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# Headspace solid-phase micro-extraction and gas chromatography with micro-electron capture detection for the measurement of p,p'-DDE in rat whole blood and hair

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**A sensitive, specific, and reproducible capillary gas chromatography (GC) with micro-electron capture detection (μ-ECD) method using headspace solid-phase micro-extraction (HS-SPME) for the quantitative analysis of p,p'-DDE in rat whole blood and hair was developed. A 100 μm polydimethylsiloxane (PDMS) phase was used for the extraction. The obtained detection limits were 0.003 ng/mg and 0.004 ng/mg in whole blood and hair, respectively, at a signal-to-noise ratio of 3 (S/N = 3). Linearity was obtained in the ranges 0.003 - 2.0 ng/mg and 0.004 - 2.0 ng/mg in whole blood and hair, respectively, and the correlation coefficients of whole blood and hair were greater than 0.998. This method was successfully applied to the analysis of p,p'-DDE in rat whole blood and rat hair after an oral dose of p,p'-DDE.**

**Key words : p,p'-DDE, Whole blood, Hair, Gas chromatography with micro-electron capture detection, Headspace solid-phase micro-extraction**

## INTRODUCTION

DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) (Fig. 1a) was first synthesized in Germany in 1874. Although DDT was used for many years against Anopheles mosquitoes in malaria control programs in various countries, it met with varying degrees of success and eventually was found to be carcinogenic. Thereafter, alternative insecticide types, such as carbamates and/or organophosphates, have been introduced against vector mosquitoes because these types were safer to the public health than DDT. However, DDT is still widely used against malaria vector mosquitoes in many countries [1].

The chemical stability of p,p'-DDE (1,1-bis(4-chlorophenyl)-2,2-dichloroethene) (Fig. 1b), a metabolite of DDT, is similar to that of DDT. Moreover, p,p'-DDE has been reported to be a potent androgen receptor antagonist [2] and has been associated with breast cancer [3].

Polychlorinated biphenyls (PCBs), DDT, and p,p'-DDE are mainly regarded as ubiquitous environmental pollutants, especially in wild fish products [4] and in the atmosphere [5]. Moreover, PCBs accumulate readily in the human body because of their chemical stability and lipophilicity [6, 7].

Several analytical methods to measure PCBs, including DDT and p,p'-DDE, in biological samples (blood, adipose tissue, liver, and breast milk) have been published [8-12]. Usually, either of two different approaches has been used to measure these types of compounds. GC with electron capture detection (ECD) seems to be the method of choice for detecting organic chlorine compounds in biological samples. Although GC-ECD is supersensitive to halogen, the linearity range of this method is narrow. Sensitivity was enhanced by using a negative-ion chemical ionization (NCI) mode of GC-mass spectrometry (GC-MS) [13]. However, GC-MS-NCI is

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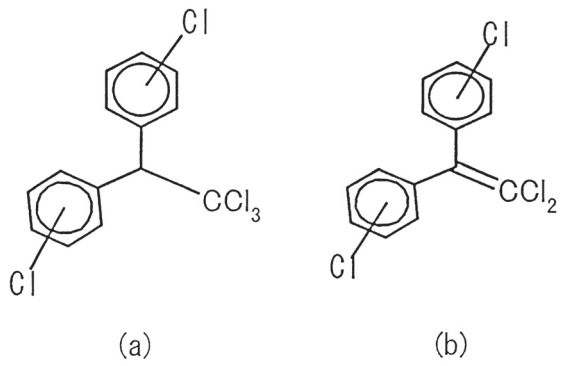


**Fig. 1** Chemical structures of DDT (a) and p,p'-DDE (b).

demanding and cannot be applied in routine analysis.

Hair analysis has proved to be a reliable way to retrospectively detect chronic drug abuse, mainly in clinical and forensic toxicology [14]. So far, only two analytical studies of organic chlorine compounds in hair have been published; one used GC-ECD [15] and the other GC-MS [16]. Knowledge of methods to analyze organic chlorine compounds in hair would be useful for retrospective screening procedures and for environmental risk assessment. To our knowledge, the HS-SPME method has yet to be applied to the measurement of p,p'-DDE in hair samples. Moreover, conventional ECD was miniaturized into $\mu$-ECD, and as a result the linearity range of detection was extended. Therefore, we have developed a method that combines HS-SPME and GC-$\mu$-ECD to measure p,p'-DDE in rat whole blood and hair. This method was used to test rat whole blood and hair for concentrations of p,p'-DDE after administration of an oral dose of p,p'-DDE.

