

**NOVEL SAMPLE PREPARATION METHOD
FOR DIOXINS ANALYSIS
EXTRACTION / DESORPTION CLEAN-UP PROCEDURE
WITH SPME CARBON FIBER**

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Introduction

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. However, current methods are time consuming and very expensive. Therefore, these methods are not satisfactory because the market requires rapid, high-throughput, low cost analyses. Under such circumstances, various fast and easy analytical methods are being developed.

Solid Phase Micro Extraction (SPME), manufactured by Supelco, utilizes various coated adsorbents and (or) absorbents on a fiber contained in a syringe like device. This tool is used widely for the extraction of pesticides, VOCs and other organic analytes primarily from aqueous based media.

We have developed an analytical method for PCDDs/PCDFs and co-planar PCBs using a SPME fiber with HRGC/HRMS. We found that SPME fibers coated with carbon material can extract selectively PCDDs/PCDFs and Co-PCBs from organic solvents such as n-nonane and can introduce a large amount of PCDDs/PCDFs and Co-PCBs into the GC without introducing a large amount of solvent.

The SPME extraction/desorption apparatus is demonstrated in Fig. 1. The carbon coated fiber has been inserted in the syringe tube. On the extraction condition, the fiber is stretched out from the syringe tube and is dipped into sample extracts. After certain extraction period, the syringe can be used as GC injection device for GC/MS analysis directly. This simple method can be promising the saving time for clean-up in dioxins analysis.

Materials and Methods

1. SPME adsorption/desorption method

We examined the optimum condition for extraction/desorption of dioxins from n-nonane solution using various types of SPME fibers. As the test solution, 7 PCDDs, 10 PCDFs and 12 Co-PCBs congeners, which were obtained from Wellington, were respectively dissolved into n-nonane at a concentration of 1 pg/ μ l. The SPME fibers examined are listed in Table 1. The recovery of the methods was verified

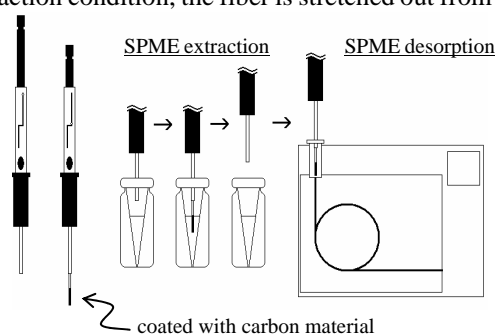


Fig. 1. The SPME extraction / desorption apparatus

Table 1

SPME Fiber	Surface area (μ g)	Density (g/mL)	pore size (cc/g)		
			Micro	Meso	Macro
Carbopack Z	220	0.18		1.74	
Carbopack B	100	0.36		Nonporous	
Carbopack C	10	0.68		Nonporous	
Carboxen 1006	715	0.4	0.29	0.26	0.23
Carboxen 1016	75	0.4		0.34	

comparing with a standard test solution.

The optimization experiments for extraction condition were conducted under the combination of extraction period (5, 10, 20, 30, 40, 60 and 80 min.) and extraction temperature (35, 60, 80, 100, 120 and 140 C). The desorption conditions, that is the temperature of GC injection port, were changed for 260, 280 and 300 C.

HRGC/HRMS analysis of dioxins was carried out with Agilent HP6890 gas chromatograph and JMS-700 mass spectrometer (JEOL Ltd). The column used for gas chromatography was BPX-5, 25m x 0.15 mm, 0.25 μm thickness (SGE) with 0.25mm I.D. x 1m guard column.

2. Application to the real soil sample

10 g (dry wt.) soil sample was extracted with toluene using a Soxhlet extraction apparatus for 16 hours. The toluene extract was divided into two portions. The one was prepared according to conventional clean-up method using multi-layer silica gel column (Supelco) and dual-layer carbon reversible column (Supelco). The other aliquot was applied to extraction/desorption method directly. As the calibration standard for dioxins analysis, DF-A5-CS6 and PCB-A5-CS6 (Wellington) were mixed at a ratio of 1:4, and then diluted with n-nonane to one fifth in concentration.

The analysis of dioxins was conducted with a same condition except for GC columns. The columns used for gas chromatography were SP-2331, 60m x 0.25mm, 0.2um (Supelco) for tetra to hexa CDD/DFs, and RH-12ms 60m x 0.25mm (INVENTX) for hepta to octa CDD/DFs and Co-PCBs respectively. Some time, a RH-12ms column was used, if it is necessary to analyze all congeners at once.

