ELSEVIER

Journal of Chromatography B, 761 (2001) 93–98

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Solid-phase microextraction and gas chromatography–mass spectrometry analysis of *p,p′*-DDE in biological samples

Takahiko Kusakabe, Takeshi Saito*, Sanae Takeichi

Department of Forensic Medicine, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa 259-1193, Japan

Received 12 February 2001; received in revised form 3 July 2001; accepted 3 July 2001

Abstract

A simple and rapid extraction method for the analysis of *p,p′*-DDE from rat whole blood and tissues was developed using headspace solid-phase microextraction (SPME). A vial containing a sample of *p,p′*-DDE, sodium hydroxide, and benzophenone as internal standard was heated at 120°C. A polydimethylsiloxane-coated SPME fiber was exposed for 15 min in the headspace of the vial. The fiber needle was then injected and desorbed by exposing the fiber in the injection port of a capillary gas chromatography–mass spectrometry system. The calibration curve demonstrated good linearity throughout the concentration range from 0.02 to 50 μg/g for rat whole blood and liver samples. The limit of detection for *p,p′*-DDE was 0.020 μg/g using 0.5 g rat whole blood and liver samples. Coefficients of variation ranged from 7.0 to 7.9%. This method was used to analyze a rat whole blood sample after administration of *p,p′*-DDE.

Keywords: Headspace solid-phase microextraction; *p,p′*-DDE

1. Introduction

p,p′-DDE, 1,1′-(2,2-dichloroethylidene)-bis(4-chlorobenzene) (Fig. 1), is a metabolite of the pesticide *p,p′*-DDT. *p,p′*-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) (Fig. 1) was used world-wide from the 1940s to the 1960s in agriculture and for sanitation purposes. However, DDT is still used in Asia. DDE is extremely stable and resistant to breakdown in the natural environment and in humans [1,2]. DDT and DDE are mainly taken up from

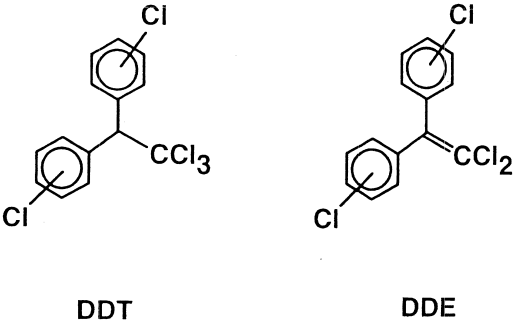

Fig. 1. Chemical structures of *p,p′*-DDT and *p,p′*-DDE.

*Corresponding author. Tel.: +81-463-93-1121; fax: +81-463-92-0284.

E-mail address: saito@is.icc.u-tokai.ac.jp (T. Saito).

0378-4347/01/$ – see front matter
PII: S0378-4347(01)00308-5

dietary products into humans, and stored in the body fatty tissue due to their high lipid solubility [3].

Recent investigations have linked *p,p′*-DDE to the development of breast cancer [4] and to decreased sperm counts [5]. Moreover, other reports have raised new concerns that PCBs and *p,p′*-DDE may disrupt normal reproduction and development through inhibition of the androgen receptor function [6]. *p,p′*-DDE, in particular, shows the most potent anti-androgenic effect among the analogues of DDE. These chemicals in our environment are reported to be environmental endocrine disrupters [7].

Several analytical methods, using either gas chromatography with electron capture detection [8–11] or gas chromatography–mass spectrometry (GC–MS) [12], have been proposed for the specific determination of *p,p′*-DDE. All of these extraction methods require a lengthy analysis time. To date, no quantitative method is available for the rapid quantification of DDT and *p,p′*-DDE in biological samples. Here, we present a simple, rapid and highly selective method for the quantitative measurement of *p,p′*-DDE in rat whole blood using headspace SPME–GC–MS.

