

Multiresidue Analysis of 18 Organochlorine Pesticides in Traditional Chinese Medicine

Lili Hao and Jian Xue*

Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100094, China

Abstract

A simple and efficient method for simultaneous gas chromatographic (GC) determination of 18 organochlorine pesticides [tecnazene, hexachlorobenzene, α -benzenehexachloride (BHC), pentachloronitrobenzene (PCNB), γ -BHC, heptachlor, aldrin, methyl pentachlorophenyl sulfide, β -BHC, δ -BHC, heptachlor epoxide, α -endosulfan, *trans*-chlorodane, *cis*-chlorodane, *p,p'*-dichlorodiphenyl dichloroethylene, *o,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDT] in Traditional Chinese Medicine (TCM) is described. The procedure involves ultrasonic extraction and sulphuric acid treatment as the cleanup method. Detection of the sample is performed by GC–electron capture detection. A series of experiments are conducted to optimize the final pretreatment conditions [acetone–petroleum ether (1:1) as the extract solvent, ultrasonication for 15 min, three steps, concentrated sulphuric acid with 10% water for sulphuric acid treatment]. Recovery studies are performed at 10, 50, and 100 parts-per-billion (ppb) fortification levels of each organochlorine pesticide, except for α -endosulfan, which are 20, 100, and 200 ppb. The percentage recoveries range from 77.9% \pm 6.4% to 114.0% \pm 8.1% (average \pm standard deviation). The simple and rapid method may be used to routinely determine organochlorine pesticides in TCM.

Introduction

Traditional Chinese Medicines (TCMs) have been used in medical practice for thousands of years and is recognized especially as a valuable and readily available resource for health care in Asian nations. A World Health Organization report indicated that approximately 70–80% of the world populations rely on nonconventional medicine, mainly of herbal sources, in their primary healthcare (1). With the ever-increasing worldwide use of herbal medicines and the rapid expansion of their global market, the safety and quality of medicinal plant materials and finished herbal medicinal products has become a major concern for health authorities, pharmaceutical industries, and the public.

TCMs have a high risk of contamination from agricultural chemicals, such as organochlorine pesticides (OCPs). Though

OCPs were banned in the 1970s because of their toxicity and persistence (2), residues can still be found today (3,4). It is an important task to propose limits and pesticide residue monitoring methods for TCMs. TCMs have very complex matrices, according to their botanical origin, and determining pesticide residue amounts in ranges below the ng/g level is difficult and complex (4). Sample preparation is a key element in pesticide residue analysis on TCMs.

Different pretreatments for pesticide analysis of various samples have been proposed. The traditional method, such as Soxhlet extraction, consumes too much time and solvent (5). A number of methods, such as ultrasonic solvent extraction (6,7), solid-phase extraction (8,9), supercritical fluid extraction (10), accelerated solvent extraction (11), microwave extraction (12,13), solid-phase microextraction (4,14,15), etc., were widely used in recent years to resolve the problem regarding time and solvent consumption. Whichever technique is used for extraction, various components with a high molecular size, such as lipids, are always present and need to be eliminated to permit a more definitive identification of pesticide residues and to minimize adverse effects on the gas chromatograph (GC) (16). Column chromatography using florisil (17,18), silica gel column (19), and gel-permeation chromatography (20) are popular nowadays.

Compared with the described cleanup methods, chemical digestion methods, namely sulfuric acid treatment, is simple to apply and is of low cost (21). However, certain OCPs, such as dieldrin, endrin, and heptachlor epoxide will be destroyed or removed by the process. By using concentrated sulfuric acid with water, the recoveries of pesticides can be increased, as the polarity of concentrated sulfuric acid increases. Thus, some OCPs will not be removed into the sulfuric acid layer (22).

In the present paper, a step-by-step evaluation study involving ultrasonic solvent extraction and sulfuric acid treatment was carried out for a simple method to analyze 18 OCPs in *Radix Codonopsis*. The methods can analyze nine more OCPs than the method recommended in the *Pharmacopoeia of the People's Republic of China* (23). Sonication provides an efficient contact between the solid and solvent, and the ultrasonic bath is simple and cost efficient to operate. The extraction procedure was optimized with regard to the solvent used and the duration of sonication. The sulfuric acid treatment was optimized with regard to the

* Author to whom correspondence should be addressed: email xuejian5076@yahoo.com.cn.

percentage of water in sulfuric acid. The pesticides were determined by GC–electron capture detection (ECD).