## EXPERIMENTAL

### Materials

p,p'-DDE was obtained from Riedel-de Haen (Seelze, Germany). The SPME device, which was coated with 100 $\mu$m polydimethylsiloxane fiber, was purchased from Supelco Inc. (Bellefonte, PA, USA). Other chemicals used were of analytical grade.

### Blank whole blood and hair Samples

Male dark Agouti rats (Nihon SLC, Hamamatsu, Japan), weighing 220-260 g, were used. Blank whole blood samples were collected after heparin as an anticoagulant. The blank whole blood was stored at approximately − 20°C until use.

The back hair of the rats was shaved with an animal electric shaver before p,p'-DDE administration. These hair samples were used to determine the optimal conditions for HS-SPME. The hair samples were soaked in 0.1 % sodium dodecyl sulfate for 1 min and then washed twice with Milli-Q water for 1 min. The washed hairs were blotted between two sheets of filter paper and allowed to dry at room temperature. All experimental procedures using the animals were reviewed and approved by the Ethical Committee (Animal Experimental Committee) of Tokai University School of Medicine.

### Gas chromatography conditions

GC-$\mu$-ECD analyses were performed on a gas chromatograph model 6890 with $\mu$-ECD (Hewlett-Packard, Palo Alto, CA, USA). The GC column was a J&W Scientific DB-1 capillary column (30 m × 0.25 mm i.d., 0.25 $\mu$m film thickness) (Folsom, CA, USA). The column oven temperature was programmed to rise at 20°C/min from an initial temperature of 150°C to 300°C, where it was maintained for the final 5.5 min. The column pressure was 14.89 psi. Splitless injection was used, with a split valve off-time of 3.0 min. The temperatures of the injector port and detector were 250°C and 280°C, respectively.

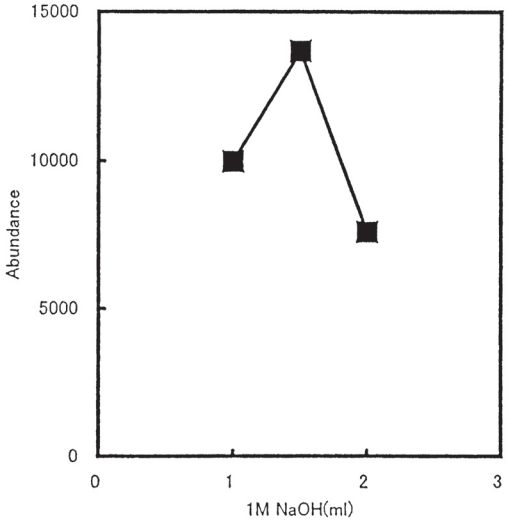


**Fig. 2** Effect of 1N NaOH volume on extraction rates in HS-SPME. Each extraction was performed for 15 min at 100°C from a 50 mg rat hair sample spiked with p,p'-DDE at 2.0 ng/mg. Pre-heating time = 0 min.

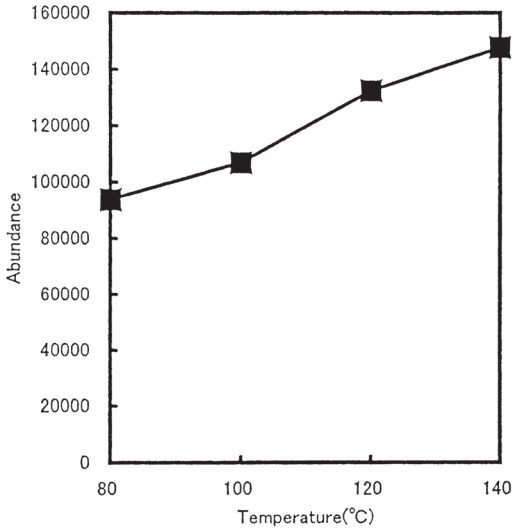


**Fig. 3** Effect of tempertature on extraction rates in HS-SPME. Each extraction was performed for 15 min from a 50 mg rat hair sample spiked with p,p'-DDE at 2 ng/mg and 1.5 ml 1N NaOH. Pre-heating time = 0 min, adsorption time = 15 min.

