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Supercritical fluid extraction and clean-up of organochlorine pesticides in Chinese herbal medicine

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Abstract

A method involving the simultaneous extraction and clean-up of 13 organochlorine pesticides (OCPs) from Chinese herbal medicines (CHMs) was developed using supercritical fluid extraction (SFE) followed by gas chromatography–electron capture detection and mass spectrometric confirmation. The pesticides in the study consisted of α-, β-, γ-, and δ-benzene hexachloride, heptachlor, aldrin, heptachlor epoxide, endosulfan I, 4,4′-DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethene), dieldrin, endrin, 4,4′-DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane), endosulfan II, 4,4′-DDT (2,2-bis(*p*-chlorophenyl)1,1,1-trichloroethane), endrin aldehyde, and endosulfan sulfate. A series of experiments was conducted to optimize the final extraction conditions [pure CO_2, 250 atm extraction pressure (1 atm=101 325 Pa), 50°C extraction temperature, 5 min static extraction time, 20 min dynamic extraction time, 2.0-g Florisil sorbent on top of 0.1-g samples, 12-ml *n*-hexane eluting at 1 ml/min, and a 10-ml extraction vessel]. Florisil sorbent was placed with the sample in the SFE vessel to provide a facile and effective clean-up approach. Mean recoveries of 78–121% with reproducibilities of 5–31% were obtained for the pesticides except for endosulfan II, endosulfan sulfate and endrin aldehyde. The simple and rapid method may be used to determine OCPs in CHMs routinely, and in fact, was used to analyze CHMs sold in Taiwan.

Keywords: Extraction methods; Pharmaceutical analysis; Organochlorine compounds; Pesticides

1. Introduction

The first medicines for mankind came from herbal plants. The effectiveness and safety claimed by most herbal medicines were generally based on historical experience, rather than on scientific and clinical evidence which are required by modern scientific medicine [1]. The therapeutic effects of some herbal remedies have recently been demonstrated in scientific investigations [2–4]. Herbal remedies, i.e., with known mild pharmaceutical effects and minimum side effects, have won respect and look promising [5–8]. Regulatory reforms are currently being undertaken in the United States [9], European countries [10], and Taiwan [11] to encourage scientific research on herbal remedies as an alternative approach to the development of new pharmaceuticals.

The practice and use of herbal remedies and medicines have been prevalent among the Chinese for thousands of years. A variety of herbal plants are consumed daily by the Chinese either as medicines or as food ingredients. Chinese herbal medicine (CHM) plays an important part in the health of the

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Chinese and has become an indispensable part of Chinese daily living. However, to meet the heavy demand of CHMs in Taiwan, half of the CHMs sold on the market are imported. The safety of the imported CHMs is generally unknown. Safety issues in herbal medicines is a subject of scientific interest [12,13] and the balance between toxicity, risk and benefit must always be considered [14,15]. The cultivation of herbal plants used for CHMs usually takes years of time and requires the use of pesticides to reduce pest damage. Improper use of pesticides not only pollutes the cultivating soil, and ground water, but also leads to accumulation of pesticides in the plants. Pesticides may therefore be present in the CHMs. The risk to health due to the incidental intake of contaminants in the CHM by the consumers is highly possible. The need to analyze residual contaminants in CHM becomes apparent. We were entrusted by the Committee of Chinese Medicine and Pharmacy, Department of Health of Taiwan to conduct research for this purpose. The organochlorine pesticides (OCPs) are the first target analytes because they are still used in CHM-exporting countries. Furthermore, OCPs possess a long half life, high accumulation potential, harmful biological effects, and detrimental impacts on the environment [16–18].

The matrices encountered in the analysis of pesticides in CHMs usually were dried plants (single-component) or mixture of dried plants (composite-description) and mostly in powder form. Some popular composite-descriptions were sold in ready-to-use form, i.e., as scientific CHM, which were manufactured from raw materials of known quality and using standard processing procedures to assure consistent therapeutic effects. The preference of composition-description CHMs over single-component CHMs is not compatible with modern medicinal practice because benefits of CHM frequently result from the interactions between various ingredients to produce synergistic therapeutic effects.

The analysis of OCPs in CHMs might therefore encounter specific problems concerning extraction, purification and detection of OCPs in complex matrices. Pesticides in matrices similar to CHM, such as fruit, vegetable, and medical plant matrices, have been determined using various techniques to separate pesticides from the matrices followed by clean-up and concentration procedures before GC determination [19–31]. The general drawbacks, such as the use of large amounts of solvents, time-consuming, labor-intensive and considerable waste production, associated with these classical extraction techniques have been reduced using matrix solid-phase dispersion [19,20] and supercritical fluid extraction (SFE) [27–31]. SFE has shown to be an efficient and rapid method for the isolation of pesticides from fruit, vegetable, and medical plant matrices [27–31]. The benefits in speed, cost, and the use of small amounts of solvents in traditional SFE are further increased by the integration of clean-up methods into SFE [31–34], which makes SFE an ideal and practical alternative to classical Soxhlet and sonication extractions.

The objective of the present study was to investigate the use of an on-line clean-up SFE method for the analysis of 16 OCPs residues [α-, β-, γ-, and δ-benzene hexachloride (BHC), heptachlor, aldrin, heptachlor epoxide, endosulfan I, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethene (4,4′-DDE), dieldrin, endrin, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (4,4′-DDD), endosulfan II, 2,2-bis(*p*-chlorophenyl)1,1,1-trichloroethane (4,4′-DDT), endrin aldehyde, and endosulfan sulfate] in CHMs followed by GC with electron capture detection (GC–ECD) at ng/g levels. The proposed SFE method has been applied to determine OCPs in CHMs sold on local markets and evaluated by the standard addition approach. GC–ECD positive results were confirmed with GC mass spectrometric detection (GC–MS).