2. Materials and methods

2.1. Materials

p,p′-DDE was obtained from Riedel-de Haën (Seelze, Germany). Benzophenone was obtained from Wako (Osaka, Japan). The SPME device coated with 100 μm polydimethylsiloxane fiber was purchased from Supelco (Bellefonte, PA, USA). Other chemicals used were of analytical grade. Rat whole blood and liver were used in the examination of the heating temperature, preheating time and exposure time conditions. The weight of the liver was measured as the wet weight.

2.2. Gas chromatography–mass spectrometry (GC–MS) conditions

A Hewlett-Packard 5890 series II GC coupled to a Model 5971 mass spectrometer was used. A 30 m×0.25 mm I.D. fused-silica capillary DB-1 column (J&W Scientific, Folson, CA, USA) with 0.25 μm film thickness was used. Splitless injection was employed (split valve off-time 3.0 min). The column oven temperature was programmed to rise from an initial temperature of 100°C to 280°C at 20°C/min and was maintained at 280°C for the final 3 min. The injection temperature and ion source temperature were 250 and 280°C, respectively. Splitless injection mode was used, and the splitter was opened after 3 min. Selected ion monitoring (SIM) was used as follows: *p,p′*-DDE, *m/z* 246; benzophenone (I.S.), *m/z* 105.

2.3. Solid-phase microextraction (SPME) procedure

Whole blood and wet liver samples (0.5 g), I.S. (1 μg/μl, 20 μl), and sodium hydroxide solution (1 *M*, 1 ml) were placed in a 12 ml vial which was rapidly sealed with a silicone septum and an aluminum cap. The liver samples weighed 0.5 g after being cut into small pieces using a scalpel. The vial was immediately 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 (10.5 ml) of the vial for 15 min. After adsorption of the compounds, the fiber was withdrawn into the needle, removed from the vial, and immediately inserted into the injection port of the GC–MS system. The fiber was exposed in the injection port for 3 min and the analytes were desorbed into the GC column.

2.4. Heating temperature, preheating time and exposure time conditions

In order to achieve the optimal conditions (the best recovery rate and C.V.), the effects of heating temperature, preheating time, and exposure time were determined using the above conditions. Rat whole blood or liver samples containing 1.0 μg/g of *p,p′*-DDE ($n = 3$) were prepared and tested. The vials were heated at four different temperatures (80, 100, 120, 140°C), for four different preheating times (0, 10, 20, 30 min), and five different exposure times (5, 10, 15, 20, 30 min) to determine the optimal conditions. These conditions were examined in the

order heating temperature, preheating time and exposure time.

3. Results and discussion

Fig. 2 shows the effects of heating temperature on the extraction of *p,p′*-DDE. There was a preheating time of 0 min and an extraction time of 15 min. The amount of adsorbed *p,p′*-DDE reached a maximum at 120°C. The best recovery rate was obtained at 120°C. In order to determine the heating temperature (120°C), the vial was preheated for four different lengths of time (0, 10, 20, 30 min). The fiber was then exposed for 15 min and analyzed. Fig. 3 shows the effects of preheating time on the extraction of *p,p′*-DDE. There was no large difference in the adsorbed amount of *p,p′*-DDE with respect to preheating time. However, the C.V. values were minimum at 0 min. Employing the above results, using a heating temperature of 120°C and a preheating time of 0 min, five different exposure times (5, 10, 15, 20, 30 min) were used to determine the optimum exposure time. The results are shown in Fig. 4. The adsorbed amount of *p,p′*-DDE exhibited no difference between 15 and 20 min, and thereafter it attained equilibrium. However, the C.V. values were about 7.9 and 16% for 15 and 20 min, respectively.

