

Multi-residue Analysis of 18 Organochlorine Pesticides in 10 Traditional Chinese Medicines by Gas Chromatography (GC)

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(Received July 13, 2006; Accepted April 22, 2007)

A rapid and simple method for the simultaneous analysis of 18 pesticides (α -(C₆H₆Cl₆)-Benzene hexachloride, β -BHC, γ -BHC, δ -BHC, heptachlor, heptachlor epoxide, tecnazene, pentachloronitrobenzene (PCNB), hexachlorobenzene, aldrin, methyl pentachlorophenyl sulphide (MPCPS), α -endosulfan, *trans*-chlorodane, *cis*-chlorodane, *p,p'*-(C₁₄H₈Cl₄)-Dichlorodiphenyl dichloroethylene, *o,p'*-(C₁₄H₉Cl₅)-Dichlorodiphenyl trichloroethane, *p,p'*-(C₁₄H₁₀Cl₄)-Dichlorodiphenyl dichloroethane, *p,p'*-DDT) by gas chromatography (GC) in 10 Traditional Chinese Medicines (TCMs) has been developed. The limit of detection was found to be 1.2×10^{-13} to 9.0×10^{-13} g with the recoveries ranging from 72.9 to 114.5% and Relative Standard Deviation being less than 17.5%. In addition, the assay was applied to monitor the residues in 10 TCMs, amongst which at least one of the above pesticides were found in 67 samples. The results showed that α -BHC was the most frequently detected with a presence of 44.6% in the tested samples, whilst heptachlor epoxide, *trans*-chlorodane, *cis*-chlorodane and *p,p'*-DDD were not detected at all. Nonetheless, none of pesticides in all samples appeared to exceed the maximum residue limits (MRLs) seen in the Chinese regulations.

Key words— multi-residue, organochlorine pesticides, Traditional Chinese Medicine, gas chromatography

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INTRODUCTION

Traditional Chinese Medicine (TCM) have been used in medical practice for thousands of years and recognized especially as a valuable and readily available resource for health care in Asian nations. A report by World Health Organization indicated that about 70–80% of the world population rely on non-conventional medicine which are mainly of herbal sources in their primary health-care.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 herbal medicinal products have become a major concern for health authorities, pharmaceutical industries and the public.

TCM have a high risk of contamination of agricultural chemicals, such as organochlorine pesticides (OCPs). Though some OCPs were banned in the 1970s in China due to their toxicity and persistence,²⁾ residues still can be found at present.^{3,4)} As a result, it is an important task to develop methods for the determination of pesticide residues for TCM. However, TCM have very complex matrixes due to a variety of botanical origin and therefore it is difficult and complex to determine the amounts of pesticide residues in the level of less than ng·g⁻¹.⁴⁾

Sample preparation is regarded as a key step in the pesticide residues analysis of TCM. To our knowledge, various components such as lipids are needed to be eliminated so as to permit a more definitive identification of pesticide residues and to minimize interfering effects on the gas chromatography (GC). To this end, various column chromatography using Florisil,^{5,6)} silica gel⁷⁾ and gel permeation⁸⁾ were popularly used in clearing procedure nowadays. However, due to the complexity in the matrixes of TCM, a better procedure may be desirable. Therefore, the aim of this paper is to develop an effective method to determine 18 OCPs residues in TCM with also a view to testing the applicability of the method to quality control of TCM in routine in terms of the residues of OCPs.

MATERIALS AND METHODS

Chemicals, Reagents and Materials— Pesticide Standard: All OCP standards [α -(C₆H₆Cl₆)-Benzene hexachloride, β -BHC, γ -BHC, δ -BHC, heptachlor, heptachlor epoxide, tecnazene, pentachloronitrobenzene (PCNB), hexachloroben-

zene, aldrin, methyl pentachlorophenyl sulphide (MPCPS), α -endosulfan, *trans*-chlorodane, *cis*-chlorodane, *p,p'*-Dichlorodiphenyl dichloroethylene, *o,p'*-Dichlorodiphenyl trichloroethane, *p,p'*-Dichlorodiphenyl dichloroethane, *p,p'*-DDT] (98%, purity were purchased from Sigma, Germany). Stock solution of pesticide mixture was prepared by dissolving accurate amounts of powdered sample in petroleum ether (pesticide grade). The concentrations were maintained at approximately 0.2 $\mu\text{g/ml}$ for all pesticides.