2. Experimental

2.1. Chemicals

All chemical standards were obtained from AccuStandard (New Haven, CT, USA). Hexachlorobenzene (HCB) was added to each sample prior to extraction to check that no leaks or other potential problems with SFE occurred during extraction, and 100 ng/g mirex was added to the extracts to serve as an internal standard. The solvents of *n*-hexane and acetone were Absolv grade from Tedia (Fairfield, OH, USA). The extraction fluid was SFC (supercritical fluid chromatography)/SFE grade carbon dioxide with helium head (2000 p.s.i.; 1 p.s.i.=6894.76 Pa) from Air Products (Plumsteadville, PA, USA). The

carbon dioxide tank was changed when the tank pressure reduced to ~700 p.s.i. The ODS (C_{18}, octadecylsilyl derivatized silica, 40 μm) was obtained from Nacalia (Tokyo, Japan). Florisil (PR grade 147–225 μm) was obtained from Janssen (Geel, Belgium). Silica gel (74–147 μm, ASTM 923 grade) was obtained from Aldrich (Milwaukee, WI, USA). Neutral alumina (64–193 μm, activity I) was obtained from Merck (Darmstadt, Germany). C_{18} was prewashed by continuously refluxing in *n*-hexane–acetone (1:1) for 24 h in a Soxhlet apparatus followed by oven drying at 60°C for 1 h. Silica gel, Florisil, and alumina were activated by drying at 150°C for 12 h before use. Sorbent activity was estimated using the Brockmann scale (based on the elution characteristics of a series of test dyes). The physiochemical properties of the 16 OCPs in the study are listed in Table 1.

2.2. CHM samples and spiking methods

Twenty single-component and 11 scientific CHMs were purchased from the local market. The CHMs were usually dried. The single-component CHMs were ground mechanically to obtain a homogeneous powder and sieved through a No. 60 mesh sieve. *Glycyrrhizae radix* samples used for fortified and control studies were obtained by extracting ~30 g of sieved powders with 250 ml of water for 8 h, followed by extracting with 250 ml of 50% methanol, and *n*-hexane, each for 8 h. The cleaned *Glycyrrhizae radix* was dried at 50°C and kept in a desiccator prior to use. Chemical analysis revealed that the thus obtained *Glycyrrhizae radix* blanks were free of OCPs residues. Aliquots of *Glycyrrhizae radix* blanks (~0.1 g) were loaded into 10-ml stainless steel extraction vessels and followed by adding a known amount of OCPs (25 μl, 3 μg/ml) and HCB (50 μl, 1 μg/ml) to prepare 75 ng/ml OCPs fortified samples. In order to assure that the analyte–matrix interactions in the fortified samples resembled those in the real-world samples, a slurry spiking method was used to prepare the fortified samples [38–40]. The solvent was evaporated to dryness at ambient temperature for ~5 min. Sorbent 2.0 g Florisil (or C_{18}, silica gel, neutral alumina) was placed on top, i.e., the exit end, of the 0.1 g CHM samples.

2.3. SFE

All extractions were performed on a Suprex (Pittsburgh, PA, USA) PrepMaster equipped with an AccuTrap collecting device. The CO_2 extracts containing the analytes were trapped by the deactivated fused-silica beads (55–105 μm; Sigma, MO, USA)

Table 1
Physiochemical properties of the 16 OCPs

Entry	Analyte	Molecular formula	Molecular weight	Vapor pressure [35] (mmHg)	Melting point [35] (°C)	Log K_{ow} [e] [37]
1	α-BHC	$C_6H_6Cl_6$	290.80	$2.5\cdot10^{-5}$ [a]	159–160	3.78–3.81
2	β-BHC	$C_6H_6Cl_6$	290.80	$2.8\cdot10^{-7}$ [a]	309–310	3.80–3.84
3	γ-BHC	$C_6H_6Cl_6$	290.80	$9.4\cdot10^{-6}$ [a]	112–113	3.66–3.72
4	Heptachlor	$C_{10}H_5Cl_7$	373.35	$3.0\cdot10^{-4}$ [b]	95–96	5.27–6.06
5	δ-BHC	$C_6H_6Cl_6$	290.80	$1.7\cdot10^{-5}$ [a]	138–139	–
6	Aldrin	$C_{12}H_8Cl_6$	364.93	$6.0\cdot10^{-6}$ [b]	104–104.5	5.66–7.40
7	Heptachlor epoxide	$C_{10}H_5Cl_7O$	389.34	$3.0\cdot10^{-7}$ [a]	157.5–159	5.40
8	Endosulfan I	$C_9H_6Cl_6O_3S$	406.96	$9.0\cdot10^{-3}$ [d] [36]	109 [36]	3.74 [49]
9	4,4'-DDE	$C_{14}H_8Cl_4$	318.05	$5.5\cdot10^{-6}$ [c] [36]	88–90 [36]	5.69–6.96
10	Dieldrin	$C_{12}H_8Cl_6O$	380.93	$1.8\cdot10^{-7}$ [b]	176–177	4.32–5.40
11	Endrin	$C_{12}H_8Cl_6O$	380.93	$2.0\cdot10^{-7}$ [b]	>200	4.56–5.20
12	4,4'-DDD	$C_{14}H_{10}Cl_4$	320.05	$6.5\cdot10^{-6}$ [c] [36]	109–110 [36]	6.22
13	Endosulfan II	$C_9H_6Cl_6O_3S$	406.96	–	213	3.74 [49]
14	4,4'-DDT	$C_{14}H_9Cl_5$	354.49	$1.5\cdot10^{-7}$ [a]	108.5	6.19–6.91
15	Endrin aldehyde	$C_{12}H_8Cl_6O$	380.93	–	–	–
16	Endosulfan sulfate	$C_9H_6Cl_6O_4S$	422.95	–	–	–