### HS-SPME procedure

In a 7.5 ml glass vial, an aliquot containing a 50 mg sample (whole blood or hair) was added to 1N NaOH and p,p'-DDE at 2.0 ng/mg. The vial was immediately sealed with a silicon septum and a screw cap, and was heated in an aluminum block heater (Dry Heat Bath EB-303, Iuchi, Osaka, Japan). The needle of the SPME device containing the extraction fiber was inserted through the septum of the vial. The fiber was pushed out through the needle and exposed to the headspace of the vial for 15 min. After adsorption of p,p'-DDE, the fiber was drawn into the needle, removed from the vial, and immediately inserted into the GC injection port. The fiber remained in the port for 3 min to allow desorption of the analyte into the GC column.

### HS-SPME optimization

To optimize conditions for HS-SPME, the effects of 1N NaOH volume, heating temperature, preheating time, and exposure time were determined using the following procedures. Three volumes of 1N NaOH (1.0, 1.5, and 2.0 ml) were added to different vials, which were heated to four different temperatures (80, 100, 120, and 140°C) under five different preheating times (0, 5, 10, 15, and 30 min) and four different exposure times (5, 10, 15, and 20 min).

## RESULTS AND DISCUSSION

### SPME optimization

#### Sodium hydroxide solution volume, heating temperature, preheating time, and exposure time conditions

Figure 2 shows the effects of 1N NaOH volume on extraction of p,p'-DDE. 1.0 ml 1N NaOH are necessary at least in order to dissolve 50 mg hair sample. The amount of adsorbed p,p'-DDE was highest when 1.5 ml of 1N NaOH was used. According to our experiences, bimodal curve was not shown 1N NaOH volume and abundance. Therefore, it is considered that 1.5 ml is an optimum volume of 1N NaOH. Figure 3 shows the effects of heating temperature on the extraction of p,p'-DDE; extraction efficiency increased as the temperature increased, so we adopted 140°C as the heating temperature. However, abundance is not achieved equilibrious at 140°C. We could not examine over 140°C, because the thermostable temperature of the vial is to 140°C. Figure 4 shows the effects of preheating time on extraction of p,p'-DDE.

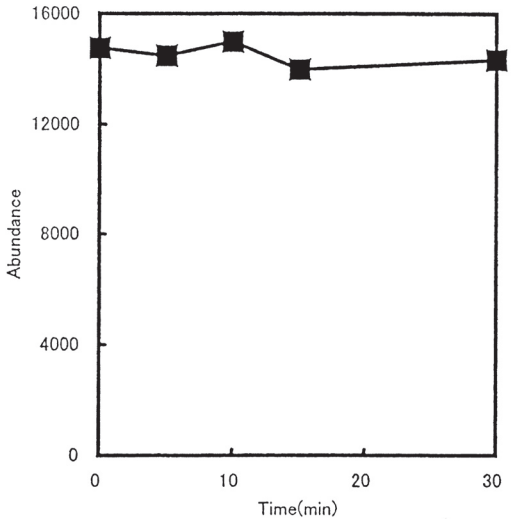


**Fig. 4** The effect of pre-heating time on extraction rates in HS-SPME. Each extraction was performed for 15 min at 140°C from a 50 mg rat hair sample spiked with p,p'-DDE at 2.0 ng/mg and 1.5 ml 1N NaOH.

