

Simple and Rapid Determination of Organochlorine Pesticide Residues in Kampo Products by Gas Chromatography/Mass Spectrometry with Negative Chemical Ionization

Takaomi Tagami,* Keiji Kajimura, Katsuhiko Yamasaki, Yoshiyuki Sawabe, Chie Nomura, Shuzo Taguchi, and Hiroataka Obana

Osaka Prefectural Institute of Public Health, 1–3–69 Nakamichi, Higashinari-ku, Osaka 537–0025, Japan

(Received October 27, 2009; Accepted November 23, 2009; Published online November 27, 2009)

A simple, rapid and inexpensive sample preparation method using extraction by shaking and sulfuric acid treatment was developed for determining eight organochlorine pesticides in kampo products by gas chromatography/mass spectrometry with negative chemical ionization. The recoveries were between 71% and 117%, and most relative standard deviations were less than 10%. The proposed method was applied to twenty-three samples of kampo formulae, and no samples were contaminated by eight organochlorine pesticides over the quantification limit.

Key words — kampo product, pesticide residue, gas chromatography/mass spectrometry

INTRODUCTION

Kampo formulae are used for various illnesses, and these formulae should be confirmed to be safe because patients generally take kampo formulae directly over the long term. α -benzene hexachloride (BHC), β -BHC, γ -BHC, δ -BHC (BHCs), *p,p'*-dichloro diphenyl dichloroethylene (DDE), *o,p'*-dichloro diphenyl trichloroethane (DDT), *p,p'*-dichloro diphenyl dichloroethane (DDD) and *p,p'*-

DDT (DDTs) have been detected in crude drugs^{1–3)} that are the ingredients of kampo formulae, and there is concern that BHCs and DDTs will move from crude drugs to kampo products. The levels of BHCs and DDTs in kampo formulae are controlled to be lower than self-imposed residual pesticide limits set by the Japan Kampo Medicine Manufacturers Association. Maximum residue levels of organochlorine pesticides were set for kampo formulae containing ginseng, senna leaf, astragalus root, polygala root, glycyrrhiza, cinnamon bark, asi-sarum root, cornus fruit, perilla herb, jujube, citrus unshiu peel, loquat leaf and/or moutan bark [BHCs: 0.2 ppm (as total BHCs), DDTs: 0.2 ppm (as total DDTs)]. However, there have been few surveys of BHCs and DDTs in kampo products distributed in Japan, and few analytical methods have been reported.^{1,4,5)} All methods are time-consuming and use gas chromatography (GC)/electron capture detection (ECD). It was reported previously that organochlorine pesticides, such as BHCs and DDTs in crude drugs, could be analyzed selectively by GC/mass spectrometry (MS) with negative chemical ionization (NCI).⁶⁾ Furthermore, a simple and rapid determination of pyrethroid pesticides in kampo products by GC/MS with NCI was also reported.⁷⁾ In this study, a simple, rapid, and inexpensive sample preparation method to determine BHCs and DDTs in kampo products was investigated using GC/MS with NCI.

MATERIALS AND METHODS

Pesticide Standards — Pesticide standards were obtained from Wako Pure Chemical Industries (Osaka, Japan) and Riedel de Haën (Hannover, Germany). Each compound was dissolved in acetone to make 0.5 mg/ml standard stock solution. From these, spiking solutions were prepared at 5 μ g/ml.

Reagents — Acetone, hexane, and anhydrous sodium sulfate of pesticide analysis grade and sulfuric acid were purchased from Wako Pure Chemical Industries.

Kampo Products — Nine kampo formulae (kamishoyosan, hochuekkito, daikenchuto, saireito, shosaikoto, bakumondoto, goshajinkigan, rikkunshito and shoseiryuto) of the top 10 by amount of importation and production⁸⁾ were used in this study. Self-imposed residual pesticide limits are applied to these 9 kampo formulae. Three kampo

*To whom correspondence should be addressed: Osaka Prefectural Institute of Public Health, 1–3–69 Nakamichi, Higashinari-ku, Osaka 537–0025, Japan. Tel.: +81-6-6972-1321; Fax: +81-6-6972-2393; E-mail: tagami@iph.pref.osaka.jp

products were obtained for each kampo formulae for which self-imposed residual pesticide limits were set. Saireito and daikenchuto consisted of 2 samples because these kampo formulae are distributed by only 2 companies. Goshajinkigan consisted of 1 sample because this kampo formula is distributed by only 1 company. Overall, 23 kampo products of 9 kampo formulae were obtained (year: 2009).