[a] At 20°C; [b] at 25°C; [c] at 30°C; [d] at 80°C; [e] octanol–water partition coefficient.
1 mmHg=133.322 Pa.

in the AccuTrap first at −30°C. They were then eluted from the trap at 30°C using an appropriate volume of *n*-hexane. The restrictor temperature was fixed at 80°C. The *n*-hexane eluate in the collection vial was then transferred into a concentration tube and purged with nitrogen to a volume of ~0.8 ml. One hundred μl of mirex quantitation standard was added. The final volume of the eluate was adjusted to 1.0 ml and subjected to GC–ECD analyses. For GC–MS confirmations, the eluates were concentrated further to meet the higher detection limit of the MS detector.

2.4. Optimization study

The SFE efficiency of analytes from solid matrices is controlled by many factors, including the affinity of the components towards the matrix, the vapor pressure of the analytes, and the solubility and diffusion coefficients of the components in the supercritical fluid. These factors are governed by a complex relationship between many experimental variables, including modifier, pressure, temperature, static and dynamic extraction time [40–42]. To our knowledge, OCPs in CHMs have not been determined using SFE before. In this study, we therefore conducted a systematic study to optimize the extraction conditions. The same 75 ng/ml concentration of fortified OCPs was used throughout the optimization study. A single variable was changed while all other variables were kept constant. Relative effects of each controlled SFE variable were evaluated based on the optimal combination of maximal overall recoveries of analytes and minimal interference.

2.5. GC

GC–ECD analyses were carried out using a HP-5890 Series II gas chromatograph equipped with a SPB-608 capillary column (30 m×0.25 mm I.D., 0.25 μm film thickness) and a ^{63}Ni electron capture detector. Samples were introduced into the GC column via an on-column injector system. The injector temperature was programmed similarly to that for the column, except for being maintained 3°C higher. The ECD was operated at 300°C. Nitrogen was used as the carrier gas at a constant flow-rate of 1.1 ml/min. Nitrogen was also used as the make-up gas at a constant flow-rate of 43 ml/min. The column temperature was initially held at 60°C, then programmed at 30 C°/min to 150°C and held for 2

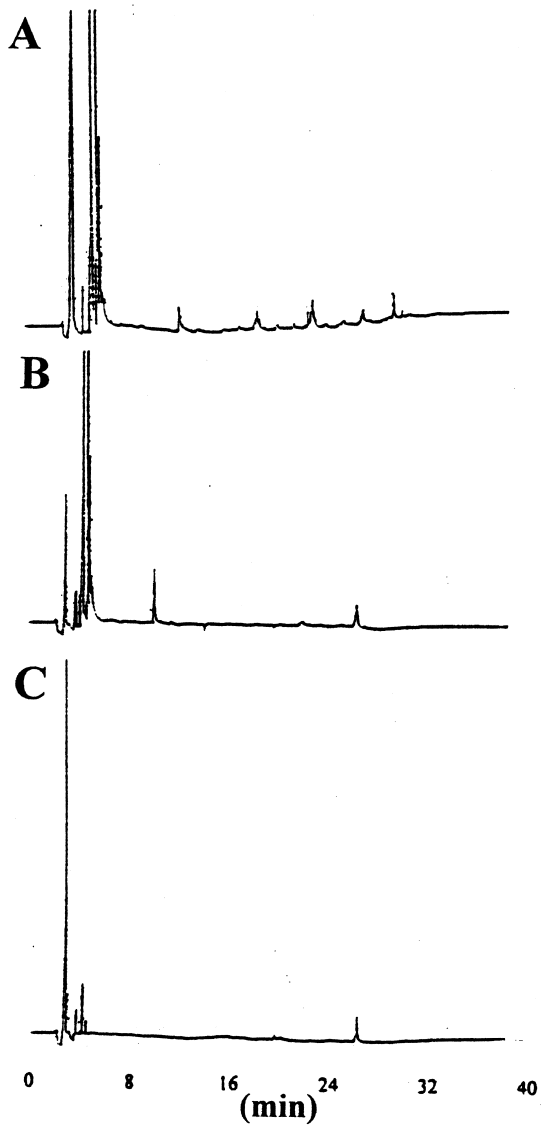

Fig. 1. Representative GC–ECD chromatograms of SFE extracts of (A) 0.1 g *Glycyrrhizae radix* blanks in the extraction vessel; (B) 2.0 g Florisil trapping sorbent on top of 0.1 g *Glycyrrhizae radix* blanks in the extraction vessel, and (C) 2.0 g Florisil trapping sorbent in the extraction vessel. Extraction conditions: modifier=null; pressure=250 atm; temperature=50°C; static extraction time=5 min; dynamic extraction time=15 min.

min, then a 7.3 C°/min to 285°C and held for 10 min.

GC–MS confirmations were carried out using a HP 5890 Series II 5972 MSD. equipped with a J and W DB-5MS (30 m×0.25 mm I.D., 0.25 μm film thickness). The injector was operated in the split mode and at 250°C. Helium was used as the carrier gas at a flow-rate of 1.0 ml/min. The column temperature was initially held at 70°C for 2 min, then programmed at 20 C°/min to 180°C and held for 2 min, then at 10 C°/min to 300°C and held for 4 min. Effluents from the GC column were transferred via a transfer line held at 250°C and fed into a 70 eV electron impact ionization source held at 250°C. OCPs were analyzed using selected ion monitoring (SIM) GC–MS as previously reported [43].