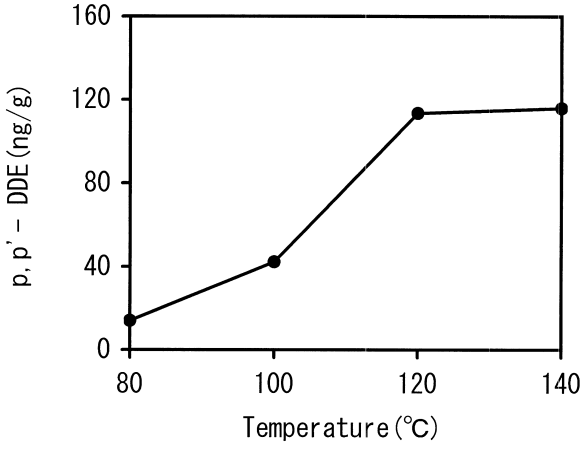

Fig. 2. The effect of temperature on the SPME sorption curve for *p,p′*-DDE extracted from a spiked rat liver sample (1.0 μg/g). The extraction conditions were: 120°C, preheating time 0 min, 15 min exposure.

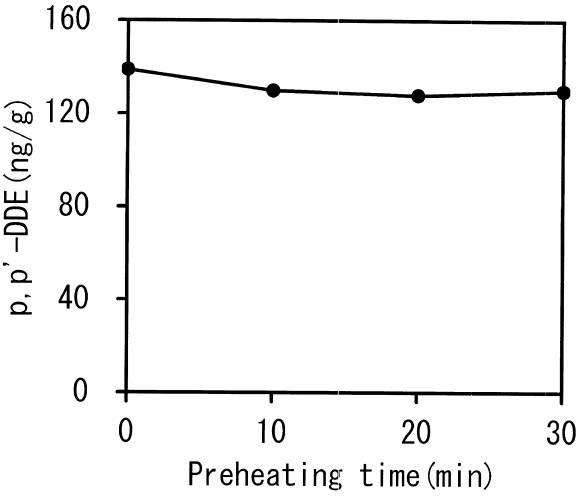

Fig. 3. The effect of preheating time on the SPME sorption curve for *p,p′*-DDE extracted from a spiked rat liver sample (1.0 μg/g).

Fifteen minutes seems to be the turning point for adsorption to the fiber and, therefore, C.V. values increase after 15 min. Therefore, 15 min was adopted for use, as the C.V. values were better at 15 min than at 20 min. These results did not differ between whole blood and liver samples. Moreover, there was no effect of the temperature of the sample on these extraction conditions.

For calibration, drug-free whole blood and liver spiked with *p,p′*-DDE at concentrations of 0.02, 0.04, 2, 5, 10, 20 and 50 μg/g in the presence NaOH

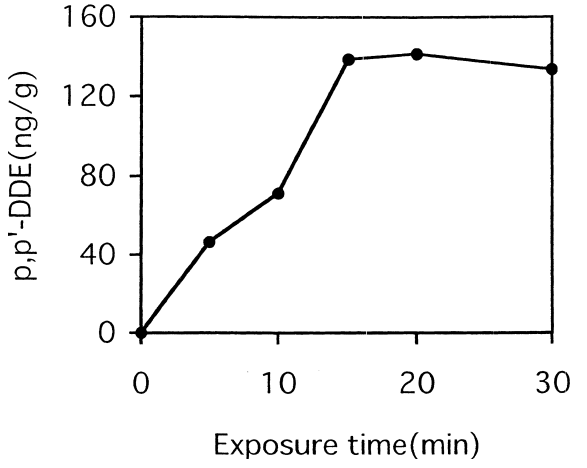

Fig. 4. The effect of exposure time on the SPME sorption curve for *p,p′*-DDE extracted from a spiked rat liver sample (1.0 μg/g).

solution (1 *M*, 1 ml) and I.S. (20 μg) were used. The calibration curves were obtained by plotting the peak-area ratio between *p,p′*-DDE and I.S. The conditions were: heating temperature 120°C, preheating time 0 min and exposure time 15 min. The seven-point standard curve showed good linearity ($r = 0.999$ for whole blood and $r = 0.997$ for liver) over the concentration range tested. The equations for the curves were: $y = 0.142x - 0.052$ for whole blood and $y = 0.119x + 0.106$ for liver. The C.V. values for the slopes were between 6.2 and 8.9%, indicating very good assay reproducibility.

