Development of pre-treatment method for Co-PCBs in biological materials

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Introduction

As for the method of pre-treatment of Co-PCBs analysis for biological materials, a lot of examination was done and a various method has been suggested. It was important point to let analysis be successful to choose a method suited for a sample among the inside of various methods, but factual knowledge and experience were demanded to do it.

We expect to the pre-treatment method that the method did not depend on the property of a material, and more the method reduce a time for processing procedures. The pre-treatment method based on the technique of acid decomposition and hot extraction with solvent we examined each condition. With using microwave decomposition device for the acid hydrolysis that the improvement to a decomposition time for shorter, and was able to change the biological material into a liquid phase. We do an experiment to establish examination of various conditions of decomposition and comparison with alkali decomposition method.

Method

Chemical reagent and devices

C13 labelled PCBs (3,3',4,4'-T4CB , 3,3',4,4',5- P5CB , 3,3',4,4',5,5'- H6CB) used from WELLINGTON LABORATORIES. And which was used diluted to 60 pg/ uL (following, C13-Standard-Solution).

Other PCBs standard reagent was supplied by WELLINGTON LABORATORIES. The solvent used dioxin measurement grade or pesticide measurement grade and the other chemical reagent used special grade from KANTO CHEMICAL CO.,INC. or WAKO PURE CHEMICAL INDUSTRIES, Ltd. MLS-1200MEGA (Milestone Inc.) microwave decomposition device was used for decomposition of biological materials. JMS-700 (JEOL CO Ltd.) was used for GC-MS.

Examination of Acid Hydrolysis Temperature

As biological materials, examination of decomposition condition was done using a pig liver. 2g of homogenised material and 50mL of 6N Hydrochloric acid were added in a reaction vials and were sealed. Reaction temperature kept 60, 80, 100,120 and 140degrees. And reaction vials were heated by microwave powered at 1000W till reached the laying temperature and continued 30minutes powered at 800W. The most suitable conditions were searched from indissoluble residue and shade of solution about reaction temperature.

Examination of Addition for Collection 1

50mL of 6N Hydrochloric acid and 100uL of C13-Standerd-Solution were added in a reaction vials and were sealed. Reaction vials were heated by microwave powered at 1000W till 100 degree

and continued 30 minutes powered at 800W. After moving liquid phase from reaction vials, 50mL extractant (Hexane, Dichloromethane, Diethyl ether, Toluene, 3:1=Dichloromethane/Hexane, 3:1=Diethyl ether/Hexane, 3:1=Toluene/Hexane, 3:1=Hexane/Acetone) were added and were heated 10minutes 100degree.

Examination of Addition for Collection 2

2g of homogenised material and 50mL of 6N Hydrochloric acid was decomposed by microwave at 100degree 30minutes and the process was repeated till 2L of liquid phase was got. And C13-Standerd-Solutions were added into liquid phase to include C13-Standerd at 60pg/50ml. 50mL of this solution, 20mL of sulphuric acid and 50mL of extractant (Hexane, Dichloromethane, Diethyl ether, Toluene, 3:1=Dichloromethane/Hexane, 3:1=Diethyl ether/Hexane, 3:1=Toluene/Hexane,) were added into the separating-funnel, and liquid-liquid extraction was done.

Examination of Addition for Collection 3

2g of homogenised material, 50mL of 6N Hydrochloric acid and 100uL of C13-Standerd-Solution were added in a reaction vials and were sealed. Reaction vials were heated by microwave powered at 1000W till 100 degree and continued 30 minutes powered at 800W. After moving liquid phase from reaction vials, 50mL 3:1= Dichloromethane/Hexane were added and were heated 10minutes 100degree. 50mL of liquid phase, 20mL of sulphuric acid and 50mL of Toluene were added into the separating-funnel, and liquid-liquid extraction was done.

Soxhlet Extraction and Alkali Decomposition Method

2g of materials were homogenised with Sodium Sulphate and were moved cylinder paper-filter. Soxhlet extraction were done with 3:1=Diethyl ether/Hexane for 7 hours. And this solution were Concentrated with KD-Concentration till the volume less than 10mL. Then, sampling spike were added to 100uL of C13-Standerd-Solution, and alkali decomposition were done with 50mL of 1N Potassium Hydroxide/Ethanol for 1 hour by reflux. Liquid-Liquid extraction was done with 50mL of Hexane.

Cleanup for GC-MS sample preparation and GC-MS measurement condition

As each experiment sample held many matrixes, both of sililicagel chromatograph and sulphuric acid extract were done. Then, concentrated till 100uL, and did provide GC-MS.

GC-MS Column was used SPB-octyle (SUPELCO 50m 0.20mm 0.25 um film). The HRMS was operated in electron impact ionisation mode.

Injection volume : 1uL
Injection temperature : 280degree
Injection method : splitless
Oven temperature: held at 100degree for 2minutes,
: then programmed to 260degree at 30degree,
: and held 30minutes
Ion Source temperature : 280degree

Result and Discussion

The temperature for acid hydrolysis

What we should pay attention to hydrolysis is that the biological material must be decomposed

till hydrophilic phase. But, even if Co-PCBs is stable for oxidation, adding powerful oxidative substante to that is not good action.