3. Results and discussion

3.1. Sample pretreatment and clean-up

Trapping sorbents were loaded on top of the CHM samples in the extraction vessels for on-line clean-up of interfering species. Trapping sorbents such as Florisil, C_{18}, silica gel, and neutral alumina which have been used in various solid matrices [32,33,44,45] were evaluated. All sorbents, except Florisil, failed to produce clean chromatograms (results not shown). The cleanest chromatogram was obtained using Florisil sorbent (Fig. 1B) and is comparable to that of the system blank (Fig. 1C). The disappearance of the matrix peaks in the chromatogram of samples without using trapping sorbents (Fig. 1A) evidenced the clean-up efficiency of Florisil sorbent. Subsequent analyses were therefore conducted with 2.0 g Florisil sorbent on top of 0.1 g samples.

3.2. Effect of modifier on the SFE of OCPs from the fortified Glycyrrhizae radix

Supercritical CO_2 is an ideal fluid for SFE except for one drawback, the limited ability to dissolve polar or high molecular weight compounds even at very high densities. The solubility can be improved by the addition of polar organic solvents, known as modifiers, to the fluid. The extent of increase in solubility and selectivity of the fluid mixture are determined by the identity and concentration of the modifiers. Fig. 2 shows the effect of adding 0.1 ml of acetone, methanol, ethyl acetate, or tetrahydrofuran (THF) to the fortified *Glycyrrhizae radix*. The mean recovery was 70% when modifier was not added, and changed to 67%, 61%, 67%, 66%, respectively, when 0.5 ml modifier of acetone, methanol, ethyl acetate, or THF was added to the fortified *Glycyrrhizae radix*. The overall recoveries appeared to be similar regardless of the addition of polar modifier. The phenomenon was ascribed to the

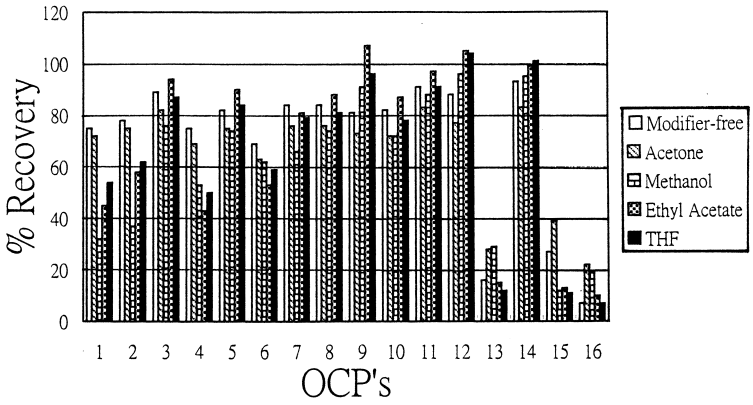

Fig. 2. Effect of modifier on the SFE of OCPs from the fortified *Glycyrrhizae radix*. Refer to Table 1 for assigned OCP numbers. Extraction conditions: modifier=specified in the figure; pressure=250 atm; temperature=50°C; static extraction time=5 min; dynamic extraction time=15 min.

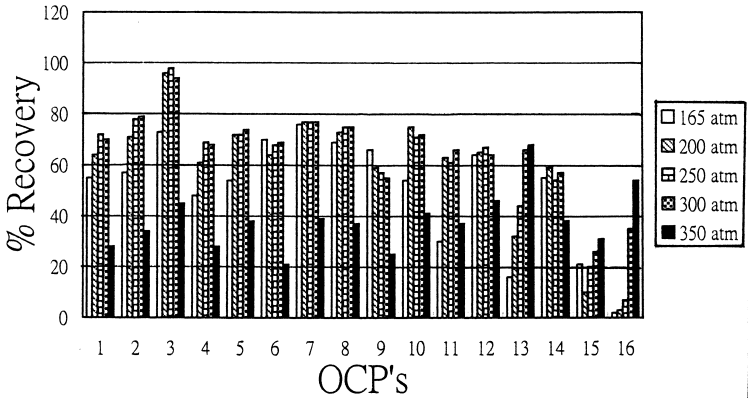

Fig. 3. Effect of pressure on the SFE of OCPs from the fortified *Glycyrrhizae radix*. Refer to Table 1 for assigned OCP numbers. Extraction conditions: modifier=null; pressure=specified in the figure; temperature=50°C; static extraction time=5 min; dynamic extraction time=15 min.

presence of intrinsic moisture, i.e., 8%, in the CHMs, which might function as polar modifier [46,47]. On average, the intrinsic moisture in the CHMs was measured to be ~10%, which was sufficient to increase the solubility power of the mixture fluid. Further addition of polar modifiers did not yield improved recoveries. Subsequent SFE was carried out without the addition of any modifier.

3.3. Effect of pressure on the SFE of OCPs from the fortified Glycyrrhizae radix

The influence of pressure on the recoveries of fortified OCPs from the fortified *Glycyrrhizae radix* and matrix interference was studied at under five conditions. As Fig. 3 shows, the recoveries of most analytes increased with increasing pressure from 165 atm to 300 atm (1 atm=101 325 Pa). Above this value, increasing pressure had no effect. The recoveries at 300 atm are a little higher than those at 250 atm, the problem of matrix interference, i.e., from the late eluting components, is more serious, however. Subsequent SFE was carried out at 250 atm extraction pressure.