Chemicals: All glassware was washed with distilled water, followed by pure acetone, and subsequently baked in the oven at 100°C for 12 hr. All the solvents used, namely ethyl acetate, petroleum ether (60–90°C), acetone, methylene chloride and concentrated sulphuric acid, were of chromatographic grade (Beijing Chemical Plant, China). Anhydrous Na_2SO_4 was also purchased from Beijing Chemical Plant. Petroleum ether of pesticide grade was purchased from Fisher Scientific (Fair Lawn, NJ). Ultrasonic bath (Tian Peng-150, 50 Hz, 220 V) was purchased from TianPeng, China. The rotary vacuum evaporator (New Rotary Vacuum Eaporator-1) was purchased from Eyela, Japan.

Samples: *Radix Astragali* (13), *Radix Glycyrrhizae* (7), *Radix Rehmanniae* (7), *Flos Chrysanthemi* (10), *Fructus Crataegi* (10), *Fructus Gardeniae* (11), *Fructus Lycii* (8), *Cortex Phellodendri* (9), *Ginkgo Leaf* (9), and *Herba Leonuri* (8) were purchased from the local market, cut into very small pieces with scissors, ground mechanically to obtain a homogeneous powder and sieved through a No.60 mesh sieve.

Sample Preparation—Extraction: An aliquot of 2.0 g sample were accurately weighed into a flask, added 30 ml acetone-*n*-petroleum ether (50+50) and sonicated for 15 min 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 acetone-*n*-petroleum ether (50+50), the extracting solutions were combined and subsequently concentrated to a few milliliters with a rotary vacuum evaporator in a water bath at a temperature of 35°C. The residue was transferred into a 10 ml test tube with 5 ml petroleum ether.

Cleanup: One ml of concentrated sulphuric acid with 10% water, were added to the sample residues and shook vigorously using a vortex-mixer for 1 min, the mixture was then centrifuged at 3000 r/min for 10 min to separate the two layers and

1 ml of the upper organic layer was transferred into a GC vial.

Recovery Test: The recoveries of the 18 OCPs in *Radix Astragali*, *Radix Glycyrrhizae*, *Radix Rehmanniae*, *Flos Chrysanthemi*, *Fructus Crataegi*, *Fructus Gardeniae*, *Fructus Lycii*, *Cortex Phellodendri*, *Ginkgo Leaf*, and *Herba Leonuri* was assessed by spiking a nominal amount of each pesticide to each sample resulting in concentrations at 0.01, 0.05, and 0.1 mg/kg respectively.

Analysis of Pesticides—The prepared sample solutions were detected by GC (Varian 6000 (Palo Alto, CA, U.S.A.)) equipped with a ^{63}Ni electron-capture detector (ECD). Fused-silica capillary column DB-1701 (30 m \times 0.32 mm i.d., film thickness 0.25 μm . Agilent Technology (Palo Alto, CA, U.S.A.)) was used. General operating conditions were as follows: inlet temperature 210°C; detector temperature, 300°C; column temperature initially 120°C for 1 min, heated to 150°C at a rate of 8°C/min and kept for 2 min, finally increased at a rate of 5°C/min to 270°C and kept for 10 min; carrier gas (nitrogen) flow 1.2 ml/min, sample injection volume 1 μl ; injection mode, splitless.

RESULTS AND DISCUSSION

Pesticide Determinations by GC

Optimal condition for the determination of OCPs was investigated. Figure 1 shows typical gas chromatograms of standards for the 18 pesticides. All pesticides were detected within 30 min with adequate separation and good sharp peaks.

Sample Extraction

Three organic solvent systems were utilized to extract 18 pesticides from TCM powder, namely ethyl acetate, acetone: *n*-petroleum ether (50+50) and acetone: *n*-methylene chloride (50+50).⁹⁾ The results showed that acetone: *n*-petroleum ether could lead to the recovery of all the pesticides being greater than 70% with limited matrix interference.