Experimental

Materials

All glassware was washed with liquid soap and rinsed properly with distilled water and then with pure acetone. They were then baked in an oven at 100°C for 12 h. All the solvents used [ethyl acetate, petroleum ether (60–90°C), acetone, methylene chloride, and concentrated sulfuric acid] were of analytical grade (Beijing Chemical Plant, Beijing, China). Pesticide-grade petroleum ether was purchased from Fisher Scientific (Fair Lawn, NJ). An ultrasonic bath (TP-150, 50HZ, 220V) was purchased from TianPeng (Beijing, China). The rotary vacuum evaporator (NE-1) was purchased from Eyela (Tokyo, Japan). All OCP standards [tecnazene,

hexachlorobenzene, α -benzenehexachloride (BHC), PCNB, γ -BHC, heptachlor, aldrin, methyl pentachlorophenyl (MPCPS), β -BHC, δ -BHC, heptachlor epoxide, α -endosulfan, *trans*-chlorodane, *cis*-chlorodane, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDT] (98% purity) were obtained from Sigma, (Munich, Germany).

Ten samples of *Radix Codonopsis* (No 1–10) were bought in a local market (Beijing, China), cut into very small pieces with scissors, ground mechanically to obtain a homogeneous powder, and sieved through a No. 60 mesh sieve (Pharmacopoeia sieve, Ejiang, Shanyin, China).

Preparation of standard solution

A stock solution of pesticide mixture was prepared by dissolving accurate amounts of powdered sample in petroleum ether (pesticide grade). Mass concentrations of compounds were 0.4 $\mu\text{g}/\text{mL}$ for α -endosulfan and 0.2 $\mu\text{g}/\text{mL}$ for all other pesticides.

GC–ECD analysis

A Varian 8000 GC equipped with a ^{63}Ni ECD and a fused-silica capillary column (DB-1701, 30 m \times 0.32-mm i.d., 0.25- μm film thickness) (Agilent, Folsom, CA) was used. The operating conditions were as follow: initial temperature, 120°C (held for 2 min), increased at 8°C/min to 180°C (held for 4 min), then increased at 8°C/min to 225°C, and then increased at 10°C/min to 270°C and held for 10 min; injector temperature, 210°C; carrier gas, nitrogen; injection volume, 1 μL ; detector temperature, 300°C; and make-up gas, nitrogen.

Preparation of spiked samples

Powdered *Radix Codonopsis* (No. 1) (2.0 g) was ground with 2.0 g anhydrous sodium sulfate until a fine powder was obtained (23). The pesticide

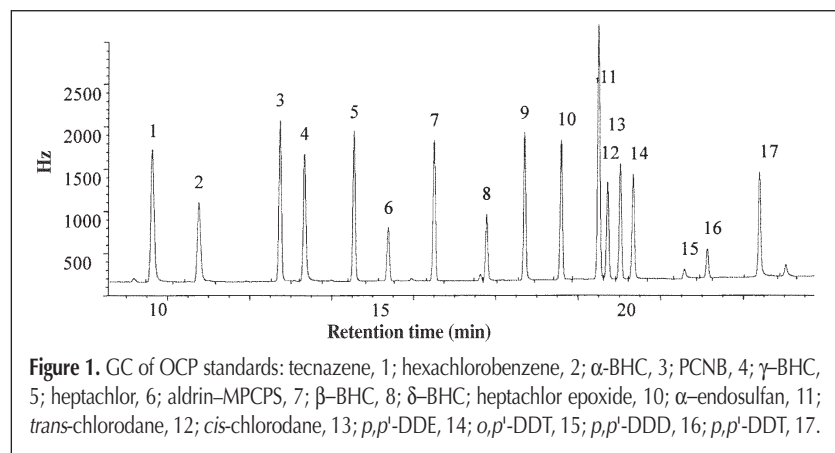


Figure 1. GC of OCP standards: tecnazene, 1; hexachlorobenzene, 2; α -BHC, 3; PCNB, 4; γ -BHC, 5; heptachlor, 6; aldrin–MPCPS, 7; β -BHC, 8; δ -BHC; heptachlor epoxide, 10; α -endosulfan, 11; *trans*-chlorodane, 12; *cis*-chlorodane, 13; *p,p'*-DDE, 14; *o,p'*-DDT, 15; *p,p'*-DDD, 16; *p,p'*-DDT, 17.

