DEVELOPMENT OF IMMUNOCHROMATOGRAPHIC TEST FOR SCREENING OF POLYCHLORINATED BIPHENYLS IN INSULATING OIL

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Introduction

Polychlorinated biphenyls (PCBs) are one of the major concerns in environmental contaminants because of their toxicity to human and wildlife, and low rate of degradation in the environment. Recently, there is a considerable concern regarding the occurrence of low level of PCB contaminations in insulating oils and transformer oils in Japan. To confirm the existence of PCB contamination in suspected oils immediately, simple and cost-effective analytical techniques as an alternative to a conventional instrumental analysis are highly desired.

One possible alternative method is an immunochemical techniques based on poly- or monoclonal antibodies. Especially, an immunochromatographic method is very simple, low-cost and needs no special equipments and skills. We recently developed an immunochromatographic assay for PCB quantification using a monoclonal antibody specific to 2,3',4,4',5-pentachlorobiphenyl (PCB 118)[1,2,3]. The objective of this study is to develop an on-site and kit-based screening method for the PCB-contaminated oils. In this paper, we report a performance of a newly developed handy clean-up method and the immunochromatographic test to determine PCBs in insulating oils.

Methods and Materials

All chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). A PCB-free insulating oil was purchased from Matsumura Oil (Osaka, Japan). Plastic centrifuge tubes, plastic syringes, glass vials, silicon tubes and other disposable apparatus were purchased from Fisher, Terumo (Tokyo, Japan), Maruemu (Osaka, Japan) or As One (Osaka, Japan).

Sample clean-up

For on-site use, the entire clean-up procedures were developed to be constituted with disposable apparatus and equipments which required no power supply.

Oil samples (10 mL) were added to 10 mL of dimethyl sulphoxide (DMSO) and vigorously agitated by hand for more than 10 seconds. After standing for more than 2 minutes, the lower DMSO phase was added to a mixture of distilled water (5 mL) and *n*-hexane (2.5 mL) in a glass vial. After vigorous shaking and phase separation, the upper *n*-hexane phase was passed though a silica gel-sulfuric acid (44%) column [silica gel (1 g), 44% sulfuric acid-impregnated silica gel (10

g) and silica gel (1 g)] and eluted with *n*-hexane. In the elution step, the first fraction (4 mL) was discarded and then the next eluate (4 mL) was added to 0.5 mL of DMSO in a glass vial. After vigorous shaking and phase separation, the lower DMSO phase was used for an assay.

Immunochromatographic test

An assay principle and a construction of a test device of the developed immunochromatographic method were described previously[3]. The assay was performed according to a previously described protocol with a little modification. Briefly, a gold colloid labeled anti-PCB antibody which was dried in a glass vial was suspended with 100 µL of tris buffered saline (TBS), followed by an addition of 10 µL of pretreated oil samples. A 75 µL of gently agitated mixture was applied on an immunochromatographic test device and moved forward on a nitrocellulose membrane by a capillary action. After keeping for 20 minutes, a color intensity of two lines on the membrane was measured by an immunoreader (Otsuka electronics Co., Ltd., Osaka, Japan). The two lines, designated a trap line and a test line, were immobilized by a hapten-BSA conjugate and an anti-mouse IgG antibody, respectively. When no PCBs existed in samples, the entire anti-PCB antibody was trapped by the hapten-BSA conjugate, which resulted in an appearance of a red color on the trap line. When samples included PCBs, a complex of PCBs and the anti-PCB antibody passed through the trap line and then reached to the test line. A ratio of color intensity of the test line to that of the trap line increased in proportion to a concentration of PCBs in the samples. The immunoreader was programmed so as to display a judgment value as the ratio calculated by a following equation: judgment value (%) = (test line color intensity / trap line color intensity) x 100. For determination, the judgment value for a blank sample (DMSO) was subtracted from each value for tested oil samples.

Results and Discussion

Clean-up method

The whole clean-up procedures could be conducted within 30 minutes and required no special equipments. Moreover, a liquid waste for each analysis was as low as 30 mL, which was much less than that for a conventional method for an instrumental analysis. Thus, the clean-up method would be acceptable to on-site testing.

Assay performance of immunochromatographic test

The performance of the immuno-chromatographic assay was determined with PCB-free insulating oil spiked with various concentrations of Kanechlor (KC) -300, 400 and 500

