IMPROVEMENT OF BROAD RANGE (BR) PCB ELISA FOR DETERMINATION OF PCBS IN INSULATING OIL

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Introduction

Insulating oils contaminated with low-level PCBs have been great concerns, because the millions of transformers, whose insulating oils are suspected to be unintentionally contaminated with PCBs, are stockpiled or still in use in Japan. The government notified that the insulating oils over 0.5 mg/kg of PCBs be considered as PCB wastes and be completely disposed within 22 years according to the POPs treaty which went into effect in 2004[1]. For determination of PCBs in insulating oil, instrumental analysis such as high-resolution gas chromatography mass spectrometry (GC-MS) and GC with an electron capture detector (GC-ECD) are generally employed[2]. These analytical methods are highly reliable. However, they have several potential drawbacks including expensive instrumentation, large sample volume, extensive purification and technical expertise in operation. Due to these shortcomings, the analysis of a large number of samples may be both cost and time prohibitive. Therefore, there is a strong need for rapid, simple, and cost-effective methods for quantitative analysis of PCBs in insulating oils, such as enzyme-linked immunosorbent assay (ELISA). We had already reported in Dioxin 2005[3] the PCB ELISA which reacted fairly equally to Kanechlor 300/400/500, and pretreatment method for determination PCBs in insulating oil. In this study, we improved the sensitivity of this ELISA as well as the simplicity of pretreatment method to obtain better accuracy and precision results on determination of around 1 mg/kg levels of PCB oil samples.

Materials and Methods

Pretreatment of insulating oil for ELISA analysis

The oil sample was added to hexane and distributed with dimethyl sulfoxide (DMSO). After a wash with hexane, the DMSO phase was diluted with aqueous solution, and then PCB was extracted with hexane. The hexane phase containing PCBs was then sulfonated and transferred to DMSO solution to analyze with ELISA.

Immunoassay Procedure

Standard (Kanechlor 400: KC-400) or pretreated PCB samples were dissolved in 100% DMSO. The DMSO sample (80 uL), distilled water (120 uL), HRP-labeled PCB (250 uL) and anti-PCB antibody coupled with magnetic particles (500 uL) were sequentially added to polystyrene tubes or disposable glass, and incubated for 30 min at room temperature. After the magnetic particles were trapped with the magnetic separator, the unbound reagents were discarded, and then the washing solution (1mL) was added to the assay tubes. After the repetition of this washing step, the color solution (500 uL) was added and incubated for 20 min at room temperature. The reaction was terminated by adding a stop solution (500 uL) and the absorbance was measured at 450 nm. The PCB concentration was calculated with commercially available software (Delta Soft®) using 4-paramter fitting regression. The PCB concentration in oil sample was calculated with the following formula:

PCB concentration in oil sample (mg/kg) = ELISA mean value (ng/mL) X dilution factor* / ELISA conversion coefficient**) /1000

* dilution factor: dilution magnification from oil sample to ELISA assay sample through the pretreatment

** ELISA conversion coefficient: The averaged ratio of ELISA value to GC-ECD values, which were determined by analyzing 20 real oils samples (data not shown). The ratio of less than 1 (around 0.5) indicated less recovery rate of pretreatment for ELISA (around 70 to 80%) and negative matrix effect on ELISA analysis (around 70 %).

Results and Discussion

Pretreatment methods

Table 1 Comparison the former and refined pretreatment methods

	Former method	Refined method
Sampling volume(μL)	50	250
hexane / DMSO distribution	once	once
wash with hexane	twice	once
hexane extraction	once	once
dehydration	once	none
sulfonation	once	once
wash with water	once	none
Dilution factor of ELISA assay sample against the oil sample	150	100
ELISA conversion coefficient	0.47	0.51

As shown in Table 1, the procedure of a refined method was simpler than that of a former one due to the reduction of hexane-washing step and omission of dehydration and water washing steps. The sampling volume of a refined method increased 5 times larger than that of a former one but the resulting dilution factor did not reduce accordingly. The solvent volume during simplified pretreatment steps was increased to have led a final

dilution factor to be 100 instead of a theoretical 30. However, the increase of sampling volume would give higher precision due to the decrease of the sample measurement error. Owing to these improvements, the time consumption for 40 oil samples pretreatment was shortened to roughly 1hour from 4-5 hours.

Lowest quantification limit (LQL)

The standard curves of Kanechlor (KC)-400 using polystyrene tubes (former method) and glass tubes (refined method) are shown in figure 1. The lowest quantification limit (LQL) defined as B/B0=80%, was lowered from 7 ng/mL to 2 ng/mL in 100% DMSO by replacing the assay tube material from polystyrene to glass. It seemed that some non-specific adsorption of PCB was occurred in a polystyrene tube, and that adsorption was alleviated using a glass tube.