The absolute recovery was determined by extracting and assaying in the SIM mode blank whole blood samples with *p,p′*-DDE at concentrations of 1.0 and 50 μg/g. The representive peak areas of these extracted samples compared with those of unextracted methanolic standards at the same concentrations gave (expressed as the mean±SD of five separate experiments/concentration) 8.02±2.0 and 8.34±6.1%, respectively. Likewise, liver samples gave 6.64±2.56 and 6.95±5.6%, respectively. The result for water is not obtained when analysis of samples containing solid or sludge is analyzed by SPME, even if examined [13]. Therefore, the recovery rate of the liver would be lower than the blood. The lower limits of detection (corresponding to a signal equivalent to three times the background noise) were 0.020 μg/g for whole blood and liver. The lower limits of quantitation were 0.020 μg/g for whole blood and liver.

The day-to-day precision (expressed as the C.V.), estimated by daily analyses of an aliquot of whole blood and liver samples with *p,p′*-DDE at 1.0 μg/g over a period of 7 days, was 7.0 and 8.4%, respectively; these results were found to be acceptable. We exchanged the fibre for a new fiber when the day-to-day precision indicated that it was required. A new fibre produces a small C.V.

Pawliszyn [13] reported that the SPME extraction process is exothermic; an increase in sampling temperature will reduce the analytic recovery. However, we found that it was possible to increase the adsorbed *p,p′*-DDE by increasing the heating temperature. In previous experimental reports [14–17], elevating the heating temperature was shown to increase the amount of adsorbed compounds. However, when the heating temperature was too high, the amount adsorbed on the fiber decreased (Fig. 2). Although *p,p′*-DDE is stable towards heat, the distribution constant decreases at high temperature. The decreasing distribution constant would reduce the amount adsorbed.

As reported by Namera et al. [17], when using headspace SPME for low volatility chemicals including tetracyclic antidepressants, a preheating procedure is not necessary. Although *p,p′*-DDE is not a volatile chemical, the result is similar. Preheating is not required in order to fill low volatility chemicals into the vial by slow gasification. The vial fills with volatile chemicals under preheating at 100°C or less in a short time. The headspace is saturated with *p,p′*-DDE, just as for volatile chemicals, in a short time, because, at high heating temperatures, vaporization equilibrium is rapidly attained.

In our method, *p,p′*-DDE was extracted from samples in the presence of sodium hydroxide. The primary objectives for the addition of sodium hydroxide were to dissolve the tissues and to increase the amount of adsorbed *p,p′*-DDE. When the addition of 1 *M* NaOH was examined, maximum recovery with 1 ml was obtained. Although this volume was the minimum in which the sample dissolved completely, this quantity efficiently gasifies *p,p′*-DDE. Moreover, *p,p′*-DDE cannot be extracted from the samples (whole blood and tissues) without adding sodium hydroxide. It has been reported [15,17,18] that the recovery is increased in headspace SPME by the addition of salts to the sample solution. Although we added salts, an increase in recovery was not obtained.

Fig. 5 shows representative chromatograms for an extracted blank rat whole blood sample in the presence of NaOH solution (1 *M*, 1 ml) (A), an extracted blank rat whole blood sample spiked with *p,p′*-DDE at 1.0 μg/g and I.S. in the presence of NaOH solution (1 *M*, 1 ml) (B), and that for extracted whole blood and *p,p′*-DDE (100 mg/kg/day) administered to a pregnant rat for 14–18 days (C). These chromatograms show that the peaks for *p,p′*-DDE and I.S. are fully separated and the endogenous peaks from rat whole blood do not interfere with the separation of the analytes.