To compare SPME method with Japanese official method, a 1μl pretreatment sample was injected with a micro syringe on three columns, SP-2331, 60m x 0.25mm, 0.2um for the analysis of Tetra~Hexa CDD/DF, a RH-17ms, 30m x 0.32mm, 0.15μm column for Hepta~Octa CDD/DF, and a DB-5ms, 60m x 0.32mm, 0.25um (Agilent) for analysis of Co-PCBs, respectively.

Results and Discussion

Firstly, Carboxen 1016-PDMS fiber was examined to optimize extraction time, extraction temperature and desorption temperature. As shown in Fig. 2, the response of PCDD/DF increased with an increase of extraction time, but the increase response of Co-PCBs was small. A standard solution of Co-PCBs with 0.4 pg/μl was used in this experiment.

Secondly, an extraction temperature was examined (Fig. 3). By increasing the extraction temperature, the response of PCDD/DF decreased; the response at 140 was about 1/7 - 1/4 of response at 60. On the other hand, the response of Co-PCBs increased proportionally with an increase of temperature; the maximum increase was 5 times greater for several Co-PCBs.

Finally, the desorption temperature was examined (Fig. 4). By increasing

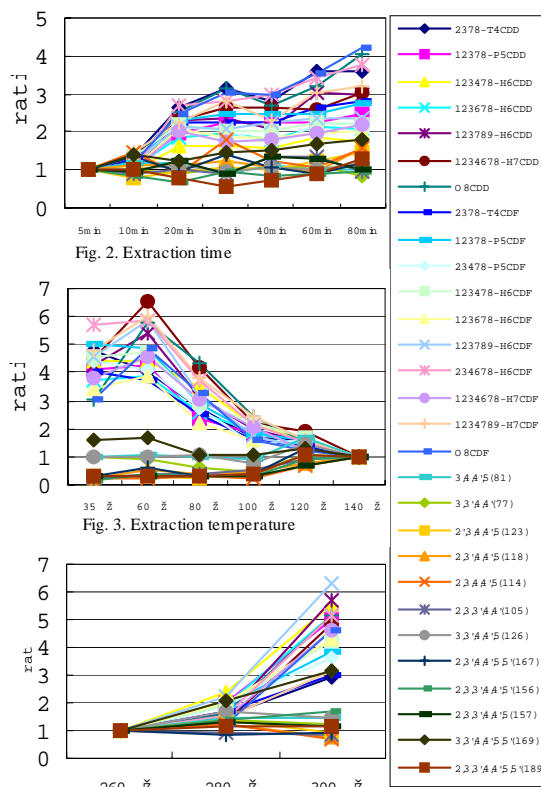


Fig. 4. Desorption temperature

desorption temperature, the response of PCDDs/ PCDFs increased. In another experiment, using Carbo-pack B SPME was examined at 300, 320, 340,360,380 ,the response of highly PCDDs/PCDFs increased . Based on these experiment, an extraction time of 40 min. under an ambient extraction temperature of 25~30 were selected. A desorption

temperature of 300 was selected by considering SPME fiber durability.

Then, 5 SPME fibers coated with Carboxen 1006, Carboxen 1016, Carbo-pack Z, Carbo-pack B or Carbo-pack C were evaluated at above conditions. The response of 5 SPME fibers compared to 1µl direct injection is shown in Fig. 5. All carbon SPME fibers except for Carboxen 1006 show about 5-16 times larger response for PCDDs/PCDFs compared to one of 1µl direct injection. Carbo-pack B showed the highest response for Co- PCBs and PCDDs/PCDFs. However, the response of Co-PCBs with most of carbon SPME fibers showed lower response compared to 1µl direct injection.

Carbo-pack B and Carboxen 1016 fibers showed higher or equal response for non-ortho Co-PCBs but lower for mono-ortho Co-PCBs than one of 1µl direct injection. A planar structure compound seems to adsorb preferentially on the carbon surface of the fiber.

Based on the results above, we evaluated Carbo-pack B SPME fiber with an actual soil sample to establish fast and easy analysis method. A calibration solution was extracted with Carbo-pack B SPME fiber and as shown in Fig. 6. It showed a high sensitivity specifically to 2,3,7,8-chlorine substituted DDs/DFs compared to 1µl direct injection.

Unknown peaks on the chromatogram with Carbo-pack B SPME fiber disappeared; on the other hand, some unknown peaks can be seen on the chromatogram with direct injection according to the official method using a multi-layer silica gel column and a carbon column to pretreatment the sample. It is considered that Carbo-pack B SPME fiber adsorb PCDDs/PCDFs and Co-PCBs selectively.