Validation Studies

The developed method was subjected to validation for the 18 pesticides in 10 TCM and one blank extract. The recovery results are showed in Table 1 which indicated that the recoveries of 18 pesticides in 10 TCM were found to be more than 72.9%. The results also showed a good reproducibility for these pesticides with Relative Standard Deviation ranging

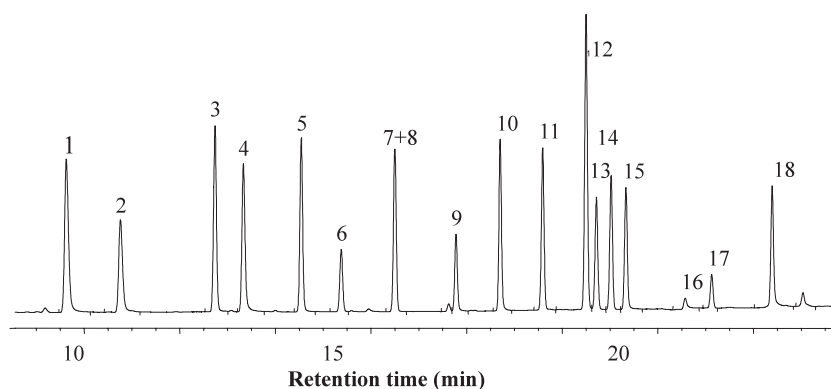


Fig. 1. Gas Chromatogram of OCP Standards

1: tecnazene, 2: hexachlorobenzene, 3: α -BHC, 4: PCNB, 5: γ -BHC, 6: heptachlor, 7+8: aldrin+MPCPS, 9: β -BHC, 10: δ -BHC, 11: heptachlor epoxide, 12: α -endosulfan, 13: *trans*-chlorodane, 14: *cis*-chlorodane, 15: *p, p'*-DDE, 16: *o, p'*-DDT, 17: *p, p'*-DDD, 18: *p, p'*-DDT.

Table 1. Recoveries of 18 Pesticides in 10 TCM at Three Fortification Levels ($n = 5$)

Sample	Average (%) [RSD was in bracket (%)]		
	0.01 (mg/kg)	0.05 (mg/kg)	0.1 (mg/kg)
<i>Radix Astragali</i>	90.7 (15.2)	99.0 (19.0)	95.2 (7.4)
<i>Radix Glycyrrhizae</i>	90.3 (14.0)	102.2 (14.8)	114.5 (9.9)
<i>Radix Rehmanniae</i>	83.6 (13.8)	83.4 (17.6)	72.9 (11.6)
<i>Flos Chrysanthemi</i>	93.2 (11.0)	93.4 (7.5)	90.7 (6.9)
<i>Fructus Crataegi</i>	90.0 (11.6)	87.4 (9.3)	87.7 (8.5)
<i>Fructus Gardeniae</i>	103.5 (14.0)	103.6 (8.5)	88.0 (14.0)
<i>Fructus Lycii</i>	82.4 (17.5)	83.1 (8.6)	78.8 (3.1)
<i>Cortex Phellodendri</i>	94.8 (5.7)	94.1 (13.4)	87.1 (7.5)
<i>Ginkgo Leaf</i>	97.2 (9.7)	89.2 (10.4)	89.0 (5.5)
<i>Herba Leonuri</i>	74.1 (13.0)	86.4 (12.2)	93.6 (9.5)

from 3 to 20% ($n = 5$).