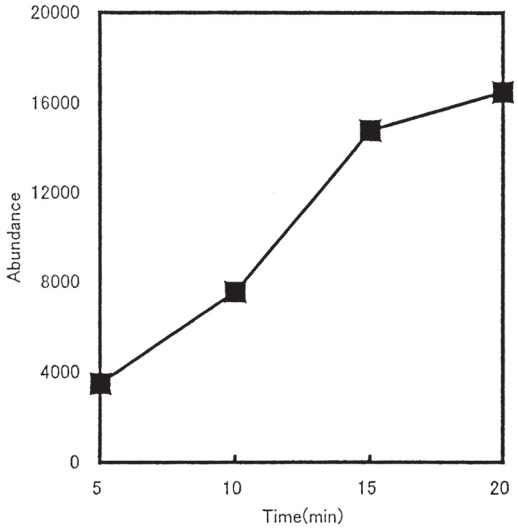


**Fig. 5** The effect of exposure time on extraction rates in HS-SPME. Each extraction was performed at 140°C from a 50 mg rat hair sample spiked with p,p'-DDE at 2.0 ng/mg and 1.5 ml 1N NaOH. Pre-heating time = 0 min.

**Table 1** Linear regression data, recovery range and detection limit for p,p'-DDE

| Sample | Concentration range (ng/mg) | Regression line | Correlation coefficient (r) | Recovery range (%) | Detection limit (ng/mg) |
|---|---|---|---|---|---|
| Whole blood | 0.003-2.0 | y = 294685x − 8540 | 0.998 | 4.5-9.5 | 0.003 |
| Hair | 0.004-2.0 | y = 156715x − 1455 | 0.998 | 2.4-7.2 | 0.004 |

It is reported that preheating is effective for some drugs [17, 18]. However, preheating did not affect the absorbed amount of p,p'-DDE, so we adopted a preheating time of 0 min. Figure 5 shows the effects of exposure time on extraction of p,p'-DDE. The adsorbed amount of p,p'-DDE was highest at 20 min and thereafter stayed at equilibrium. However, the mean C.V. values were 6.9 % and 9.8 % with 15 min and 20 min, respectively. The vapour phase, liquid phase and fiber phase of p,p'-DDE are in equilibrium in head space vial at 15 min, because C.V. was a minimum value. Thus, the equilibrium between these phases is destroyed after 20 min. Therefore, we adopted 15 min as the exposure time.

**Calibration**

The validation data on whole blood and hair sample are presented in Table 1. HS-SPME was performed at a temperature of 140°C, with 0 min preheating time and 15 min exposure time. The calibration curves were obtained by plotting the peak area versus the sample size of p,p'-DDE. Linear correlations were verified over the ranges of 0.003-2.0 ng/mg (r = 0.998) and 0.004-2.0 ng/mg (r = 0.998) in whole blood and hair, respectively. The equations for the curve were: y = 294685x-8540 for whole blood; y = 156715x-1455 for hair. The C.V. values for the slopes were between 7.3-8.5 %, indicating very good assay reproducibility. To determine the limit of detection (LOD) and the limit of quantitation (LOQ), a separate calibration curve in the range of LOD

**Table 2** Inter-day and Intra-day precision for determination of p,p'-DDE in rat whole blood and hair (n = 8)

| | Spiked concentration (ng/mg) | Inter-day | Intra-day |
|---|---|---|---|
| Whole blood | 0.004 | 8.5 | 9.3 |
| | 0.2 | 8.0 | 8.5 |
| | 2.0 | 7.5 | 8.3 |
| Hair | 0.004 | 9.1 | 8.4 |
| | 0.2 | 6.2 | 7.9 |
| | 2.0 | 6.9 | 8.5 |

**Table 3** Recoveries of p,p'-DDE in rat whole blood and hair at different spiked concentrations, after application of the presented SPME method (n = 8)

| | Spiked concentration (ng/mg) | Mean recoveries (%) | CV (%) |
|---|---|---|---|
| Whole blood | 0.004 | 4.5 ± 0.09 | 8.5 |
| | 0.2 | 5.5 ± 0.39 | 8.0 |
| | 2.0 | 9.5 ± 0.59 | 7.5 |
| Hair | 0.004 | 3.60 ± 0.03 | 9.1 |
| | 0.2 | 2.40 ± 0.15 | 6.2 |
| | 2.0 | 7.15 ± 0.49 | 6.9 |

(0.002-0.01 ng/mg) was established. The LOD and LOQ were calculated for signal-to-noise ratios of 0.003 ng/mg and 0.004 ng/mg in whole blood and hair, respectively. The day-to-day precision (expressed as the C.V.) was estimated by daily analysis of an aliquot of whole blood and hair samples with p,p'-DDE at 0.004, 0.2, and 2.0 ng/mg over a period of 7 days. The inter-day and intra-day precision at these three concentrations is shown in Table 2.