3.4. Effect of temperature on the SFE of OCPs from the fortified Glycyrrhizae radix

The variation of temperature during the SFE will effect the density of fluid, the volatile property of the

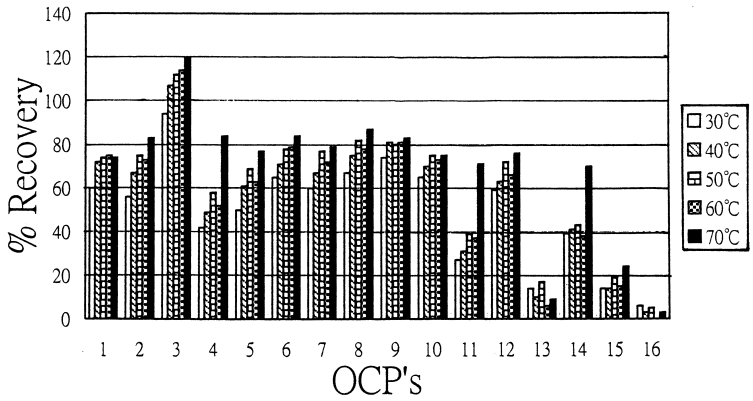

Fig. 4. Effect of temperature on the SFE of OCPs from the fortified *Glycyrrhizae radix*. Refer to Table 1 for assigned OCP numbers. Extraction conditions: modifier=null; pressure=250 atm; temperature=specified in the figure; static extraction time=5 min; dynamic extraction time=15 min.

analytes, and the desorption of the analytes from the matrix. The analytes become more volatile at elevated temperatures but the supercritical CO_2 density decreases with increasing temperature at fixed pressure. The influence of temperature on the recoveries of fortified OCPs from the fortified *Glycyrrhizae radix* and matrix interference was studied at five different temperatures (30, 40, 50, 60, and 70°C).

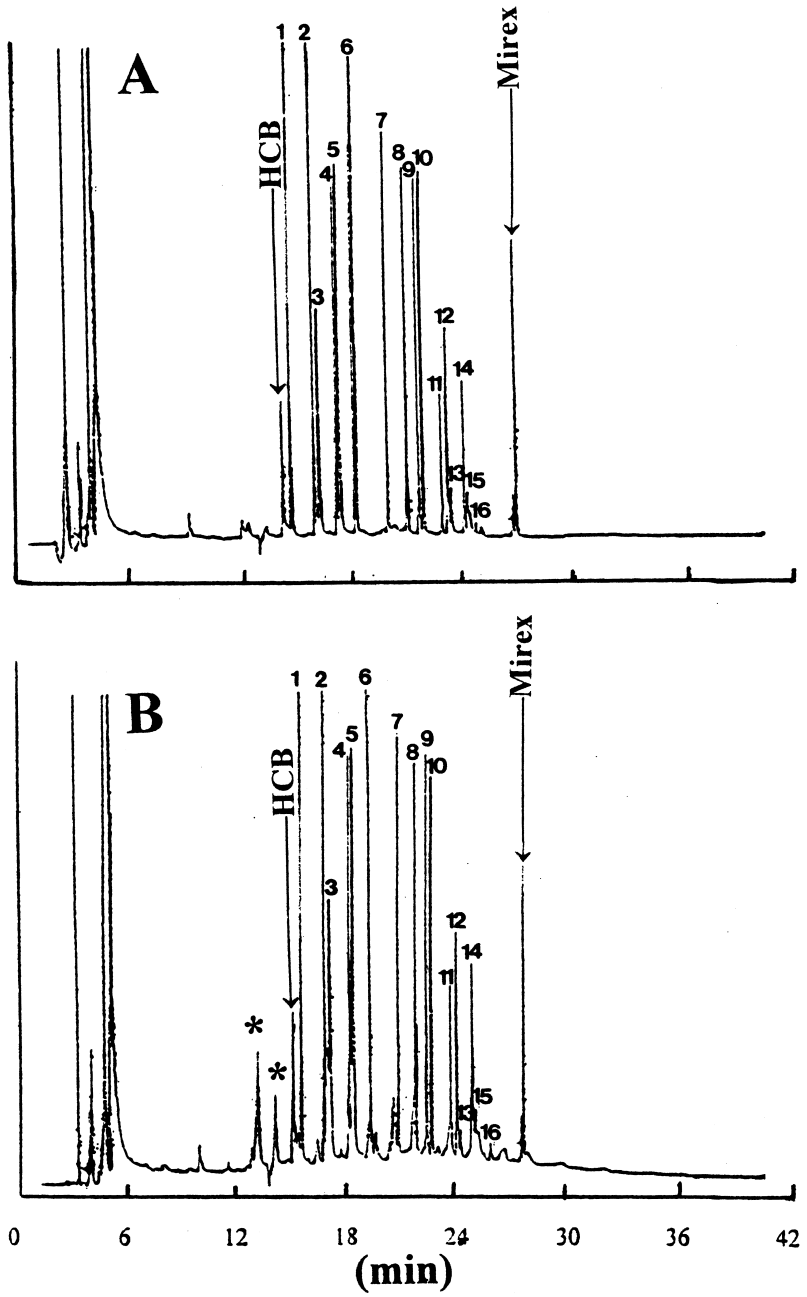

Fig. 5. GC–ECD chromatograms of SFE extracts of fortified *Glycyrrhizae radix* at (A) 50°C, and (B) 70°C. Refer to Table 1 for assigned OCP numbers. Extraction conditions: modifier=null; pressure=250 atm; temperature=specified in the figure; static extraction time=5 min; dynamic extraction time=15 min.