Application to Actual Sample

Using the method developed, 92 specimens of 10 different TCMs were analyzed and data shown in Table 2. The pesticides were detected in specimens including tecnazene, hexachlorobenzene, α -BHC, PCNB, heptachlor, aldrin, MPCPS, β -BHC, δ -BHC, α -endosulfan, *p, p'*-DDE, *o, p'*-DDT and *p, p'*-DDT, and result showed that 72.8% of samples contained at least one of the above pesticides. Amongst detected pesticides, α -BHC, PCNB and hexachlorobenzene were found in 44.6, 34.8, and 33.8% of samples, respectively. The content of pesticide residues were found to range from 0.71 to 78.3 ng·g⁻¹. Although many samples were found the presence of α -BHC, the contents were found be well lower than maximum residue limits (MRLs) seen in Chinese regulations. The content of residues appeared to be material species dependent, for example, some TCM materials such as *Radix As-*

tragali, *Flos Chrysanthemi* and *Fructus Gardeniae* were found a relatively high content of residues, due to either the environmental soil origin or the necessity of the pesticide administration for the purpose of controlling pests, whilst *Cortex Phellodendri* had not been detected any of the 18 pesticides.

The pharmacopoeia of the People's Republic of China 2005 specifically requires that the MRLs^{10,11)} of 9 pesticides to *Radix Astragali* and *Radix Glycyrrhizae*. According to the requirement, PCNB should be less than 0.1 ppm, whilst a total amount of α -BHC, γ -BHC, β -BHC and δ -BHC, and the total level of DDT, including *p, p'*-DDE, *o, p'*-DDT, *p, p'*-DDD and *p, p'*-DDT should be less than 0.2 ppm. In the present study, the results showed that although at least one of pesticides were detected in most of specimens, no sample was found to violate the Chinese MRLs. Nonetheless, 19.6% of the analyzed samples were found to exceed European MRLs¹²⁾ in terms of the pesticide level of hexachlorobenzene, BHC (α , γ , β , δ), PCNB,

Table 2. Results of Monitoring of 92 TCM Samples Using Proposed Methods

Pesticide	Positive			
	Sample	No.	Detection Ratio ^{a)} (%)	Concentration (ng/g)
α -BHC	<i>Radix Astragali</i>	8	8.7	1.1– 5.6
	<i>Radix Glycyrrhizae</i>	4	4.3	1.9– 3.7
	<i>Radix Rehmanniae</i>	3	3.3	1.2– 5.0
	<i>Flos Chrysanthemi</i>	6	6.5	2.8– 9.9
	<i>Fructus Crataegi</i>	3	3.3	1.7– 2.5
	<i>Fructus Gardeniae</i>	8	8.7	3.1–54.6
	<i>Fructus Lycii</i>	1	1.1	2.8
	<i>Ginkgo Leaf</i>	1	1.1	3.4
	<i>Herba Leonuri</i>	7	7.6	4.7–15.4
Total	41	44.6		
β -BHC	<i>Radix Astragali</i>	4	4.3	2.8– 3.8
	<i>Fructus Gardeniae</i>	1	1.1	78.3
	Total	5	5.4	
γ -BHC	<i>Radix Astragali</i>	5	5.4	1.8– 4.3
	<i>Radix Glycyrrhizae</i>	2	2.2	1.1– 2.1
	<i>Radix Rehmanniae</i>	1	1.1	0.7
	<i>Flos Chrysanthemi</i>	1	1.1	11.4
	<i>Fructus Crataegi</i>	1	1.1	1.8
	<i>Fructus Gardeniae</i>	1	1.1	15.2
	<i>Fructus Lycii</i>	2	2.2	2.1–19.4
	<i>Ginkgo Leaf</i>	2	2.2	1.7
	<i>Herba Leonuri</i>	3	3.3	5.9– 7.4
Total	18	19.7		
δ -BHC	<i>Radix Astragali</i>	4	4.3	1.4–13.0
	<i>Radix Rehmanniae</i>	1	1.1	8.5
	<i>Fructus Gardeniae</i>	1	1.1	27.2
	<i>Ginkgo Leaf</i>	3	3.3	1.5– 5.4
	<i>Herba Leonuri</i>	1	1.1	4.9
Total	10	10.9		
Heptachlor	<i>Radix Astragali</i>	2	2.2	2.6– 5.1
	<i>Radix Glycyrrhizae</i>	1	1.1	6.9
	<i>Fructus Crataegi</i>	7	7.6	2.5– 8.8
	<i>Fructus Gardeniae</i>	2	2.2	29.3–73.0
Total	12	13.1		
Tecnazene	<i>Radix Astragali</i>	2	2.2	2.4– 2.9
	<i>Flos Chrysanthemi</i>	5	5.4	2.6–13.7
	<i>Fructus Crataegi</i>	1	1.1	4.1
	<i>Fructus Gardeniae</i>	6	6.5	3.6–16.0
	<i>Herba Leonuri</i>	5	5.4	3.6–30.3
Total	19	20.6		
PCNB	<i>Radix Astragali</i>	1	1.1	3.7
	<i>Radix Glycyrrhizae</i>	1	1.1	1.5
	<i>Radix Rehmanniae</i>	1	1.1	1.6
	<i>Flos Chrysanthemi</i>	6	6.5	7.8–20.2
	<i>Fructus Crataegi</i>	4	4.3	4.0–41.0
	<i>Fructus Gardeniae</i>	9	9.8	4.1–22.6
	<i>Fructus Lycii</i>	2	2.2	5.6–23.5
	<i>Herba Leonuri</i>	8	8.7	8.9–59.5
Total	32	34.8		