Table I. Retention Time and Limit of Detection of the Studied Pesticides

Pesticide	t_R (min)	LOD* (10–1 ng)
Tecnazene	9.800	1.9
Hexachlorobenzene	10.567	2.5
α -BHC	12.367	1.8
PCNB	12.917	4.3
γ -BHC	14.300	1.6
Heptachlor	15.083	2.2
Aldrin–MPCPS [†]	16.145	1.6
β -BHC	17.500	7.5
δ -BHC	18.233	1.3
Heptachlor epoxide	18.850	1.5
α -endosulfan	19.533	9.0
<i>trans</i> -Chlorodane	19.750	1.2
<i>cis</i> -Chlorodane	20.000	1.8
<i>p,p'</i> -DDE	20.250	1.4
<i>o,p'</i> -DDT	21.567	3.8
<i>p,p'</i> -DDD	22.517	3.6
<i>p,p'</i> -DDT	22.933	3.8

* LOD = limit of detection.
[†] Value of the LOD was the sum of the data for both pesticides.

Table II. Recoveries of the Pesticides Obtained by Sulfuric Acid Treatment

Pesticide	Average (%) \pm SD ($n = 3$) for concentrated sulfuric acid with different water content		
	0%	5%	10%
Tecnazene	89.4 \pm 4.1	90.4 \pm 13.2	89.4 \pm 3.4
Hexachlorobenzene	88.9 \pm 5.0	88.9 \pm 6.3	88.1 \pm 15.4
α -BHC	85.6 \pm 6.2	85.3 \pm 5.2	84.6 \pm 5.5
PCNB	79.8 \pm 4.5	80.0 \pm 5.3	79.1 \pm 10.6
γ -BHC	88.6 \pm 7.1	88.9 \pm 6.3	87.7 \pm 9.6
Heptachlor	88.9 \pm 4.9	89.5 \pm 7.4	88.5 \pm 7.3
Aldrin+MPCPS	89.1 \pm 4.2	90.1 \pm 5.5	89.3 \pm 6.3
β -BHC	88.5 \pm 6.2	88.9 \pm 4.3	88.0 \pm 5.5
δ -BHC	87.3 \pm 5.6	87.9 \pm 4.1	87.0 \pm 7.7
Heptachlor epoxide	73.6 \pm 3.0	75.0 \pm 4.9	81.4 \pm 6.1
α -Endosulfan	86.7 \pm 4.2	95.4 \pm 2.3	95.4 \pm 5.7
<i>trans</i> -Chlorodane	91.4 \pm 5.8	91.7 \pm 6.3	90.4 \pm 4.0
<i>cis</i> -Chlorodane	93.3 \pm 3.2	93.5 \pm 5.5	91.9 \pm 6.0
<i>p,p'</i> -DDE	93.3 \pm 12.8	93.6 \pm 4.5	92.1 \pm 5.5
<i>o,p'</i> -DDT	99.4 \pm 3.9	99.7 \pm 6.7	98.8 \pm 6.7
<i>p,p'</i> -DDD	101.5 \pm 4.3	101.3 \pm 4.3	99.8 \pm 5.0
<i>p,p'</i> -DDT	133.9 \pm 9.1	123.7 \pm 5.9	122.6 \pm 17.5

mixture solution (0.5 mL) was added, and the mixture was shaken and left overnight to attain homogeneity.

Optimization of sulfuric acid treatment

The efficiency of the sulfuric acid treatment was checked by recovery experiments. The sulfuration efficiencies of concentrated sulfuric acid, concentrated sulfuric acid with 5% H₂O, and

10% H₂O were compared. The standard pesticides mixture (0.5 mL) and 5 mL of petroleum ether were added to a 10-mL test tube, then 1 mL of differently concentrated sulfuric acid was added to it, and it was shaken vigorously using a vortex mixer for 1 min. The mixture was centrifuged at 3000 rpm for 10 min to separate the two layers, and 1 mL of the upper organic layer was transferred into a GC vial. The pesticides were determined by GC-ECD, and the recoveries were calculated.

