiency of the cleanup process. ISS(Internal Standard Spiking Solution, Wellington lab Inc.) was composed of  $^{13}\text{C}_{12}$ -1,2,3,4-TCDD and  $^{13}\text{C}_{12}$ -1,2,3,7,8,9-HxCDD in n-nonane(Sigma, USA) and diluted by a factor of 10 with n-nonane.

### Extraction

Corn oil was used as lipid simulant and diluted PAR and LCS spiking solution were spiked with 100ul and 200ul, respectively. After spiking, the sample aliquots were extracted using a soxhlet, dry oven and microwave oven as an apparatus for extraction of meat sample, respectively. Soxhlet extraction solvents was used n-hexane(Wako, for dioxin analysis, Japan) : dichloromethane(J.T.Baker, for organic residue analysis, USA)(1:1) for 18 hrs. Forced convection dry oven extraction was carried out at 80 °C for 12 hrs. Microwave oven extraction was carried out at 700W for 5 mins. Obtained extracts were concentrated and diluted by n-hexane.

**Cleanup** : The Power-prep<sup>TM</sup>(Fluid Management System, Inc., USA) that was composed of silica column, alumina column and carbon column was used as cleanup system. Disposable prepacked silica, alumina and carbon column made of Teflon, manufactured by Fluid Management System Inc. The computer controlled Power-prep<sup>TM</sup> system performs simultaneous sample processing automatically. The cleanup solvents were as follows ; n-hexane, 2%(V/V) n-hexane : dichloromethane, 50%(V/V) n-hexane : dichloromethane, 50%(V/V) ethyl acetate(J.T.Baker, , for organic residue analysis, USA) : benzene(Wako, for pesticide residue analysis, Japan), toluene(J.T.Baker, for organic residue analysis, USA), and then eluted with 70ml of toluene into a pear shape flask. The toluene fraction was evaporated to about 1 ml by Automatic Environmental Speed Vac(SAVANT Instrument Inc., USA). And then, evaporated to dryness under a nitrogen blowdown apparatus(Organomation Associates Inc., USA) and made up

exactly 90ul with n-nonane. 10ul of syringe standard(ISS ; Internal standard spiking solution, Wellington lab Inc.) was spiked to obtained solution.

Instrument analysis : HRGC/HRMS measurement was carried out over 10,000 resolution at 10% valley by Autospec ultima(Micromass Ltd, UK) interfaced with an HP 6890 series plus gas chromatograph(Agilent, USA) equipped with DB5-MS column(J&W Scientific ; 60m length×0.25mm ID×0.25um thick) following general procedures of EPA method 1613(Tetra-through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS). The mass spectrometer was operated electron impact(EI) mode and selected ion monitoring(SIM) mode.

### Results & Discussion

The results obtained based on extraction method such as soxhlet extraction, dry oven extraction and microwave oven extraction of meat sample were shown in Fig. 1 and Table 1.

In the case of soxhlet extraction, the average recovery of PCDD and PCDF was 110.7 and 102.0%, respectively. Coefficiency of variability(C.V.) of recovery of 17 dioxin congeners in 5 replication was in the range of 3.2 – 23.2 and average C.V. was 8.4.

Secondly, when forced convection dry oven was used as extraction apparatus, the average recovery of PCDD and PCDF was 97.8 and 97.4%, respectively. Coefficiency of variability(C.V.) of recovery of 17 dioxin congeners in 5 replication was in the range of 1.8 – 18.2 and average C.V. was 7.6.

Finally, In the case of microwave oven extraction at 700W for 5 mins, the average recovery of PCDD and PCDF was 126.7 and 117.2%, respectively. Coefficiency of variability(C.V.) of recovery of 17 dioxin congeners in 5 replication was in the range of 2.5 – 20.5 and average C.V. was 10.3.

In all cases, average recovery of PCDD was higher than that of PCDF. And it was seemed that the average recovery and C.V. of 17 dioxin congeners were slightly different from each extraction method. In point of just recovery and C.V., forced convection dry oven extraction was better than any other extraction method. But considering all aspects including economy and experiment time, microwave oven could be most efficiently extracted PCDD and PCDF from lipid sample in this experiment.