The lowest temperature, 30°C, was below the critical temperature and yielded the lowest recoveries (Fig. 4). The recoveries increased from 30°C to 50°C for most analytes, probably because the maximum solubility was reached at 50°C. Further increase in temperature, i.e., to 60°C, lowered the solubility, although the difference was not significant. The abrupt increase in recoveries at 70°C was surprising. A detailed inspection of the GC chromatogram (Fig. 5) indicates that this is presumably due to the increase of matrix interference, i.e., from the early eluting components. Subsequent SFE was carried out at 50°C extraction temperature.

3.5. Effect of extraction time on the SFE of OCPs from the fortified Glycyrrhizae radix

The length of extraction time would influence the extraction efficiency and selectivity of the fluid. The static mode allowed a better penetration of the matrix by the fluid than the dynamic mode [38]. The recoveries versus the static extraction time are plotted in Fig. 6. An increase in recoveries was observed when the static extraction time increased from 1 min to 5 min. Above this value, increasing extraction time had no effect. A static extraction time of 5 min allowed the supercritical fluid to access readily the analytes. The dynamic mode allowed a higher analyte's solubility in the supercritical fluid. The recoveries versus the dynamic extraction time are plotted in Fig. 7. An increase in recoveries was observed when the dynamic extraction time increased from 5 min to 20 min. The recoveries were observed to decrease when the dynamic extraction time increased further to 25 min. Presumably, the analytes were completely extracted from the matrix after 20 min of dynamic extraction and trapped on the surface of low temperature (−30°C) silica beads. Prolonged dynamic extraction time would generate an excess of clean supercritical fluid at the late extraction period, i.e., after 20 min. This clean supercritical fluid would desorb part of the trapped analytes, which had no strong interactions with the silica beads, and evolved into the ambient. Subsequent SFE was carried out with 5 min static and 20 min dynamic extraction time.

3.6. Quantitation

Based on the above results, the optimal working extraction condition obtained was: pure CO_2, 250 atm extraction pressure, 50°C extraction temperature, 5 min static extraction time, 20 min dynamic extraction time, 2.0 g Florisil sorbent on top of 0.1 g samples, 12-ml *n*-hexane eluting at 1 ml/min, and a 10-ml extraction vessel. This condition was used throughout the study, unless otherwise specified.

The fortified levels used in this study were designed to encounter the trace contaminants possibly found in CHMs sold on the market. These values were much lower than the tolerable ranges of US FDA Action Levels, i.e., 0.3 μg/g for heptachlor,

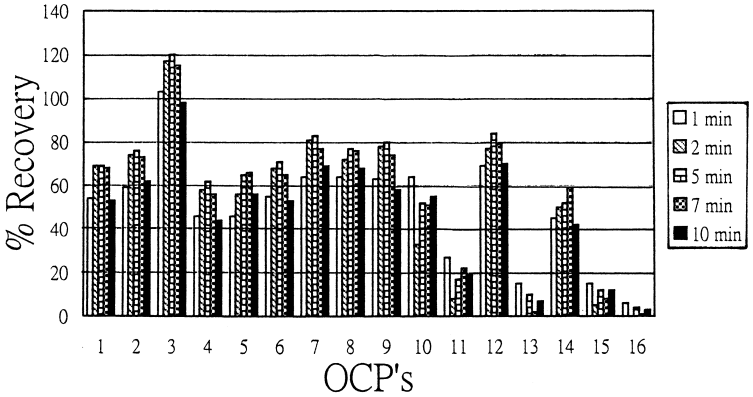

Fig. 6. Effect of static extraction time on the SFE of OCPs from the fortified *Glycyrrhizae radix*. Refer to Table 1 for assigned OCP numbers. Extraction conditions: modifier=null; pressure=250 atm; temperature=50°C; static extraction time=specified in the figure; dynamic extraction time=15 min.

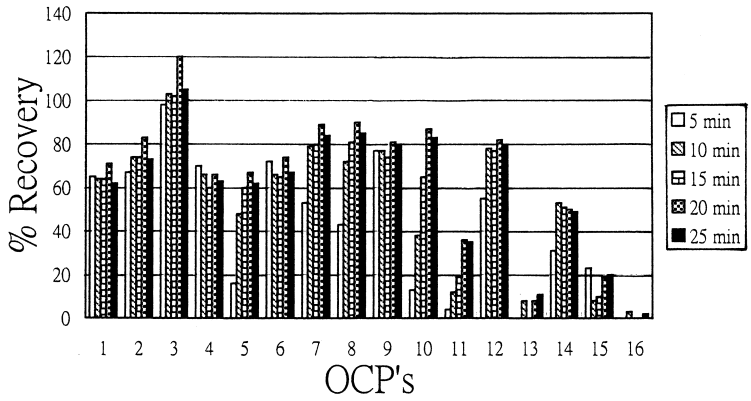

Fig. 7. Effect of dynamic extraction time on the SFE of OCPs from the fortified *Glycyrrhizae radix*. Refer to Table 1 for assigned OCP numbers. Extraction conditions: modifier=null; pressure=250 atm; temperature=50°C; static extraction time=5 min; dynamic extraction time=specified in the figure.

aldrin, heptachlor epoxide, and endrin, 5 μg/g for total DDT, in human food and animal feed [48]. The results obtained from this study would therefore be useful for monitoring OCPs in herbs of similar matrix. The recovery, the spiked level and the method detection limits (MDLs) for 16 OCPs in fortified *Glycyrrhizae radix* are summarized in Table 2.