Table III. Recoveries of the Pesticides Obtained by Ultrasonic Extraction

Pesticide	Average (%) \pm SD ($n = 3$) for three given organic solvents		
	Ethyl acetate	Acetone-petroleum ether (1:1)	Acetone-methylene chloride (1:1)
Tecnazene	93.5 \pm 11.9	87.2 \pm 6.0	78.0 \pm 6.2
Hexachlorobenzene	91.1 \pm 5.3	78.7 \pm 8.0	120.3 \pm 7.4
α -BHC	91.7 \pm 6.3	86.5 \pm 5.8	81.7 \pm 6.1
PCNB	93.3 \pm 4.5	87.0 \pm 5.8	79.0 \pm 6.9
γ -BHC	110.7 \pm 7.1	101.1 \pm 6.7	102.5 \pm 5.1
Heptachlor	86.0 \pm 4.2	79.0 \pm 5.1	76.0 \pm 4.1
Aldrin-MPCPS	94.3 \pm 3.0	85.3 \pm 5.3	80.9 \pm 6.6
β -BHC	159.9 \pm 4.1	107.7 \pm 5.9	96.6 \pm 4.4
δ -BHC	78.4 \pm 6.7	82.7 \pm 3.1	61.7 \pm 4.3
Heptachlor epoxide	69.4 \pm 4.8	74.8 \pm 6.5	60.0 \pm 5.1
α -Endosulfan	91.6 \pm 6.1	95.4 \pm 5.7	74.4 \pm 3.1
<i>trans</i> -Chlorodane	74.6 \pm 7.8	85.8 \pm 10.4	63.9 \pm 4.2
<i>cis</i> -Chlorodane	74.0 \pm 3.7	80.0 \pm 3.9	64.0 \pm 5.2
<i>p,p'</i> -DDE	73.7 \pm 3.2	79.7 \pm 2.4	63.2 \pm 6.2
<i>o,p'</i> -DDT	72.8 \pm 5.1	95.6 \pm 6.3	92.9 \pm 7.5
<i>p,p'</i> -DDD	78.9 \pm 7.2	75.9 \pm 3.4	66.3 \pm 5.1
<i>p,p'</i> -DDT	101.6 \pm 2.5	83.5 \pm 8.4	76.3 \pm 9.6

Table IV. Recoveries of Pesticides Obtained by Ultrasonic Extraction

Pesticide	Average (%) \pm SD ($n = 3$) for the three-step extraction using acetone-petroleum ether (1:1) as the solvent			
	5 min	10 min	15 min	20 min
Tecnazene	87.0 \pm 5.7	99.5 \pm 7.2	93.5 \pm 5.7	98.2 \pm 6.3
Hexachlorobenzene	73.1 \pm 5.2	81.4 \pm 2.0	76.6 \pm 3.8	81.2 \pm 11.2
α -BHC	94.4 \pm 5.8	108.4 \pm 1.2	100.9 \pm 5.4	106.0 \pm 3.4
PCNB	111.3 \pm 6.9	104.8 \pm 5.8	107.4 \pm 6.7	101.5 \pm 6.5
γ -BHC	111.2 \pm 5.6	118.6 \pm 1.4	108.4 \pm 3.8	117.2 \pm 4.7
Heptachlor	80.2 \pm 4.9	88.5 \pm 11.5	86.7 \pm 4.8	85.4 \pm 7.1
Aldrin+MPCPS	92.5 \pm 4.3	103.2 \pm 10.6	99.3 \pm 4.7	103.4 \pm 6.8
β -BHC	102.5 \pm 10.3	133.9 \pm 2.6	95.1 \pm 5.8	84.3 \pm 4.7
δ -BHC	84.6 \pm 2.3	88.6 \pm 1.8	88.5 \pm 2.3	91.4 \pm 7.4
Heptachlor epoxide	67.4 \pm 11.0	68.2 \pm 1.2	80.5 \pm 3.7	70.2 \pm 0.8
α -Endosulfan	86.5 \pm 8.2	84.3 \pm 5.8	96.6 \pm 5.6	91.4 \pm 8.1
<i>trans</i> -Chlorodane	98.4 \pm 7.5	102.3 \pm 7.9	105.3 \pm 3.9	107.2 \pm 7.7
<i>cis</i> -Chlorodane	79.7 \pm 6.2	80.6 \pm 8.2	85.0 \pm 3.9	85.8 \pm 4.3
<i>p,p'</i> -DDE	88.1 \pm 8.4	86.3 \pm 9.3	94.6 \pm 4.1	95.1 \pm 3.6
<i>o,p'</i> -DDT	90.7 \pm 4.2	98.2 \pm 1.2	87.5 \pm 3.7	85.8 \pm 12.9
<i>p,p'</i> -DDD	71.9 \pm 2.1	73.4 \pm 1.6	75.7 \pm 5.8	78.7 \pm 7.6
<i>p,p'</i> -DDT	122.8 \pm 9.0	98.5 \pm 6.6	93.1 \pm 11.2	110.0 \pm 5.4