Sample Preparation—Kampo products were powdered, 5 g was weighed out, and spiking solution (each pesticide: 1 µg) was added. After 30 min, a total of 10 ml of acetone and 20 ml of hexane were added. The mixture was shaken for 30 min, centrifuged, and the supernatant was recovered. Water (20 ml) was added to the supernatant. The mixture was shaken for 5 min. A portion (5 ml) of the upper layer was transferred to a glass-stoppered test tube, and 1 ml of sulfuric acid was added and shaken carefully. A portion (4 ml) of the upper layer was taken, transferred to a glass-stoppered test tube, and 2 ml of water was added and shaken gently. A portion (3 ml) of the upper layer was taken, transferred to a tube, and dried with 1 g of anhydrous sodium sulfate and the supernatant was used as the sample solution.

NCI Mode GC/MS—A 5973MSD was connected to a GC6890 (Agilent, Wilmington, DE, U.S.A.). GC conditions: column, DB-1701 capillary column 30 m × 0.25 mm × 0.25 µm (J&W Scientific, Folsam, CA, U.S.A.); helium carrier gas flow, 1 ml/min; injection temperature, 200°C; interface temperature, 270°C; ion source temperature, 180°C; ion mode, NCI/selected ion monitoring (SIM) mode; reaction gas, methane; oven temperature program, 50°C for 1 min, +30°C/min to 100°C, and then +20°C/min to 270°C and held for 10 min; injection mode, splitless; injection volume, 2 µl.

RESULTS AND DISCUSSION

The monitoring ions selected for SIM detection are shown in Table 1. Values of 71 (*m/z*: BHCs) and 35 (*m/z*: DDTs) were used for quantification. The analytical method was investigated using kamishoyosan. Sample preparation method was based on the previous report.⁷⁾ When the recovery test was conducted according to the previous report,⁷⁾ a matrix enhancement effect was observed. So the sample preparation method was slightly changed and sulfuric treatment of the extraction liquid was supplemented. It was reported that sulfuric treatment is effective for purifying BHCs and DDTs.^{9,10)} One of the previous reported methods for the analysis of BHCs and DDTs in kampo products also used sulfuric acid treatment in combination with column clean-up and liquid-liquid partition.⁴⁾ Sulfuric acid treatment purified extraction liquid by degradation of organic substances. BHCs and DDTs were not decomposed by sulfuric acid treatment because they are acid-stable. Sulfuric acid treatment was suitable for this study because it was simple and inexpensive. Intra-day recovery study (BHCs and DDTs: 3 spike concentrations)

Table 1. Monitoring Ions Selected for SIM

Compound	Monitoring ion (<i>m/z</i>)	
α -BHC	71	35
γ -BHC	71	35
β -BHC	71	35
δ -BHC	71	35
<i>p,p'</i> -DDE	35	282
<i>o,p'</i> -DDT	35	71
<i>p,p'</i> -DDD	35	71
<i>p,p'</i> -DDT	35	71

Table 2. Recoveries of BHCs and DDTs from Kamishoyosan

Spiked level (ppm)	Intra-day						Inter-day	
	0.04		0.2		1.0		0.2	
Compound	Average (%)	RSD (%)	Average (%)	RSD (%)	Average (%)	RSD (%)	Average (%)	RSD (%)
α -BHC	83.6	2.7	91.4	1.9	103.3	3.9	93.3	1.8
γ -BHC	82.8	7.1	91.8	1.9	101.8	3.7	92.8	1.7
β -BHC	82.0	6.3	91.7	0.9	102.4	3.1	95.0	3.5
δ -BHC	84.1	5.6	92.7	0.2	101.9	3.9	94.3	3.0
<i>p,p'</i> -DDE	85.1	6.5	96.9	1.1	104.9	3.6	96.9	2.1
<i>o,p'</i> -DDT	92.0	9.3	101.1	10.2	111.4	3.2	104.2	3.8
<i>p,p'</i> -DDD	96.8	3.9	105.7	3.4	109.4	4.1	105.1	2.9
<i>p,p'</i> -DDT	88.0	10.5	103.3	13.3	111.4	3.3	106.7	5.1

Inter-day recovery study was carried out over 3 working days.

and inter-day recovery study (over 3 working days) were conducted for kamishoyosan. Intra-day recovery rates of BHCs and DDTs when spiking solution was spiked at 0.2, 1, and 5 μg were between 82.0% and 111.4%, and the relative standard deviations (RSDs) were between 0.2% and 13.3% (Table 2). Inter-day recovery rates of BHCs and DDTs were between 92.8% and 106.7%, and for RSD were between 1.7% and 5.1% (Table 2), indicating these values seemed satisfactory for residue analysis.