For OCPs, the average recoveries (n=5) were greater than 70%, except for endosulfan II, endrin aldehyde and endosulfan sulfate. These results are in agreement with the previous study that endosulfan II and endosulfan sulfate were not recovered from Florisil [49]. The mean recovery for the 13 OCPs was 98% (not including endosulfan II, endrin aldehyde and endosulfan sulfate). The reproducibilities expressed as standard deviation (SD) were smaller than 20%, except for β-BHC and 4,4′-DDT. The

Table 2
The recovery, the spiked level and the method detection limits for 16 OCPs in fortified CHM *Glycyrrhizae radix*

Analyte	Recovery±SD[a] 75 ng/g Spk	Spiked level (ng/g)	MDL[b] (ng/g)
α-BHC	92±7	4	1
γ-BHC	101±5	3	1
β-BHC	121±31	5	2
Heptachlor	92±8	2	1
δ-BHC	88±15	5	2
Aldrin	92±5	4	6
Heptachlor epoxide	107±5	2	1
Endosulfan I	106±6	2	1
4,4'-DDE	99±14	3	1
Dieldrin	96±16	3	1
Endrin	102±17	8	1
4,4'-DDD	104±11	8	1
Endosulfan II	32±17	–[c]	–[c]
4,4'-DDT	78±27	8	5
Endrin aldehyde	33±15	–[c]	–[c]
Endosulfan sulfate	11±4	–[c]	–[c]

[a] n=5.
[b] 3.14×SD of seven replicate analyses of *Glycyrrhizae radix* blanks fortified at the spiked level.
[c] Not applicable.

mean reproducibility for the 13 OCPs expressed as average SD was 11% (not including endosulfan II, endrin aldehyde and endosulfan sulfate). The MDLs varied from 1 to 6 ng/g. The correlation coefficients exceeded 0.997, except for endrin aldehyde which was 0.988. The results indicate that the proposed SFE method yields satisfactory extraction and determination of 13 OCPs (not including endosulfan II, endrin aldehyde and endosulfan sulfate) in CHMs at the ng/g levels.

3.7. Analysis of real samples

Among the 20 single-component CHMs and 11

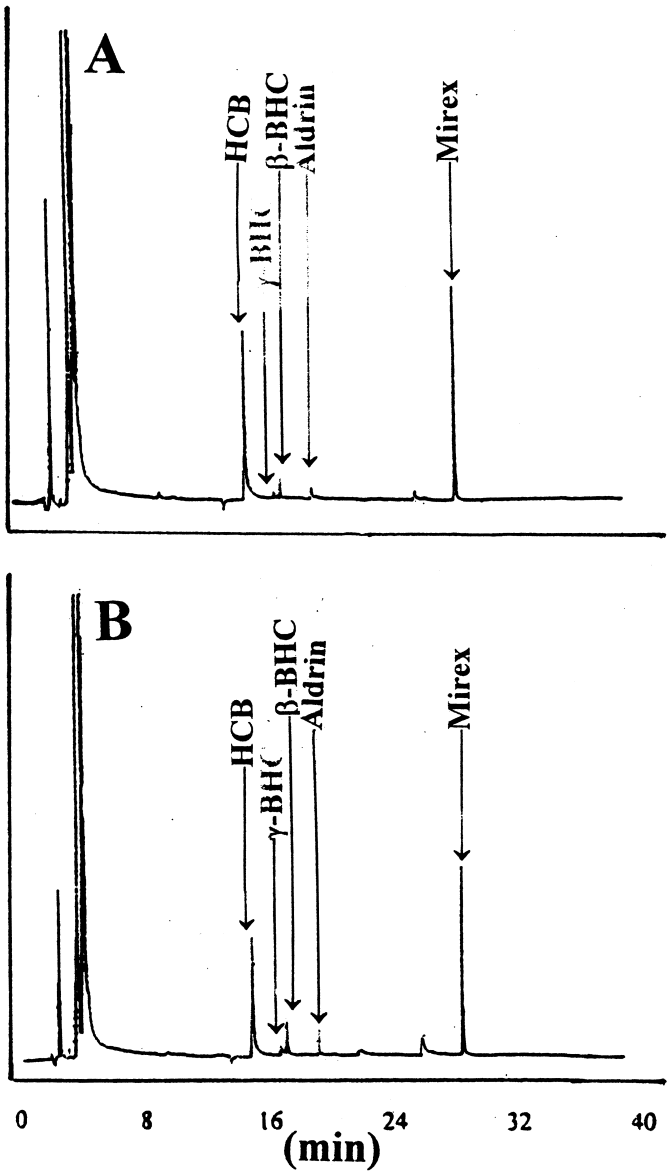

Fig. 8. GC–ECD chromatograms of SFE extracts of (A) *Paeonian radix*, and (B) *P. radix* added with 25 ng/g of γ-BHC, 250 ng/g of β-BHC and 75 ng/g of aldrin.

scientific CHMs being analyzed for OCPs, BHC-series and aldrin pesticides were mostly found in single-component CHMs, such as *Paeonian radix, Atractylodis rhizoma, Cnidii rhizoma* and *Ginseng radix*. The concentration of total OCPs is 228 ng/g (BHCs and aldrin) in *P. radix*, 82 ng/g (BHCs only) in *A. rhizoma*, 62 ng/g (BHCs and aldrin) in *C. rhizoma* and 1191 ng/g (BHCs only) in *Ginseng radix*, respectively. The contamination levels found were much higher than those found in the previous study of OCPs in soil and mussel samples [32,33]. In conjunction with the absence of the most popular