**Recovery**

Table 3 shows the rates of extraction recovery from whole blood and hair at each of the three concentrations. The same concentrations were used for spiked control samples of whole blood and hair. The recovery rates using HS-SPME were calculated by comparing the un-extracted peak areas of the directly injected p,p'-DDE to GC with extracted blank whole blood and hair samples spiked with the corresponding concentrations of p,p'-DDE. The mean recovery at three concentrations is shown Table 3.

It is well known that GC-ECD is a highly effective method to screen for organic chlorine compounds. A number of gas chromatographic methods have been proposed for the detection of PCBs, including DDT and p,p'-DDE, in blood and hair [8-13, 15, 16, 19]. The extraction methods typically used for these compounds include liquid-liquid extraction with a clean-up step [8, 9, 11, 15, 16], solid-phase extraction [10, 19], and solid-phase micro-extraction [12]. Since a blood sample undergoes dilution during protein precipitation, sample pre-concentration steps involving evaporation and reconstitution are often required prior to analysis. These steps are tedious and time-consuming. In hair samples, meanwhile, keratin must

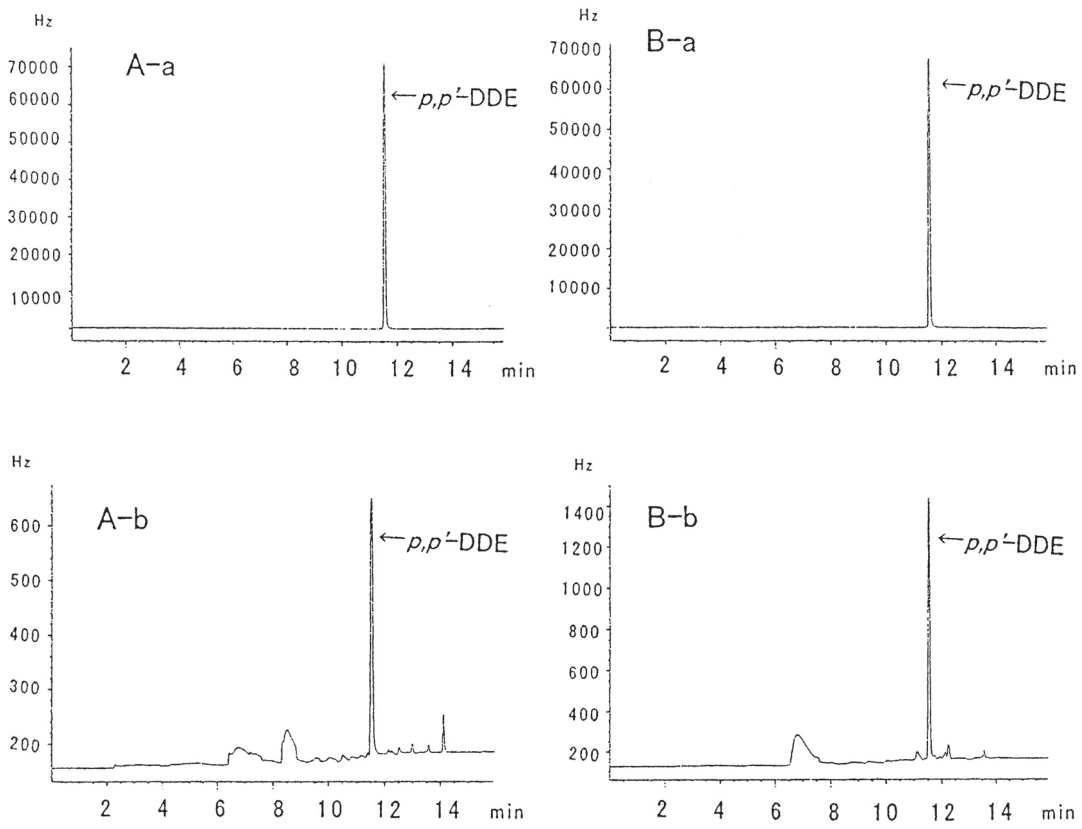


**Fig. 6** Typical GC-$\mu$-ECD chromatograms of rat whole blood obtained by HS-SPME: (A-a) blank whole blood spiked with 2.0 ng/mg p,p'-DDE; (A-b) whole blood sample 4 weeks after oral administration of 100 mg/kg p,p'-DDE. (B) Typical GC-$\mu$-ECD chromatograms of rat hair obtained by HS-SPME: (B-a) blank hair spiked with 2.0 ng/mg p,p'-DDE; (B-b) hair sample 4 weeks after oral administration of 100 mg/kg p,p'-DDE. Each extraction was performed for 15 min at 140 °C from a 50 mg rat whole blood or hair sample present in 1.5 ml 1N NaOH. Preheating time = 0 min.

**Table 4** Mean ( ± SD) whole blood and hair concentrations of p,p'-DDE in 6 rats after single administration of 100 mg p,p'-DDE

| | Sampling period (Weeks) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Whole blood (ng/mg) | 0.084 ± 0.020 | 0.092 ± 0.045 | 0.114 ± 0.044 | 0.121 ± 0.035 |
| Hair (ng/mg) | 0.150 ± 0.033 | 0.175 ± 0.045 | 0.185 ± 0.043 | 0.190 ± 0.040 |

be decomposed prior to extraction of the chlorine compounds. Our previous study demonstrated a method for analyzing biological samples by HS-SPME and GC electron ionization mass spectrometry (GC-MS) [20]. Both LOD and LOQ were 0.02 ng/mg. That method, however, is probably not preferable for analysis of p,p'-DDE in human blood and/or hair samples because of the possibility of sensitivity problems, such as LOD and LOQ. We therefore attempted to develop a method that combines GC-$\mu$-ECD with a useful HS-SPME, to save extraction time and extraction solvent. LOD and LOQ of GC-$\mu$-ECD were higher than GC-MS about 10 times.