The correlation coefficients of linearity are

Table 3. Linear Ranges, Correlation Coefficients and Quantification Limits of BHCs and DDTs

Compound	Range (ppb)	Correlation coefficient (γ)	quantification limit (ppb)
α -BHC	5–500	0.9999	1
γ -BHC	5–500	0.9999	1
β -BHC	5–500	0.9999	2
δ -BHC	5–500	1.0000	1
<i>p,p'</i> -DDE	5–500	0.9998	1
<i>o,p'</i> -DDT	5–500	0.9974	3
<i>p,p'</i> -DDD	5–500	0.9997	2
<i>p,p'</i> -DDT	5–500	0.9961	4

shown in Table 3 and varied from 0.9961–1.0000, showing good linearity.

It was attempted to apply the proposed method to 8 other kampo formulae for which self-imposed residual pesticide limits are set. The recovery values of BHCs and DDTs were between 71.4% and 116.7%, and most RSD values were less than 10% (Table 4). SIM chromatograms of shosaikoto (blank sample) and standard solution (0.05 ppm) are shown in Fig. 1. There were no interfering peaks around the pesticide peaks. There were also no interfer-

Table 4. Recoveries of BHCs and DDTs in 8 Other Kampo Products ($n = 3$)

Kampo formulae	Average (%)	RSD (%)
Hochuekkito	97.1–111.8	0.9– 4.6
Daikenchuto	71.4– 99.5	3.5– 7.0
Saireito	92.3–111.6	0.7–17.6
Shosaikoto	87.5–104.2	0.1– 4.4
Bakumondoto	87.6–100.1	0.7– 4.0
Goshajinkigan	99.9–116.7	1.3– 3.3
Rikkunshito	91.2–106.4	2.0–11.7
Shoseiryuto	88.4–105.7	0.5– 3.4

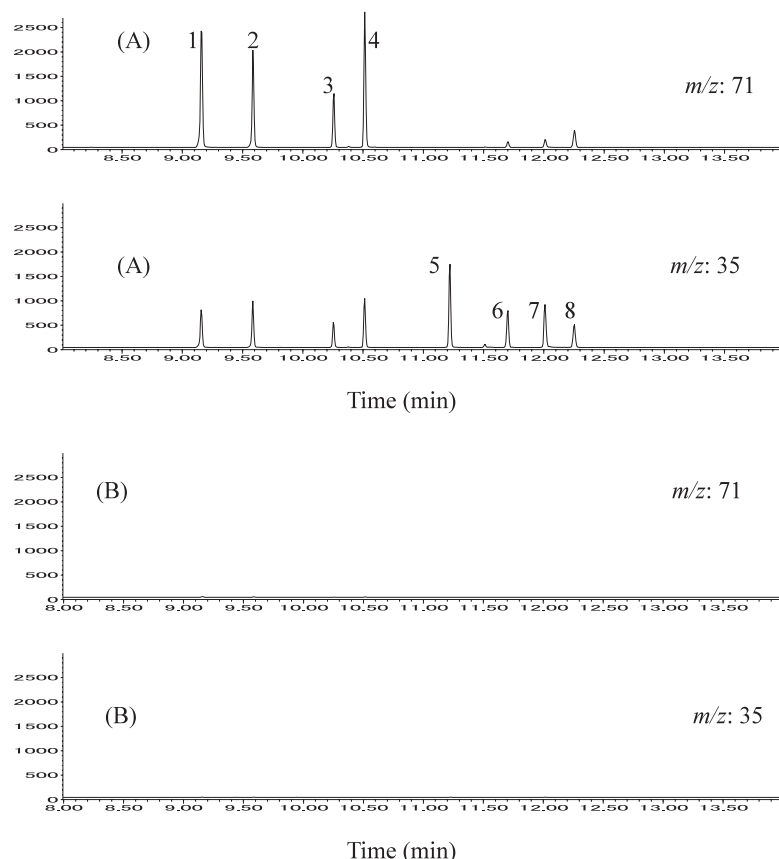


Fig. 1. SIM Chromatograms of (A) Standard Solution (Each Pesticide: 0.05 ppm), (B) Extract from Shosaikoto (Blank Sample)
1: α -BHC, 2: γ -BHC, 3: β -BHC, 4: δ -BHC, 5: *p,p'*-DDE, 6: *o,p'*-DDT, 7: *p,p'*-DDD, 8: *p,p'*-DDT.

ing peaks around the pesticide peaks in the sample solutions of other 8 kampo products including kamishoyosan.

