A SIMPLIFIED MEASURING METHOD OF PCBs IN TRANSFORMER OIL.

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Abstract
Our recent development simplified the method to measure the levels of PCB contained in transformer oils. The measurement results by our method were well correlated to those by the conventional HRMS and LRMS methods. Our method is effectively applicable to PCB screening at a regulatory PCB level of as low as 0.5 ppm (mg PCB/kg oil).

Introduction

Since the reveal of unexpected contamination of insulating oils used in PCB-free transformers with low-content PCBs, effective measures have highly been demanded to treat the possibly contaminated transformers. The current official procedure of PCB measurement is the conventional HRGC-HRMS (high-resolution gas chromatography, high-resolution mass spectrometry) method. This conventional method, however, requires troublesome pretreatment (sulfate treatment) and takes time and cost. The current official procedure is accordingly unsuitable and unsatisfactory for inspection of an extremely large amount of specimens as over 3 million transformers. Screening the transformer oils with the high potential of contamination with PCBs is essential to save the total treatment cost. An inexpensive simplified assay is required for this purpose. All the existing simplified assays have drawbacks: some having only the insufficient sensitivity, some requiring the special skills for analytical instrumentation, and some using toxic and harmful reagents for assay. Any of these measuring methods is thus not comparable to the official HRGC-HRMS method. In this project, we modified the silver nitrate – silica gel column, which had been proven to be effective for dioxin analyses, and used the modified silver nitrate – impregnated silica gel column for pretreatment, in place of the troublesome sulfate treatment. We also succeeded in enhancing the sensitivity of the simplified immuno-chromatographic system to more than 10 times and combined the enhanced simple immuno-chromatography with a scanner for accurate quantification of output signals. Our simplified immunoassay has overcome all the three challenges 'safety', 'simplicity', and 'low cost'.
Our simplified measuring method of PCBs was verified to have the required screening performances through the common verification tests by the immunoassay working group in measurement of PCB concentrations in transformer oils.

Materials and Methods

Silver Nitrate – impregnated Silica Gel Column

A silver nitrate – impregnated silica gel column was designed to have no possibility of exposure and was prepared by sealing 1.2 g of 10% silver nitrate – impregnated silica gel in a polypropylene housing. Eluted fractions of PCBs were analyzed according to the protocol given below.

Measuring Method

The following protocol was adopted to measure PCBs contained in transformer oils.
The protocol first weighed 0.2 g of each transformer oil sample in a microtube, added 0.5 mL of DMSO to the microtube, and vigorously shook the microtube for 15 seconds. After 30-second centrifugation, 0.4 mL of a lower DMSO phase was collected and was added to a mixture of saline and n-hexane (0.2 mL / 0.5 mL) in a microtube. After 15-second vigorous shaking and subsequent 30-second centrifugation, 0.4 mL of an upper

n-hexane phase was collected and was added dropwise to the 10% silver nitrate – impregnated silica gel column. The protocol wasted a first elution from the column by addition of a first 2 mL portion of n-hexane and fully recovered a next elution from the column into a glass vial by addition of a subsequent 6 mL portion of n-hexane. After addition of 0.15 mL of DMSO to the elution, the glass vial was heated on a hot plate at a temperature of 65°C. The hexane content was vaporized in approximately 1 hour.

The protocol mixed 2 μL of the DMSO residue with 98 μL of an Alkaline Phosphatase(AP)-labeled anti-PCB antibody solution at a mixing ratio of 1:50. The mixed solution was stood still for about 30 minutes and was added dropwise to an immuno-chromatographic system. The AP-labeled anti-PCB antibody unreacted with PCB was combined with a simulating antigen at a point A, while the AP-labeled anti-PCB antibody reacted with PCB was combined with an anti-mouse antibody at a point B. After elapse of 20 minutes, dropwise addition of 75 μL of a BCIP/NBT mixture developed chromogenic reactions corresponding to the respective binding amounts at the point A and at the point B.

After elapse of 15 minutes, the chromogenic intensities at the respective points A and B were read from the immuno-chromatography by a specific scanner, and the PCB concentration was estimated from the ratio A/B of the measured chromogenic intensities.

This protocol efficiently enables simultaneous treatment of 24 samples. A known marker may be adopted for 1 or 2 samples among the simultaneously treated 24 samples for the purpose of correction of a temperature-based variation or an operator-oriented variation.

Results and Discussion

The silver nitrate – impregnated silica gel column was evaluated.

Each eluted fraction from the silver nitrate – impregnated silica gel column was analyzed. Fig. 1 shows the analysis of sequential 1 mL fractions eluted with hexane from the silver nitrate – impregnated silica gel column after extraction of several Kanechroles at a fixed concentration of 2 ppm. According to the fraction distribution of Fig. 1, collection of the 3rd to the 8th eluted fractions attains 95% or higher recovery. The requirement or non-requirement for recovery of the 2nd eluted fraction was examined with a PCB sample having a known concentration of 0.7 ppm. Table 1 shows comparison between the measurement results (n =3) of the recovery of the 2nd to the 8th fractions and the recovery of the 3rd to the 8th fractions. Addition of the 2nd fraction caused reaction interference by approximately 20%. The 3rd to the 8th fractions were thus used for the measurement.