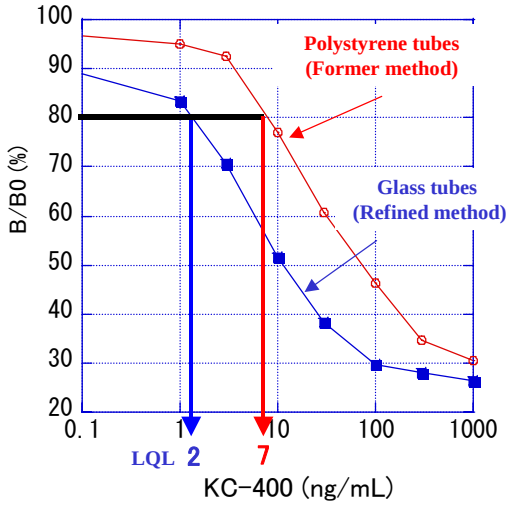

Figure 1. Standard curves of KC-400 (comparison the assay tubes)

Comparison between GC-ECD and ELISA values

PCB concentrations of twenty insulating oils were determined with a former and a refined ELISA method, respectively, as well as with GC-ECD, according to Notification No.192 (1992) of the Ministry of Health and Welfare of Japan. As shown in Table 2, the PCB values of both methods of ELISA were well correlated to those of GC-ECD both in lower and higher concentration. A refined method ELISA was more approximated to GC-ECD values, indicating ELISA / GC ratio to be 1.01 on average (SD: 0.15, max: 1.3 and min: 0.75), while a former method showing ELISA / GC ration to be 1.15 on average (SD: 0.36, max: 2.0 and min: 0.71).

As shown in figure 2, however, on the analysis of less than 5 mg/kg of PCB oil samples, the refined method ELISA showed more accurate values whose the ELISA / GC ratio ranging from 0.89 to 1.1 while the former method's ranging from 0.86 to 2.0. These data indicated that a refined method gave better accuracy than a former one, especially on the analysis of low concentration PCB samples (around 1 mg/kg), because the

ELISA sensitivity of the refined method was increased about 3 times higher than that of former one. Furthermore, the simplification in the refined method contributed to not only rapidness and cost-effectiveness but also preciseness of ELISA determination, because simplification in operational steps decreased the human error. Further improvement of pretreatment method is in progress to achieve the lowest quantification limit of 0.5 mg/kg PCB in oil samples.

Table 2 Comparison of ELISA and GC-ECD in determination of PCBs in insulating oils

SampleNo.	GC-ECD	Former method		Refined method	
		ELISA	ELISA /	ELISA	ELISA /
	mg/kg	mg/kg	GC	mg/kg	GC
1	0.0	0.0	–	0.0	–
2	0.8	0.90	1.1	0.82	1.0
3	0.8	1.6	2.0	0.71	0.89
4	0.9	1.3	1.4	0.99	1.1
5	1.0	1.1	1.1	0.93	0.93
6	1.3	2.1	1.6	1.2	0.91
7	1.6	2.1	1.3	1.6	1.0
8	2.1	3.5	1.7	2.6	1.2
9	2.2	3.3	1.5	2.1	0.95
10	2.6	2.2	0.86	2.9	1.1
11	4.1	4.3	1.0	3.9	0.95
12	5.1	6.3	1.2	6.4	1.3
13	7.0	6.4	0.91	6.4	0.91
14	7.5	7.0	0.93	7.2	0.96
15	15	12	0.83	17	1.1
16	18	16	0.87	24	1.3
17	21	15	0.71	16	0.79
18	21	17	0.78	18	0.87
19	28	22	0.81	21	0.75
20	31	31	0.99	33	1.1
		average	1.15	average	1.01
		SD	0.36	SD	0.15

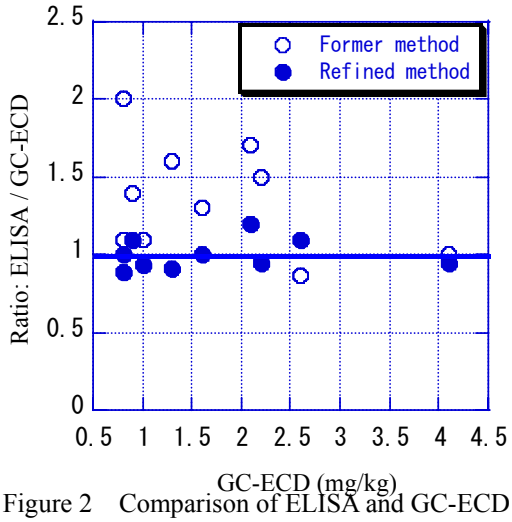

Figure 2 Comparison of ELISA and GC-ECD (PCB conc. were less than 5 mg/kg)

Acknowledgement

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