Optimization of ultrasonic extraction

The efficiency of the extraction procedure was checked by recovery experiments. In the first set of experiments, the extraction efficiencies of three organic solvents [ethyl acetate, acetone-petroleum ether (1:1), and acetone-methylene chloride (1:1)] (24) were compared. An accurately weighed spiked sample was sonicated for 15 min with 30 mL of various solvents in an ultrasonic bath. The extract was filtered into a clean 100-mL round-bottom flask. The extraction was repeated twice with two additional 20-mL portions of the solvent. The combined extract was evaporated in a rotary vacuum evaporator to dryness at 35°C. The residue was transferred into a 10-mL test tube with 5 mL petroleum ether. The extract was cleaned with concentrated sulfuric acid with 10% water, which gave the highest recovery, and analyzed by GC-ECD.

In the second set of experiments, the optimum time of sonication was determined. The spiked sample was sonicated for 5, 10, 15, and 20 min, with 30 mL acetone-petroleum ether (1:1), which gave the highest recovery rate for the previous pesticide studies. The extraction was repeated twice with two additional 20-mL portions of acetone-petroleum ether (1:1). The extract was cleaned with sulfuric acid treatment and analyzed by GC-ECD.

Recovery studies

Recovery studies were carried out at 10, 50, and 100 ppb (ng/g) fortification levels of each OCP, except for α -endo-sulfan, which was 20, 100, and 200 ppb. The samples were left overnight to attain homogeneity. They were extracted and analyzed as described previously. Recoveries were calculated by GC-ECD.

Results and Discussion

The GC of a mixture of the 18 OCP standards is shown in Figure 1. All except two of the 18 OCPs were well resolved and eluted within a reasonable amount of time (~30 min) under the optimized GC conditions. The retention times and limits of detection of the 18 OCPs are summarized in Table I.

As a traditional method, the clean-up effect of sulfuric acid treatment was good, especially when applied to complicated samples such as TCMs (23). In this work, solutions of the target compounds have been treated with concentrated sulfuric acid with different proportions of water in order to determine the possible loss of pesticides.

As shown in Table II, the best recoveries for all 18 pesticides were obtained by sulfuric acid treatment using concentrated sulfuric acid with 10% water. The three sulfuric acid treatments gave good recoveries overall. However, 10% water treatment gave a better recovery for heptachlor epoxide, which was 81.4%, and the other two treatments gave recoveries that were less than 80.0%. The data indicated that 10% water-sulfuric acid treatment can increase the recovery of heptachlor epoxide.