a) Number of positive samples/number of total TCMs (92).

Table 2. Continued

Pesticide	Sample	Positive		
		No.	Detection Ratio ^{a)} (%)	Concentration (ng/g)
Hexachlorobenzene	<i>Radix Astragali</i>	2	2.2	2.9– 4.4
	<i>Radix Glycyrrhizae</i>	3	3.3	1.4– 1.8
	<i>Flos Chrysanthemi</i>	6	6.5	3.9– 8.4
	<i>Fructus Crataegi</i>	3	3.3	2.6– 4.4
	<i>Fructus Gardeniae</i>	8	8.7	5.9–51.5
	<i>Fructus Lycii</i>	2	2.2	7.9–14.4
	<i>Herba Leonuri</i>	7	7.6	4.7–18.8
Total		31	33.8	
Aldrin+MPCPS	<i>Flos Chrysanthemi</i>	5	5.4	5.5–17.1
	<i>Fructus Crataegi</i>	2	2.2	1.2– 1.7
	<i>Fructus Lycii</i>	1	1.1	8.7
	<i>Ginkgo Leaf</i>	6	6.5	4.2–12.0
Total		14	15.2	
α -Endosulfan	<i>Fructus Lycii</i>	1	1.1	1.2
	<i>Ginkgo Leaf</i>	1	1.1	3.2
Total		2	2.2	
<i>p, p'</i> -DDE	<i>Radix Astragali</i>	4	4.3	2.7–19.0
	<i>Radix Glycyrrhizae</i>	1	1.1	3.1
	<i>Radix Rehmanniae</i>	1	1.1	3.7
	<i>Flos Chrysanthemi</i>	4	4.3	3.6–26.6
	<i>Fructus Crataegi</i>	2	2.2	3.6– 4.4
	<i>Fructus Lycii</i>	2	2.2	5.2–10.3
	<i>Ginkgo Leaf</i>	6	6.5	1.0–14.9
	<i>Herba Leonuri</i>	4	4.3	7.6–13.3
Total		24	26.0	
<i>o, p'</i> -DDT	<i>Flos Chrysanthemi</i>	1	1.1	42.3
	<i>Ginkgo Leaf</i>	3	3.3	1.0–18.1
Total		4	4.4	
<i>p, p'</i> -DDT	<i>Ginkgo Leaf</i>	1	1.1	12.6

heptachlor and DDT (*p, p'*-DDE, *o, p'*-DDT, *p, p'*-DDD, *p, p'*-DDT).

In conclusion, a practical analytical method has been described for the determination of residues of 18 OCPs in 10 Traditional Chinese Medicine by GC. Pre-treatment with acetone-*n*-petroleum ether (50 + 50) extraction and concentrated sulphuric acid with 10% water was used for cleanup and found to allow the determination without interference. The high recovery, lower limits of detection and better reproducibility ensured that the method could serve as a monitoring protocol for the determination of OCPs in TCM on routine basis.

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

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