In India, Bhatnagar et al. [10] reported the DDE and DDT levels in 31 healthy male subjects from a rural area. The serum DDE and DDT levels were

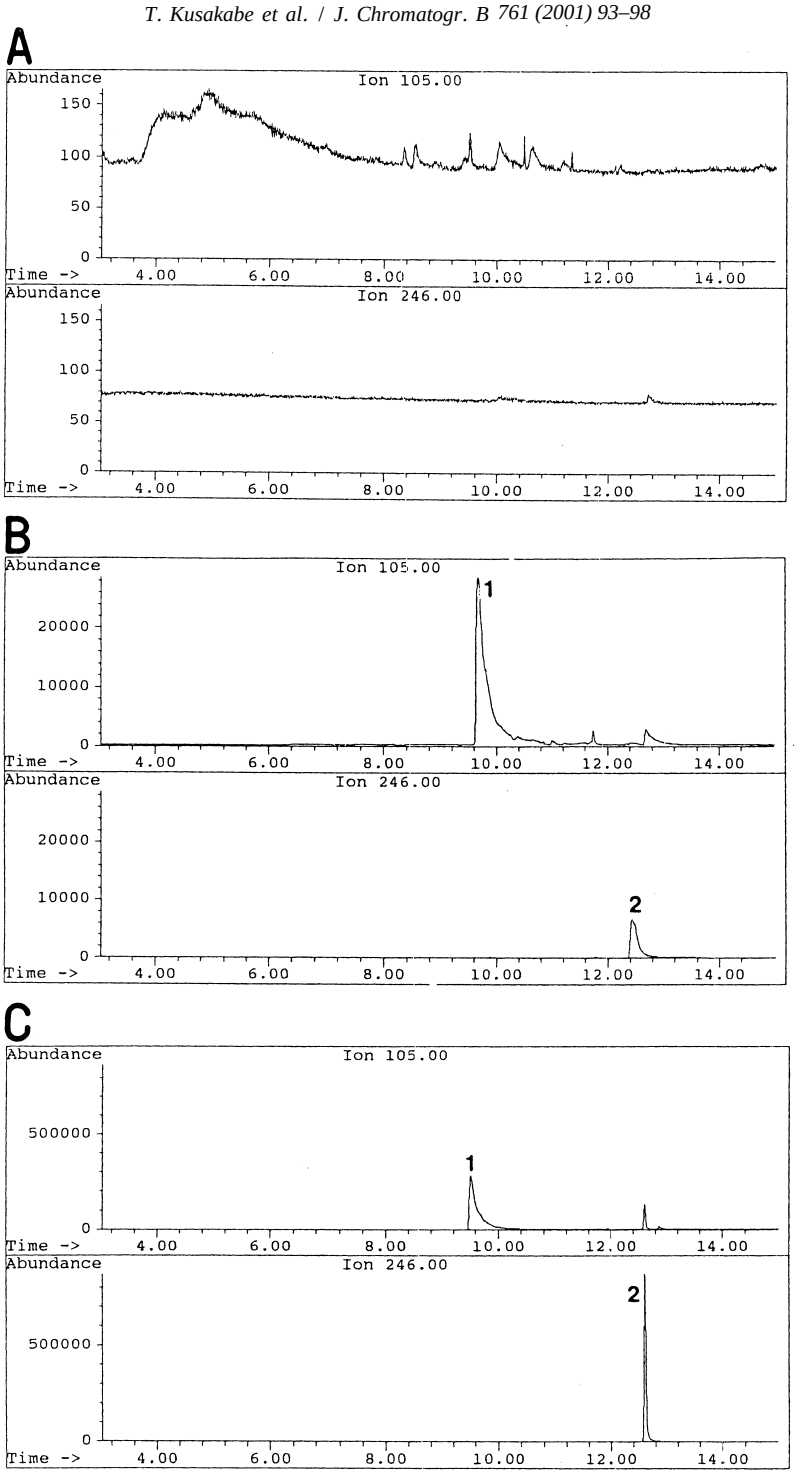

Fig. 5. Representative selected ion monitoring chromatograms for *p,p′*-DDE and internal standard. (A) Blank rat whole blood. (B) Extracted blank rat whole blood spiked with *p,p′*-DDE (1.0 μg/g) and internal standard (40 μg/g). (C) Extracted whole blood and administered *p,p′*-DDE to a pregnant rat for 14–18 days. Measured *p,p′*-DDE concentration: 20.5 μg/g whole blood. Peaks: 1= benzophenone (internal standard), 2=*p,p′*-DDE.