The best way to cope with sample matrix effects is to use a stable analogue compound as an internal standard. Such a compound should match the chromatographic retention time and recovery properties with the matrix of p,p'-DDE. Unfortunately, no suitable internal standard for our HS-SPME was available in our laboratory.

The incorporation of drugs into hair was reported to correlate highly with the hair's melanin affinity [21]. Although Gygi et al. concluded that hair color greatly affects the incorporation of weak base, they also found that color did not affect the incorporation of weak acids [22]. Pigmentation may not affect the incorporation of p,p'-DDE into hair, because p,p'-DDE is chemically stable.

Covaci *et al*. reported that p,p'-DDE levels in hair samples in Greece were 37.6 pg/mg [15]. Dauberschmidt and Wennig reported that p,p'-DDE levels in hair samples in western Europe were 39 pg/mg [16]. Longnecker *et al*. reported that p,p'-DDE concentrations in human blood were about 25 ng/g [23].

The limit of quantitation in our method would allow successful measurement of p,p'-DDE concentrations in human blood and hair.

### Application of the analytical method in pharmacokinetic studies

After a single oral administration of 100 mg/kg p,p'-DDE to each of 6 dark Agouti rats, newly grown hairs on the back were collected after 1, 2, 3, and 4 weeks in the same way as described above. Whole blood samples were collected from the tail artery at the same times. The p,p'-DDE concentrations of blood and hair were analyzed by the proposed GC-$\mu$-ECD method as described. Figure 6 shows typical GC-$\mu$-ECD chromatograms of rat whole blood and hair after this application. The mean whole blood and hair concentrations obtained are illustrated in Table 4. The whole blood concentrations were estimated on the basis of the hair concentrations.

## CONCLUSION

In conclusion, a GC-$\mu$-ECD method using HS-SPME was developed to measure p,p'-DDE in rat whole blood and hair. This method demonstrated good sensitivity and linearity. This procedure was then used to investigate rat whole blood and hair profiles after a single oral dose of p,p'-DDE. p,p'-DDE was determined in both whole blood and hair 4 weeks after administration. Therefore, the present method of whole blood and hair analysis may be useful for assessing past exposure to p,p'-DDE in a subject.

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