We determined whether the biological material has decomposed enough for hydrolysis by liquid phase that included insoluble matter. At reaction temperature below 80 degree, the insoluble matter existed in liquid phase. So we judged that the hydrolysis was not enough. As reaction temperature overed 120 degrees, and liquid phase was blackish brown from brown, we judged that oxidation reaction progressed. As a result it was assumed that we did 100 degrees, heat for 30 minutes as acid hydrolysis condition of the biological material used microwave.

An extract solvent from reaction vial and liquid phase

After moving liquid phase from reaction vial, extractant that extract Co-PCBs in the empty vial was examined from 8 solvents. The highest collection rate was shown 3:1=Diethyl Ether/Hexane and, the next was 3:1=Dichloromethane/Hexane (Table 1). 3:1=Diethyl Ether/Hexane was boiled and scattered when we added sulphuric acid, so the collection rate was shown wide range. As our conclusion, 3:1=Dichloromethane/Hexane is the best extract solvent.

Because liquid phase include Co-PCBs, we experimented solvent of liquid-liquid extraction. The solvent that collection rate was the heighest was Toluene and, the next was 3:1=Dichloromethane/Hexane. Therefore, Toluene was the best extract solvent.

Table 1. The extract efficiency of Co-PCBs for each solvent from a reaction vial and liquid-liquid extaraction method.

Solvent	Collection Rate (%) Valuues are Average ± S.D. (n=3)					
	Extract from Vial			Liquid-liquid-Extract		
	T4	P5	H6	T4	P5	H6
Hexane	68±20	60±22	55±18	8.3±5.2	7.7±4.0	5.4±4.4
Dichloromethane	54±9.6	49±19	43±17	23±22	18±20	15.3±19
Diethyle ether	55±21	58±30	55±25	9.2±4.8	10±6.8	7.7±4.6
Toluene	42±11	38±17	47±9.9	**59±0.4**	**77±17**	**100±6.0**
Dichloromethane/Hexane	**90±6.8**	**90±5.4**	**89±6.9**	47±8.1	49±7.7	44±13
Diethyle ether/Hexane	148±31	146±29	155±42	10±2.7	8.8±3.9	7.6±1.2
Toluene/Hexane	40±17	40±18	40±15	17±13	19±11	19±18
Hexane/Acetone	32±16	32±13	29±13			

T4 : 3,3',4,4'-TetraChloroBiphenyl P5 : 3,3',4,4',5-PentaChloroBiphenyl
H6 : 3,3',4,4',5,5'-HexaChloroBiphenyl

An advantage to use vial having hydrophobic surface

An interesting result that Co-PCBs from the organic material by hydrolysis was concentrated on vial surface was provided. Because reaction vial was made by PFA, the surface was hydrophobic. So, the reaction vial had ability that can keep on Co-PCB at its surface, and separate from other matrix. According to the result that did fixed-quantity of liquid phase and reaction vial separately, Co-PCBs from biological materials of 55% was measured from the hydrophobic surface. As for Co-PCBs added to internal standard, it was absorbed 90% on the hydrophobic surface. It may become

a problem that there is a big difference between two ratios, but wants to utilize this interesting property more.

We show the comparative result for the developed method and "Soxhlet-extraction alkali-decomposition" method (Figure 1). There was equilateral correlation between two methods, and the developed method had high response. The purpose of shorten analytical time is achieved to 1/10 by using this method. And we can protect the sample from contamination, because that decomposition and extraction was done under sealing.

This method is very useful as pre-treatment method for biological materials, but has to examine more.

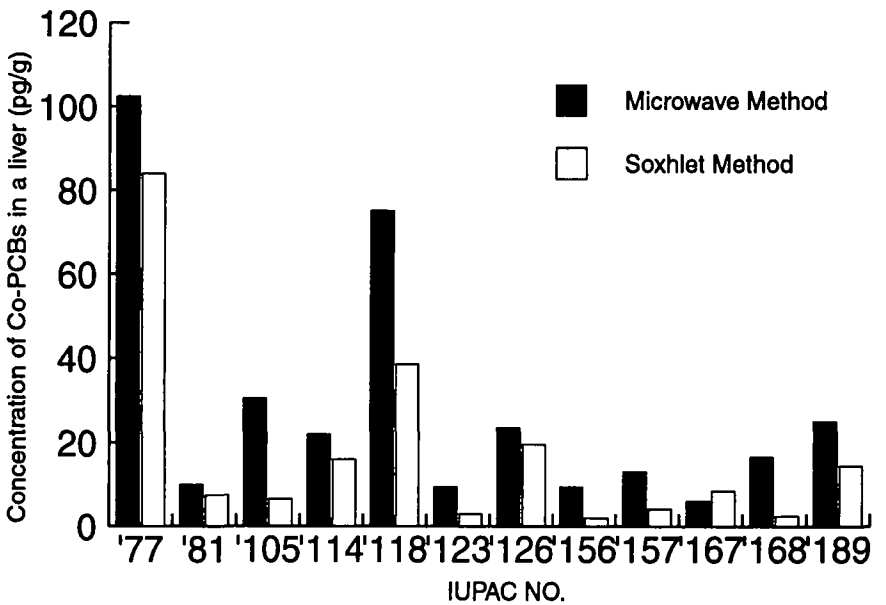

Figure 1. Relation to the Microwave decomosition Method and the Soxhlet alkali decomposed Method for Co-PCBs concentrtion in a pig liver.

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