Twenty-three samples of kampo products [9 kampo formulae (kamishoyosan, hochuekkito, daikenchuto, saireito, shosaikoto, bakumondoto, goshajinkigan, rikkunshito and shoseiryuto)] were analyzed according to the proposed method. No samples were contaminated by BHCs or DDTs over the quantification limit (as shown in Table 3: concentration in the injected solution; peak height was over 10 times the noise level of the major ions).

In conclusion, a simple, reliable, rapid, and inexpensive sample preparation method was developed to determine BHCs and DDTs in kampo products. Twenty-three samples of kampo products were analyzed according to the proposed method and no samples contained BHCs or DDTs over the quantification limit.

REFERENCES

- 1) Shioda, H., Takano, I., Seto, T., Yasuda, I., Hamano, T., Sato, K., Nagayama, Y., Onishi, K., Fujita, S., Ohno, T. and Kajikazawa, T. (1997) Pesticide residues in crude drugs and kampo pharmaceuticals. *Annual Report of Tokyo Metropolitan Research Laboratory of Public Health*, **48**, 67–70.
- 2) Yoshioka, N., Akiyama, Y., Mitsuhashi, T., Hatanaka, H., Tsuji, M. and Matsushita, S. (2000) Analysis of pesticide residues in Ginseng and Senna. *Iyaku hin Kenkyu*, **31**, 225–231 (in Japanese).
- 3) Nakazato, M., Nagayama, T., Kan, K., Tabata, S., Tateishi, Y. and Yasuda, I. (2000) Survey of harmful substances in crude drugs (III)—Ginseng radix—. *Annual Report of Tokyo Metropolitan Research Laboratory of Public Health*, **51**, 34–39.
- 4) Tamura, M., Endo, Y., Isozaki, T., Sugimoto, C., Maruta, J., Ohashi, S., Yoshikawa, S., Okonogi, A., Shimizu, K. and Sasaki, H. (2008) Pesticide residues in kampo formulations, crude drug formulations and crude drugs (1). *Iyaku hin Kenkyu*, **39**, 63–75 (in Japanese).
- 5) Shioda, H., Hamano, T., Nakajima, J. and Yasuda, I. (2007) Determination of organochlorine and pyrethroid pesticides in preparations of crude drug extract. *Annual Report of Tokyo Metropolitan Institute of Public Health*, **58**, 123–125.
- 6) Tagami, T., Kajimura, K., Takagi, S., Satsuki, Y., Nakamura, A., Okihashi, M., Akutsu, K., Obana, H. and Kitagawa, M. (2007) Simultaneous analysis of 17 organochlorine pesticides in natural medicines by GC/MS with negative chemical ionization. *Yakugaku Zasshi*, **127**, 1167–1171.
- 7) Tagami, T., Kajimura, K., Yamasaki, K., Sawabe, Y., Nomura, C., Taguchi, S. and Obana, H. (2009) Simple and rapid determination of cypermethrin and fenvalerate residues in kampo products by gas chromatography/mass spectrometry with negative chemical ionization. *J. Health Sci.*, **55**, 777–782.
- 8) Japan Kampo Medicine Manufacturers Association (2004), Kanpouseizainadonoseisandoutai (in Japanese), http://www.nikkankyo.org/publication/movement/h16pdf/04_05.pdf.
- 9) Kajimura, K., Sakagami, Y., Yokoyama, H. and Doi, S. (1994) Analytical method for organochlorine pesticide residues in crude drug containing essential oil. *Proceeding of Osaka Prefectural Institute for Public Health ED. of Pharmaceutical Affairs*, **28**, 63–68.
- 10) Terasaki, S. and Tsuno, T. (2004) Analytical method for organochlorine pesticide residues in crude drugs. *Annual Report of Toyama Prefectural Institute for Pharmaceutical Research*, **31**, 38–40.