Utilizing carbon SPME fiber characteristics, we tried SPME fiber to extract soil sample without pretreatment process. As shown in Fig. 7, a good chromatogram without any

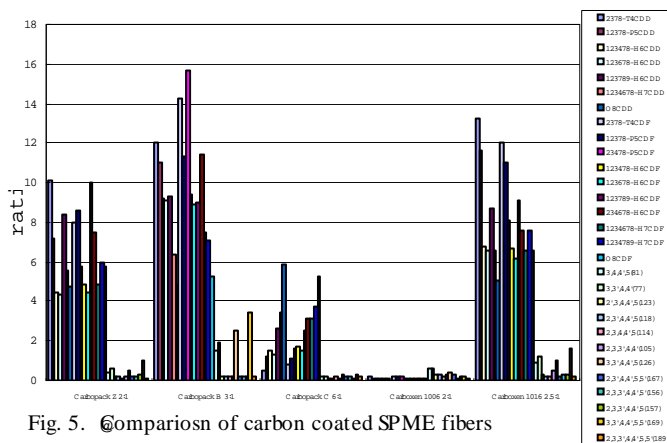


Fig. 5. Comparison of carbon coated SPME fibers

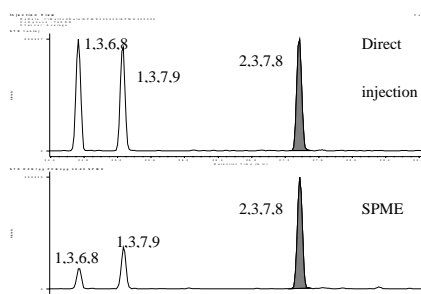


Fig. 6. STD chromatogram (TeCDD) on RH-12ms

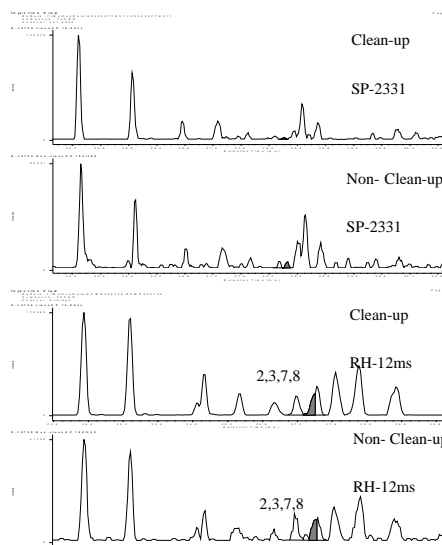


Fig. 7. Sample chromatogram (TeCDD)

unknown interference peaks was obtained. In order to quantify PCDDs/PCDFs and Co-PCBs concentration in soil samples, a calibration curve was made with SPME method and direct injection. As shown in table 2, both quantitative results of PCDDs/ PCDFs and Co-PCBs concentration with SPME method and direct injection method are very close. These result show calibration by direct injection can be applied to SPME extraction.

Quantitative results showed the concentration of PCDDs/PCDFs and Co-PCBs with the SPME method was higher than the concentration with a 1µl direct injection. This is probably due to insufficient separation of 2,3,7,8-chlorine substituted CDDs/ DFs with RH-12ms capillary column. However, SPME method can be used successfully as a simple and fast method for quantifying PCDDs/PCDFs and Co-PCBs.

Conclusion

It was determined that SPME fibers coated with carbon can extract selectively PCDDs/PCDFs and Co-PCBs, all of which possess a planar structure, from n-nonane solution. Another advantage of this SPME method is that a large volume of solvent is not introduced into GC/MS. Also, because of the selective extraction capability of SPME fibers, less nonvolatile residue from sample is introduced into the injection port. This reduces the build-up of residue in the injection port that can interfere with future injections.

A simple and fast method using the advantage of carbon SPME fiber with selective extraction and without a large solvent injection was established. The high-resolution capability of the HRMS used in this experiment was an important factor for quantification of PCDDs/PCDFs and Co-PCBs. We are going investigate to determine whether a method with low-resolution mass spectrometry (LRMS) can be applied to actual samples.

At this time the focus has been on developing a rapid and simple method for analysis of toxic 12 Co-PCBs, 10 PCDDs and 17 PCDFs. For this purpose, it was determined using a RH-12ms column that 29 components could be analyzed with one injection. In order to analyze 29 components in one extraction method, the separation of some Tetra Hexa CDD/DF congeners is sacrificed. If needed, the better separation is possible to gain using a SP-2331 column.

A limit of detection (LOD) for Co-PCBs is higher than LOD for PCDDs/PCDFs due to the lower extraction efficiency with carbon SPME fiber for Co-PCBs. We are going to improve this point by changing the solvent in sample solution.

Soxhlet extraction for 16 hours was used for soil sample in our experiment. In order to shorten analysis time, an ASE or reflux extraction instead of Soxhlet will be used.