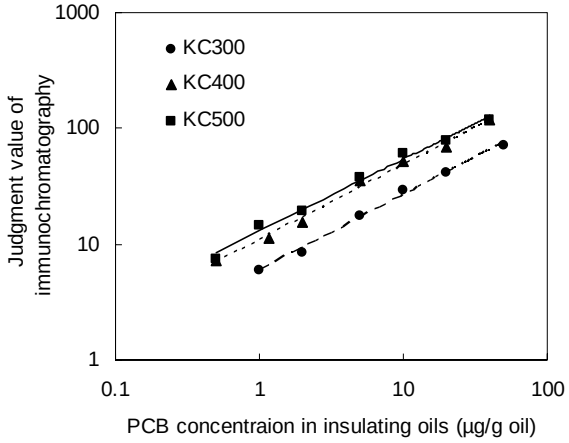

Figure 1. Dose-response curves for each Kanechlor in insulating oils

(KC-300: 1, 2, 5, 10, 20, 50 µg/g oil, KC-400 and KC-500: 0.5, 1, 2, 5, 10, 20, 40 µg/g, n=8). Dose-response curves for each KC in the oil were fitted by a linear regression after log-log transformation (Figure 1, Table 2). The limit of detection (LOD) of the assay for oil samples was defined as the PCB concentration corresponding to two times of standard deviation about the mean judgment value of the PCB-free oil (n=8). As shown in table 2, the assay was most sensitive to KC-500 and showed reduced sensitivity with decreasing degree of chlorination. This result seems to be reasonable if taking a reactivity of the antibody into consideration. The antibody shows cross-reactivity against seven PCB congeners out of 27 predominant congeners contained in Kanechlor as follows: PCB #28, 3.5; #31, 12.9; #33, 2.6; #66, 15.2; #70, 14.9; #105, 2.5; #110, 0.88 (in comparison with PCB #118 as 100). The composition percent of PCB #118 and the cross reactants of the antibody is higher in the order of KC-500, KC-400 and KC-300[4], which resulted in sensitivity difference of the assay among those three KCs.

Table 2. The regression equation and the sensitivity of the immunochromatographic assay for Kanechlor in insulating oils

Kanechlor	Regression equation ($\log y = a + b \times \log x$)	LOD (µg/g oil)
300	a: 0.77, b: 0.65, r^2=0.996	2.0
400	a: 1.03, b: 0.65, r^2=0.993	0.8
500	a: 1.12, b: 0.62, r^2=0.990	0.6

In order to evaluate an inter-assay precision of the immunochromatographic test, the assay was performed with pretreated oil samples on four separate days. Figure 2 shows a precision profile of insulating oil spiked with a range of concentrations of KC-500. The coefficient of variation (% CV) for each PCB concentration was within 30% although relatively higher CV was observed at lower limit of the assay range.

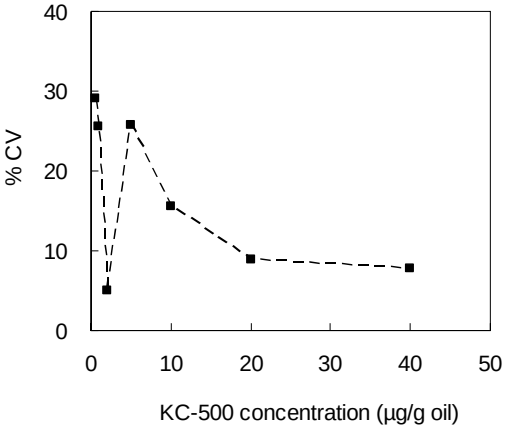

Figure 2. Inter-assay precision profile

Analysis of real oil samples

A comparison between the immunochromatographic test and GC-ECD was conducted for ten used insulating oil samples. The clean-up and assay procedures were repeated 3 times for each oil sample. The judgment value for each oil sample was converted into PCB concentration using the regression equation of KC-500 in Table 2. The converted values, so-called KC-500 equivalents, were similar in trend to GC-ECD results (Table 3). As shown in Figure 3, a good correlation was observed between immunochromatographic test and GC-ECD results, which indicates that the immunochromatographic assay would offer a practical method for predicting PCB contamination levels in insulating oils. The slope of 0.4943 from the regression equation in Figure 2 indicated that

Table 3. Immunochromatographic test results in real oil samples

Sample	KC-500 equivalents (μg/g) (mean±SD, n=3)	GC-ECD (μg/g)
A	7.2±1.5	10.4
B	13.2±1.7	20.2
C	0.7±0.3	1.1
D	3.7±0.8	5.0
E	7.6±3.5	15
F	0.7±0.6	1.2
G	7.4±2.5	26.1
H	16.7±4.1	30
I	2.3±1.2	3.4
J	0.2±0.1	0.8

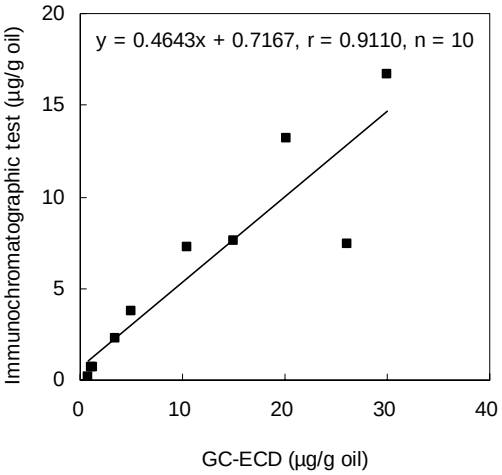

Figure 3. Correlation of immunochromatography and GC-ECD results for real insulating oil samples

the immunochromatographic test results were underestimated compared with GC-ECD results. A possible explanation is that a source of PCB contamination, namely a composition of PCB congeners in tested real oil samples was different from that in KC-500, which was used for the conversion of the immunochromatographic judgment values into the PCB concentrations (KC-500 equivalents). For example, for the oil sample G in which KC-500 equivalent was 7.4 μg/g, the equivalent became closer to GC-ECD results by using the regression equation of KC-300 in Table 2 (i.e., KC-300 equivalent was 22.4 μg/g whereas GC-ECD result was 26.1 μg/g). For practical use of the developed assay, it is reasonable to describe a measured PCB concentration as a value with some range corresponding to the most and the least sensitive KC standard.

In conclusion, combination of the newly developed clean-up method and the immunochromatographic assay had a good performance for semi-quantitative analysis of PCB-contaminated insulating oils. The assay would be acceptable to on-site testing in view of simplicity of the assay procedures, total assay time within 60 minutes and no requirements of special equipments and a power supply.

Acknowledgements

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