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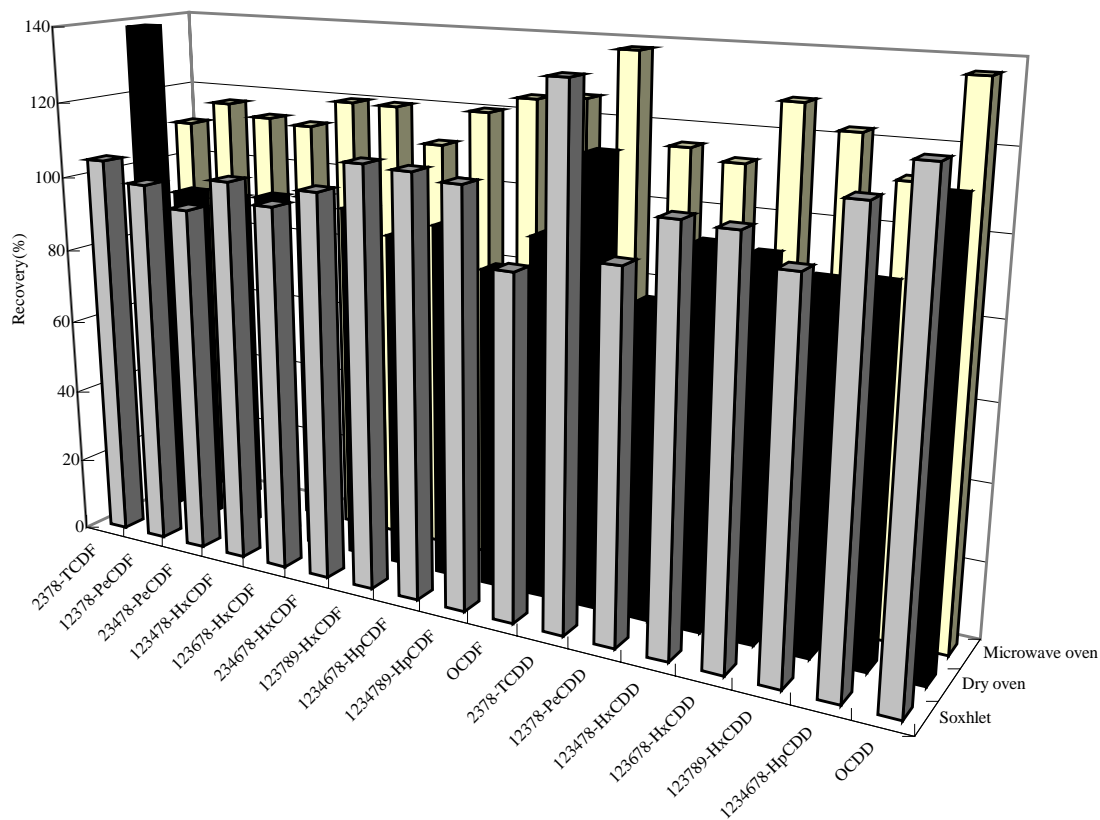


Fig. 1. The average recovery of PCDD and PCDF according to the extraction method using soxhlet, dry oven and microwave oven. The experiment was performed in 5 replications.

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Table 1. The recovery range and C.V. of PCDD and PCDF according to the extraction method using soxhlet, dry oven and microwave oven. The experiment was performed in 5 replications.

	Soxhlet (n=5)		Dry oven (n=5)		Microwave oven (n=5)	
	Recovery (%)	C.V.(% )	Recovery (%)	C.V.(% )	Recovery (%)	C.V.( %)
2378-TCDF	71.1-124.9	19.8	135.5- 140.0	1.8	103.3- 120.7	7.3
12378-PeCDF	91.3-106.9	5.9	88.2-96.2	4.7	112.6- 120.0	2.5
23478-PeCDF	87.4-103.1	6.5	88.8-99.3	5.0	103.0- 129.3	8.5
123478-HxCDF	96.0-110.4	5.9	88.9- 100.3	4.4	100.4- 123.4	8.3
123678-HxCDF	89.0-101.8	5.3	93.2- 100.5	2.8	115.6- 129.3	4.7
234678-HxCDF	99.4-107.3	3.3	87.3- 102.0	7.0	102.5- 141.3	12.3
123789-HxCDF	100.2- 122.2	7.2	77.6-98.0	10.4	101.5- 125.8	9.9
1234678-HpCDF	99.1-122.2	10.1	83.0-99.3	6.8	104.4- 133.0	9.9
1234789-HpCDF	99.0-122.0	8.4	75.6-92.1	7.1	113.3- 145.2	10.5
OCDF	55.6-107.7	23.2	78.8- 105.8	12.7	121.9- 142.3	9.4
PCDF average	89.1-111.0 (102.0)	9.6	83.8- 138.4 (97.4)	6.3	110.1- 125.4 (117.2)	8.3
2378-TCDD	119.8- 150.2	9.6	96.0- 130.9	18.2	134.4- 141.3	9.3
12378-PeCDD	90.0-97.2	3.2	73.5-86.2	6.7	106.3-	9.0

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123478-HxCDD	94.3-121.8	9.6	86.1-106.5	7.6	132.8 101.9-132.3	12.2
123678-HxCDD	99.5-110.3	4.6	92.7-101.4	3.8	104.4-157.1	15.7
123789-HxCDD	85.8-111.4	9.6	82.0-103.9	9.9	99.8-130.0	11.6
1234678-HpCDD	107.7-121.4	5.0	79.3-106.6	14.7	78.9-139.9	20.5
OCDD	116.0-123.4	5.1	103.3-122.3	6.3	131.9-146.3	10.6
PCDD average	93.9-135.2 (110.7)	6.7	80.6-115.3 (97.8)	9.6	112.8-137.9 (123.7)	12.0
<b>Total average</b>	<b>89.1-135.2 (105.6)</b>	<b>8.4</b>	<b>80.6-138.4 (97.5)</b>	<b>7.6</b>	<b>110.1-137.9 (121.1)</b>	<b>9.9</b>

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