References

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Table 2 Concentration of PCDDs and co-PCBs in soil

(ng-TEQ/(gdry))	clean-up			clean-up			Not clean-up		
	STD Direct Inj	STD Direct Inj	STD (SPME)	STD (SPME)	STD Direct Inj	STD (SPME)	STD Direct Inj	STD (SPME)	
	Direct Inj	SPME(SP-2331 & RH-12ms)	SPME (RH-12ms)	SPME (RH-12ms)	SPME(SP-2331 & RH-12ms)	SPME (RH-12ms)	SPME(SP-2331 & RH-12ms)	SPME (RH-12ms)	
2,3,7,8-TCDF	0.57	0.55	0.72	0.67	0.83	0.97	0.91	0.91	
1,2,3,7,8-PeCDF	0.90	0.80	0.475	0.60	1.45	0.65	0.80	0.80	
2,3,4,7,8-PeCDF	9.0	9.0	7.5	8.0	9.0	12	12.5	12.5	
1,2,3,4,7,8-HxCDF	2.4	2.3	1.9	1.9	2.7	3.7	3.7	3.7	
1,2,3,6,7,8-HxCDF	2.1	1.9	6.5	6.6	2.9	9.4	9.5	9.5	
1,2,3,7,8,9-HxCDF	0.19	0.25	0.14	0.15	0.16	0.27	0.25	0.25	
2,3,4,6,7,8-HxCDF	3.0	2.8	3.2	3.6	4.2	7.2	8.0	8.0	
1,2,3,4,6,7,8-HxCDF	1.1	0.99	0.99	1.0	1.4	1.4	1.5	1.5	
1,2,3,4,7,8,9-HxCDF	0.10	0.13	0.13	0.13	0.15	0.15	0.16	0.16	
OCDF	0.0061	0.0052	0.0052	0.0054	0.0073	0.0073	0.0072	0.0072	
2,3,7,8-TCDD	0.60	0.10	1.0	1.0	1.1	1.9	2.0	2.0	
1,2,3,7,8-PeCDD	3.7	2.8	5.6	5.2	3.6	14	13	13	
1,2,3,4,7,8-HxCDD	0.56	0.82	2.2	2.1	1.5	2.4	2.0	2.0	
1,2,3,6,7,8-HxCDD	1.3	1.6	1.4	1.4	1.4	2.5	2.2	2.2	
1,2,3,7,8,9-HxCDD	0.90	1.0	1.8	2.2	0.84	1.7	2.0	2.0	
1,2,3,4,6,7,8-HxCDD	1.0	1.2	1.2	1.6	2.3	2.3	3.0	3.0	
OCDD	0.14	0.11	0.11	0.11	0.12	0.12	0.12	0.12	
2,3,4,6,8-TCB i 81 j	0.0036	0.0013	0.0013	0.0020	0.0017	0.0017	0.0027	0.0027	
3,3',4,4'-TeCB i 77 j	0.0020	0.0015	0.0015	0.0013	0.0016	0.0016	0.0015	0.0015	
3,3',4,4',5-PeCB i 126 j	1.4	0.81	0.81	0.64	2.2	2.2	2.0	2.0	
3,3',4,4',5,5'-HxCB i 169 j	0.053	0.030	0.030	0.050	0.12	0.12	0.16	0.16	
2,3,4,4',5-PeCB i 123 j	0.00018	0.00031	0.00031	0.00039	0.00014	0.00014	0.00012	0.00012	
2,3,4,4',5-PeCB i 118 j	0.0051	0.0027	0.0027	0.0047	0.0035	0.0035	0.0062	0.0062	
2,3,3',4,4'-PeCB i 105 j	0.0033	0.0025	0.0025	0.0034	0.0028	0.0028	0.0037	0.0037	
2,3,4,4',5-PeCB i 114 j	0.00125	0.00155	0.00155	0.00285	0.00125	0.00125	0.00255	0.00255	
2,3,4,4',5,5'-HxCB i 167 j	0.000071	0.000036	0.000036	0.000042	0.000029	0.000029	0.000026	0.000026	
2,3,3',4,4',5-HxCB i 156 j	0.0080	0.012	0.012	0.0038	0.0105	0.0105	0.0049	0.0049	
2,3,3',4,4',5-HxCB i 157 j	0.0036	0.0060	0.0060	0.0038	0.0070	0.0070	0.00485	0.00485	
2,3,3',4,4',5,5'-HpCB i 189	0.00097	0.00033	0.00033	0.00035	0.00042	0.00042	0.00045	0.00045	
TOTALTEQ	29	27	36	37	36	63	64	64	