0.0373±0.0046 and 0.0088±0.0013 μg/g, respectively. Luo et al. [11] reported that the serum DDT level in Singapore was 0.0029 μg/g for males and 0.0015 μg/g for females, and serum DDE was 0.0081 μg/g for males and 0.0134 μg/g for females. These measurements were made using GC–ECD [15,16]. Using our method, it was difficult to detect DDE and/or DDT in most cases, however the sharp peaks might be detected by GC by using a much more sensitive detection method, such as electron capture detection.

To our knowledge, this is the first study to employ headspace SPME for the extraction of *p,p′*-DDE from whole blood and liver samples. SPME is a simple method that requires no solvents. Our method can selectively detect *p,p′*-DDE by GC–MS. The present headspace SPME method can be recommended for use before the analyses of other PCBs and dioxin by GC and GC–MS, particularly in environmental toxicology.

References

[1] R.D. Kimbrough, A.A. Jensen, Topics in Environmental Health, Vol. 4, Elsevier, Amsterdam, 1989.
[2] X. Guardino, C. Serra, J. Obiols, M.G. Rosell, M.J. Berenguer, F. López, J. Brosa, J. Chromatogr. 719 (1996) 141.
[3] World Health Organization, DDT and Its Derivatives — Environmental Aspects, Environmental Health Criteria, Vol. 83, WHO, Geneva, 1989.
[4] M.S. Wolff, P.G. Toniolo, E.W. Lee, M. Rivera, N. Dubin, J. Natl. Cancer Inst. 85 (1993) 648.
[5] IEH, IEH Assessment on Environmental Oestrogens: Consequences to Human Health and Wildlife, IEH, Leicester, 1995.
[6] W.R. Kelce, C.R. Stone, S.C. Laws, L.E. Gray, J.A. Kemppainen, E.M. Wilson, Nature 375 (1995) 581.
[7] J.A. Mclachlan, Environ. Health Perspect. 101 (1993) 386.
[8] D.P. Morgan, C.C. Roan, Arch. Environ. Health 29 (1974) 14.
[9] H. Bouwman, R.M. Cooppan, P.J. Becken, S. Ngxongo, J. Toxicol. Environ. Health 33 (1991) 141.
[10] V.K. Bhatnagar, J.S. Patel, M.R. Variya, M.P.K. Venkaiah, M.P. Shah, S.K. Kashyap, Bull. Environ. Contam. Toxicol. 48 (1992) 302.
[11] X.W. Luo, S.C. Foo, H.Y. Ong, Sci. Total Environ. 207 (1997) 97.
[12] C. Weistrand, K. Norén, Environ. Health Perspect. 105 (1997) 644.
[13] J. Pawliszyn, Solid Phase Microextraction, Theory and Practice, Wiley–VHC, New York, 1997.
[14] M. Yashiki, T. Kojima, T. Miyazaki, N. Nagasawa, Y. Iwasaki, K. Hara, Forensic Sci. Int. 76 (1995) 169.
[15] T. Kumazawa, X.P. Lee, M.C. Tsai, H. Seno, A. Ishii, K. Sato, Jpn. J. Forensic Toxicol. 13 (1995) 25.
[16] X.P. Lee, T. Kumazawa, K. Sato, O. Suzuki, J. Chromatogr. Sci. 35 (1997) 302.
[17] A. Namera, T. Watanabe, M. Yashiki, Y. Iwasaki, T. Kojima, J. Anal. Toxicol. 22 (1998) 396.
[18] D.B. Page, G. Lacroix, J. Chromatogr. 648 (1993) 199.