2～8mL	0.58ppm
3～8mL	0.71ppm

Table 1 Comparison between determination results of two fraction ranges

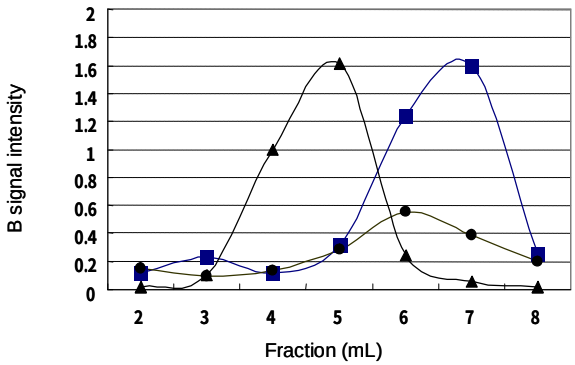

Fig.1
Eluted fractions from silver nitrate – silica gel column
▲ : KC500 ● : KC400 ■ : KC300

The purification performance of the silver nitrate – impregnated silica gel column was evaluated with used transformer oils.

Seven transformer oils stored after collection from transformers were provided as measurement samples. The PCB concentrations of these transformer oils were determined to be less than the detection limit by the conventional HRGC-HRMS measurement. PCB was added to the seven transformer oil samples to a fixed final concentration of 0. 4 ppm, and the respective samples were pre-treated and analyzed. As shown in Fig. 2, the analysis gave the determination results of the respective oil samples approximate to the known concentration of 0.4 ppm.

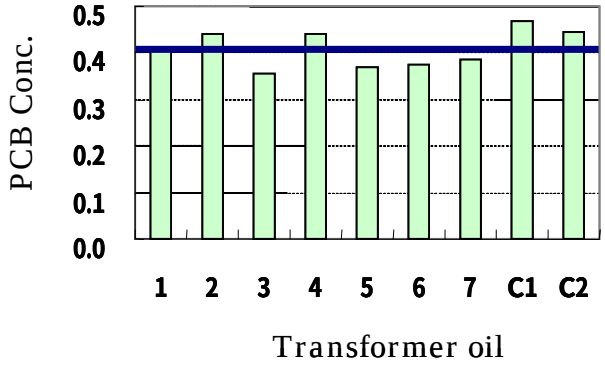

Fig.2 Determination of PCB level in transformer oils

Table 2 shows the pretreatment recovery rate.
The repeated liquid-liquid extraction lowered the recovery rate to the 30% level, but the coefficient of variation Cv was approximately 5%. This proves the sufficient level of determination reproducibility.

Pretreatment recovery rate	30.6%
Coefficient of variation（Cv）	4.6%

Table.2 Pretreatment recovery rate and Coefficient of variation

The cross reactivity was measured for evaluation of the performance of the PCB antibody. Table 3 shows the measured cross reactivities to various Kanechlors. The cross reactivity was slightly low for Kanechlor KC600 but was in an agreeable range of ±20% for Kanechlors KC300, KC400, and KC500.

	Cross Reactivity (IC50)
KC300	85% (0.477)
KC400	100% (0.406)
KC500	65% (0.623)
KC600	49% (1.183)

Table.3 Cross reactivities to various Kanechlors

For evaluation of an immunoassay system with immuno-chromatographic chips based on the enzyme chromogenic reaction, the detection limit and the effective determination range were estimated from the Cv value. Fig. 3 shows the detection limit and the effective determination range as the result of an N =5 test. The detection limit was 0.06 ppm, and the effective determination range was 0.12 ppm to 4 ppm. These results prove the sufficient stability of the immunoassay system.

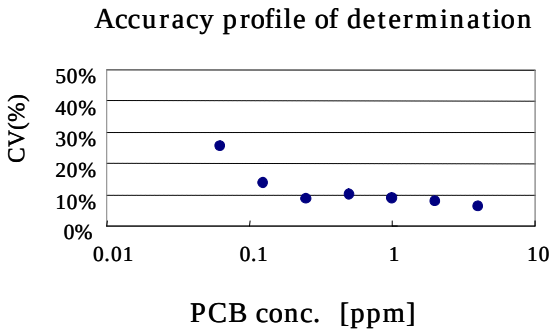

Fig.3 Cv-based effective determination range

Real samples simulating the actual PCB concentration levels were immunoassayed by the system having the above basic functions. Fig. 4 shows the correlation of the measurement results with this immunoassay system to the results of instrumental analysis.

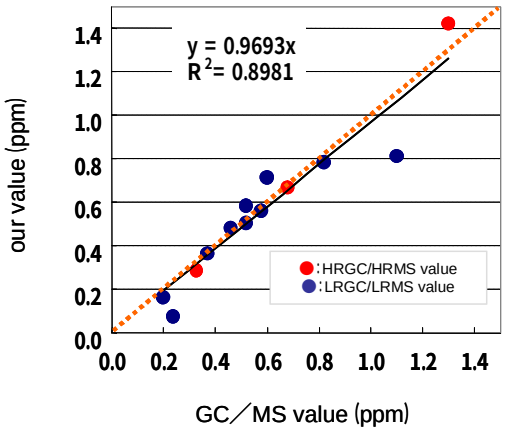

Fig.4 Correlation of measurement results with our immunoassay system to results of instrumental analysis
Abscissa: Measurement results by LRGC-LRMS and HRGC-HRMS methods
Ordinate: Measurement results by our method

Conclusions

Application of the silver nitrate – impregnated silica gel, in place of conventional sulfate treatment, to the solid phase safely and effectively eliminated the impurities to a sufficient level from transformer oils. The combined enzyme amplification effectively enhanced the detection limit of the immuno-chromatographic system from the ppm level to be lower than 0.1 ppm without changing the operability. These results verify the sufficient performance of our measuring method as complementary to the official PCB measurement method.
Our immunoassay system is effectively usable to determine the PCB concentration of as low as 0.5 ppm in transformer oils.