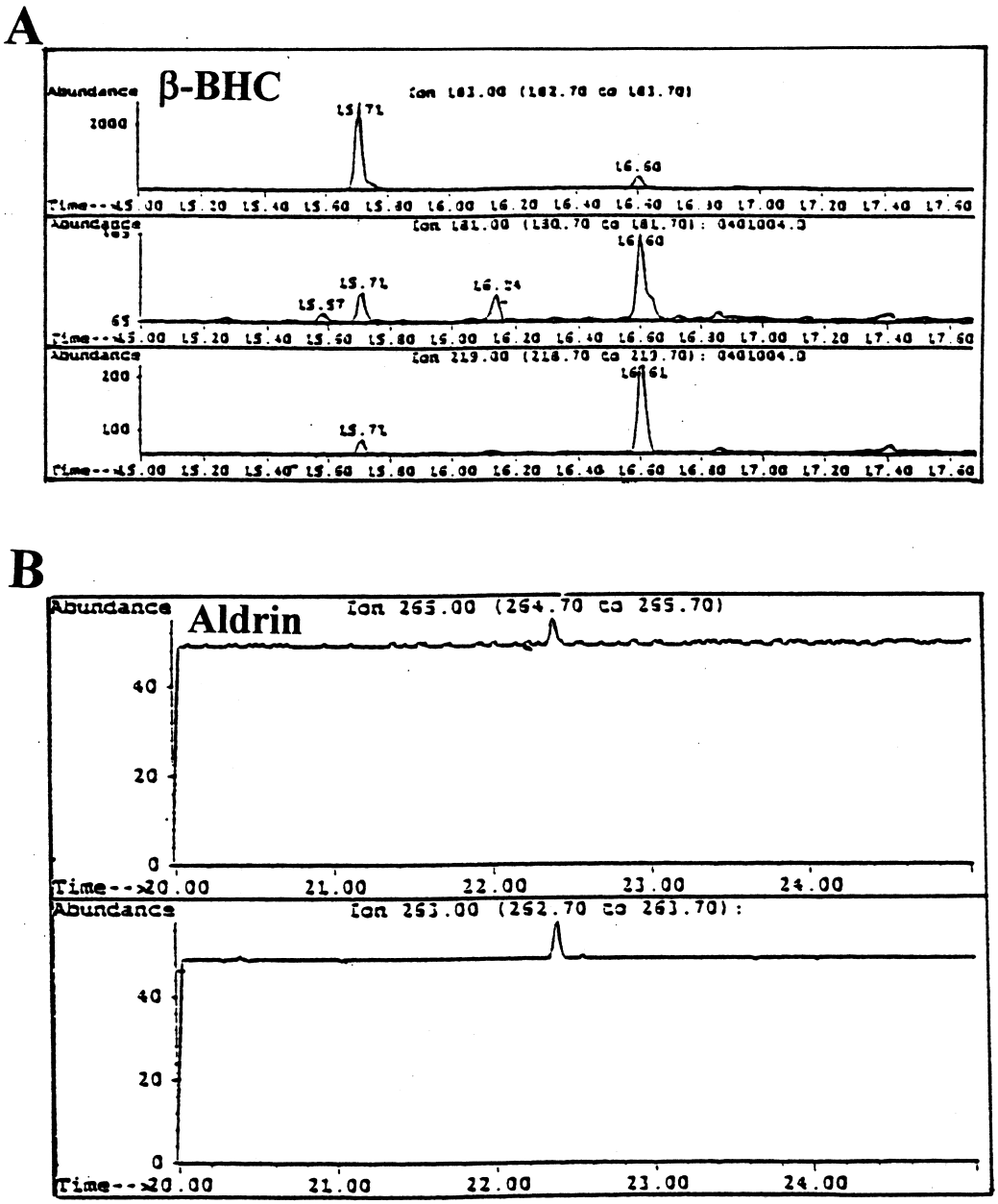

Fig. 9. GC–MS-SIM chromatograms of SFE extracts of (A) β-BHC and (B) aldrin from *Paeonian radix*. Time scale in minutes.

DDT-series pesticides, the OCPs found in these CHMs presumably came from spraying operations rather than from environmental deposition.

The extent of eliminating interfering species by the treatment with Florisil sorbent depends on the sample matrix as in the two representative chromatograms shown in Fig. 8. The absence of complicated background peaks in the chromatogram of *P. radix* (Fig. 8A) verified the feasibility of the proposed SFE method. Standard addition approaches were carried out to further check the feasibility of the proposed on-line clean-up SFE method. The recoveries of β-BHC, γ-BHC and aldrin were 87%, 106% and 43% when 25 ng/g of γ-BHC, 250 ng/g of β-BHC and 75 ng/g of aldrin were added to the *P. radix* (Fig. 8B). These chromatograms are typical of the CHMs investigated in this study. The representative GC–MS-SIM chromatograms of BHC and aldrin from the *P. radix* shown in Fig. 9 confirmed the presence of these contaminants.

4. Conclusions

A SFE method for the simultaneous extraction and clean-up of 13 OCPs in CHMs has been developed. The method loaded 2.0 g Florisil sorbent on top of 0.1 g sample in a 10-ml extraction cell, followed by GC–ECD detection and GC–MS confirmation. The MDLs vary from 1 to 6 ng/g. Mean reproducibilities between 5% and 31% and mean recoveries between 78 and 121% are obtained. The proposed SFE method is simple, rapid and requires only a small amount of samples and solvents. The total amount of time needed from SFE extraction to GC–ECD analysis is less than 2 h. Preliminary results indicate that the proposed SFE method can be successfully applied to fortified CHM (*Glycyrrhizae radix*) and real CHMs contaminated with OCPs at ng/g levels. The method may serve a screening protocol for the determination of OCPs in CHMs on a routine basis.

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