Table V. Recoveries of 18 Pesticides at Three Fortification Levels

Pesticide	Average (%) \pm SD (n = 5) for the three fortification levels (20, 100, and 200 ppb for α -endosulfan)		
	10 ppb	50 ppb	100 ppb
Tecnazene	114.0 \pm 8.1	91.9 \pm 11.2	85.1 \pm 3.9
Hexachlorobenzene	82.5 \pm 10.0	83.4 \pm 10.7	86.4 \pm 5.6
α -BHC	111.0 \pm 3.8	91.9 \pm 10.9	90.2 \pm 3.8
PCNB	105.9 \pm 13.3	97.1 \pm 14.9	111.9 \pm 6.9
γ -BHC	94.3 \pm 12.2	106.9 \pm 11.6	93.6 \pm 10.0
Heptachlor	108.8 \pm 14.5	85.6 \pm 10.5	82.4 \pm 3.9
Aldrin+MPCPS*	111.3 \pm 4.7	86.4 \pm 7.3	84.6 \pm 4.4
β -BHC	107.0 \pm 10.8	97.3 \pm 12.2	89.1 \pm 9.8
δ -BHC	91.0 \pm 7.4	83.9 \pm 4.7	89.4 \pm 5.6
Heptachlor epoxide	81.9 \pm 10.1	82.6 \pm 8.0	84.7 \pm 4.4
α -Endosulfan	109.7 \pm 15.2	98.1 \pm 7.8	84.1 \pm 8.6
<i>trans</i> -Chlorodane	92.9 \pm 9.5	87.6 \pm 5.1	92.7 \pm 6.5
<i>cis</i> -Chlorodane	95.4 \pm 11.1	81.3 \pm 6.3	89.1 \pm 6.1
<i>p,p'</i> -DDE	97.5 \pm 5.3	81.4 \pm 6.3	83.8 \pm 5.0
<i>o,p'</i> -DDT	81.0 \pm 3.3	83.4 \pm 13.1	86.9 \pm 5.6
<i>p,p'</i> -DDD	79.7 \pm 5.7	81.1 \pm 10.3	77.9 \pm 6.4
<i>p,p'</i> -DDT	85.4 \pm 8.9	96.0 \pm 10.5	88.6 \pm 7.5

* Concentrations of the respective 10, 50, and 100 ppb spiked for each pesticide.

Ultrasonic solvent extraction was used as a simple and inexpensive method applicable to a wide range of samples. The goal of optimization of ultrasonic extraction was to improve the extraction efficiency with the most efficient solvent and minimum time needed for the extraction procedure.

In this work, OCPs were extracted from *Radix Codonopsis* samples by different organic solvents, which are recommended by organizations, such as United States Environmental Protection Agency (24). The ultrasonic extraction efficiency of each solvent was checked by recovery experiments. The results are summarized in Table III. The results show that ultrasonic extraction using acetone-petroleum ether (1:1) gave the best recovery rates, ranging from 74.8% to 101.1% for the 18 OCPs.

Ultrasonic extraction efficiency of different sonic durations was checked by recovery experiments. The results are summarized in Table IV. The results show that the best recovery of the pesticides was obtained by sonication for 15 min in three steps. Shorter or longer sonication caused a decrease in the recoveries of heptachlor epoxide, which were 67.4% for 5 min, 68.2% for 10 min, and 70.2% for 20 min, probably because of insufficient sonication and the degradation of the compound.

The results of the recovery experiments of 18 OCPs at three concentration levels, namely at 10, 50, and 100 ppb (20, 100, and 200 ppb for α -endosulfan), are summarized in Table V. Four fortified samples and one procedural blank were analyzed simultaneously. The procedural blank gave no response for the pesticides analyzed, except for α -BHC, the value of which was 0.5 ng/g. However, it was subtracted when we calculated the recoveries. The percentage recoveries ranged from 77.9% to 114.0%. The reproducibility of an analytical method is characterized by the standard deviation (SD). All SDs reported in Table V were less than 15.2% for all three concentration levels.

The data fully illustrate the reliability of the method for the multiresidue analysis of 18 OCPs in *Radix Codonopsis*.

Table VI. OCPs Levels in 10 *Radix Codonopsis* Samples*

Pesticide	ng/g \pm SD (n = 3)									
	1	2	3	4	5	6	7	8	9	10
Tecnazene	ND [†]	ND	ND	ND	ND	ND	1.4 \pm 4.5	ND	ND	ND
Hexachlorobenzene	ND	ND	ND	ND	ND	0.7 \pm 5.4	2.3 \pm 6.2	ND	1.3 \pm 3.2	ND
α -BHC	0.5 \pm 2.3	0.5 \pm 3.5	1.0 \pm 6.3	1.0 \pm 5.4	1.2 \pm 7.4	1.1 \pm 6.3	2.5 \pm 2.4	1.1 \pm 1.2	0.6 \pm 6.4	ND
PCNB	ND	ND	ND	1.1 \pm 4.5	1.2 \pm 3.2	ND	9.2 \pm 5.6	ND	ND	ND
γ -BHC	ND	ND	ND	1.3 \pm 5.3	2.0 \pm 1.5	1.1 \pm 5.3	2.8 \pm 4.5	2.0 \pm 6.4	1.2 \pm 3.8	ND
Heptachlor	ND	ND	ND	ND	ND	ND	0.4 \pm 5.9	ND	ND	ND
Aldrin+MPCPS	ND	ND	6.6 \pm 6.5	1.0 \pm 6.4	ND	0.5 \pm 6.8	2.9 \pm 5.2	ND	0.8 \pm 6.4	ND
β -BHC	ND	ND	ND	3.3 \pm 3.7	11.0 \pm 5.7	2.7 \pm 6.8	ND	9.9 \pm 5.3	ND	2.9 \pm 5.6
δ -BHC	ND	ND	ND	ND	ND	ND	1.0 \pm 5.7	ND	ND	ND
Heptachlor Epoxide	ND	1.1 \pm 5.6	ND	ND	ND	ND	ND	ND	ND	ND
α -Endosulfan	ND	ND	ND	ND	ND	ND	ND	2.6 \pm 7.5	ND	ND
<i>trans</i> -Chlorodane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>cis</i> -Chlorodane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>p,p'</i> -DDE	ND	0.6 \pm 6.7	1.0 \pm 5.2	ND	ND	ND	1.3 \pm 4.3	ND	ND	ND
<i>o,p'</i> -DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>p,p'</i> -DDD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>p,p'</i> -DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

* Acetone-petroleum ether (1:1) as the extract solvent, ultrasonication for 15 min, three steps; concentrated sulfuric acid with 10% water for sulfuric acid treatment.
[†] ND = Not determined.

Analysis of real samples

Ten samples of *Radix Codonopsis* from different regions bought in a local market were analyzed. To carry out this analysis, the samples were prepared as described previously. The results (Table VI) show the presence of α -BHC, γ -BHC, β -BHC, aldrin, and MPCPS in most samples. The GC–MS system was used to confirm the identification of aldrin and MPCPS.

At present, there is no limiting specification for *Radix Codonopsis*, but the quantities of BHCs, DDTs, and PCNB in the 10 samples were less than the limits set for *Radix Glycyrrhizae* and *Radix Astragali* in the *Pharmacopoeia of the People's Republic of China* (25,26)

Conclusion

A method has been developed for the simultaneous extraction and cleanup of 18 OCPs in the TCM *Radix Codonopsis*. The optimum conditions for extraction were 15 min for ultrasonic duration, with acetone–petroleum ether (1:1) as the extraction solvent: 30, 20, and 20 mL for each step. Concentrated sulfuric acid with 10% water was used for sulfuric acid treatment. The proposed method is simple, rapid, and inexpensive. The total amount of time needed from ultrasonic extraction to GC–ECD analysis is less than 3 h. Preliminary results indicate that the proposed method can be successfully applied to fortified *Radix Codonopsis* samples and real *Radix Codonopsis* samples contaminated with OCPs at the ng/g level. The method may serve as a screening protocol for the determination of OCPs in TCMs on a routine basis. With greater worldwide use of TCMs, the limit standards for OCPs in TCMs need to be improved, and the limit standard for more species of TCMs and more varieties of OCPs should be established.

Acknowledgments

Financial support by the Ministry of Science and Technology of the People's Republic of China under grant 2001BA701A57 is gratefully acknowledged.

References

- O. Akerele. Nature's medicinal bounty: don't throw it away. *World Heal. For.* **14**: 390–95 (1993).
- J. Font and A. Marsal. Determination of organochlorine pesticides in skins and leather by gas chromatography. *J. Chromatogr. A* **811**: 256–60 (1998).
- Y.-C. Ling, H.-C. Teng, and C. Cartwright. Supercritical fluid extraction and clean-up of organochlorine pesticides in Chinese herbal medicine. *J. Chromatogr. A* **835**: 145–57 (1999).
- B.-H. Hwang and M.-R. Lee. Solid-phase microextraction for organochlorine pesticide residues analysis in Chinese herbal formulations. *J. Chromatogr. A* **898**: 245–56 (2000).
- S. Barbic, M. Petrovic, and M. Kastelan-Macan. Ultrasonic solvent extraction of pesticides from soil. *J. Chromatogr. A* **823**: 3–9 (1998).
- D.A. Lambropoulou, T.A. Albanis. Determination of the fungicides vinclozolin and dicloran in soils using ultrasonic extraction coupled

- with solid-phase microextraction. *Anal. Chim. Acta* **514**: 125–30 (2004).
- M. Yasin, P.J. Baugh, G.A. Bonwick, D.H. Davies, P. Hancock, and M. Leinoudi. Analytical method development for the determination of synthetic pyrethroid insecticides in soil by gas chromatography–mass spectrometry operated in negative-ion chemical-ionization mode. *J. Chromatogr. A* **754**: 235–43 (1996).
- J. Tekel and J. Shatrik. Pesticide residue analyses in plant material by chromatographic methods: clean-up procedures and selective detectors. *J. Chromatogr. A* **754**: 397–410 (1996).
- G.R. van der Hoff and P. van Zoonen. Trace analysis of pesticides by gas chromatography. *J. Chromatogr. A* **843**: 301–22 (1999).
- J. Hajslova. *Environmental Contaminants in Food*. C.F. Moffat and K.J. Whittle, Eds. Sheffield Academic Press, Sheffield, UK, 1999, p. 215.
- L. Wennrich, P. Popp, G. Koller, and J. Breuste. Determination of organochlorine pesticides and chlorobenzenes in strawberries by using accelerated solvent extraction combined with sorptive enrichment and gas chromatography/mass spectrometry. *J AOAC Int.* **84**: 1194–1201 (2001).
- M. Numata, T. Yarita, Y. Aoyagi, and A. Takatsu. Evaluation of a microwave-assisted extraction technique for the determination of polychlorinated biphenyls and organochlorine pesticides in sediments. *Anal. Sci.* **20**: 793–98 (2004).
- S.B. Singh, G.C. Foster, and S.U. Khan. Microwave-assisted extraction for the simultaneous determination of thiamethoxam, imidacloprid, and carbendazim residues in fresh and cooked vegetable samples. *J. Agric. Food Chem.* **52**: 105–109 (2004).
- S.H. Brondi and F.M. Lancas. Optimization of a methodology for the determination of organochlorine pesticides in surface water by SPME-GC/MS. *J. Environ. Sci. Health B* **40**: 513–23 (2005).
- M. Correia, C. Delerue-Matos, and A. Alves. Multi-residue methodology for pesticide screening in wines. *J. Chromatogr. A* **889**: 59–67 (2000).
- Farid E. Ahmed. Analyses of pesticides and their metabolites in foods and drinks. *Trends Anal. Chem.* **20(11)**: 649–61 (2001).
- M. Yasin, P.J. Baugh, G.A. Bonwick, D.H. Davies, P. Hancock, and M. Leinoudi. Analytical method development for the determination of synthetic pyrethroid insecticides in soil by gas chromatography–mass spectrometry operated in negative-ion chemical-ionization mode. *J. Chromatogr. A* **754**: 235–43 (1996).
- Method 3620B, Revision 2. *Florisil Cleanup*. United States Environmental Protection Agency, Washington, D.C., 1996.
- S. Thompson, H. Budzinski, K. LeMenach, M. Letellier, and P. Garrigues. Multi-residue analysis of polycyclic aromatic hydrocarbons, polychlorobiphenyls, and organochlorine pesticides in marine sediments. *Anal. Bioanal. Chem.* **372**: 196–204 (2002).
- G.G. Rimkus, M. Rummeler, and I. Nausch. Gel permeation chromatography–high performance liquid chromatography combination as an automated clean-up technique for the multiresidue analysis of fats. *J. Chromatogr. A* **737**: 9–14 (1996).
- L. Berdie, and J.O. Grimalt. Assessment of the sample handling procedures in a labor-saving method for the analysis of organochlorine compounds in a large number of fish samples. *J. Chromatogr. A* **823**: 373–80 (1998).
- Y. Zhou and W. Zhang. Determination of residue of organochlorine containing pesticides in fruits by GC with HP-50 +530um column. *J. Inst. Anal. Chin.* **13**: 58–61 (1994).
- Pharmacopoeia of the People's Republic of China*, Appendix Q. Chemical Industry Press, Beijing, China, 2000.
- Method 3550C, Revision 3. *Ultrasonic Extraction*. United States Environmental Protection Agency, Washington, D.C., 2000.
- Pharmacopoeia of the People's Republic of China*, Chemical Industry Press, Beijing, China, 2000, p. 65.
- Pharmacopoeia of the People's Republic of China*, Chemical Industry Press, Beijing, China, 2000, p. 249.

Manuscript received August 3, 2005;
revision received March 27, 2006.