Decontamination of Organochlorine Pesticides Residue and Heavy Metal in *Rehmannia glutinosa Libosch* by SFE

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Abstract

A method involving the simultaneous extraction and decontamination of 12 organochlorine pesticides (OCPs) and seven heavy metals (HM) from *Rehmannia glutinosa Libosch* was established using supercritical fluid extraction (SFE). A gas chromatography (GC) method with electron capture detection was employed for the determination of the OCPs. The quantitative determination of active constituents (iridoid glycoside and catalpol) in *Rehmannia glutinosa Libosch* was detected by high-performance liquid chromatography (HPLC). An atomic absorption spectrometry (AAS) was designed for the determination of seven HM, including lead (Pb), cadmium (Cd), copper (Cu), iron (Fe), zinc (Zn), arsenic (As), and mercury (Hg) in *Rehmannia glutinosa Libosch*. Recovery of the determination of the 12 organochlorine pesticides in *Rehmannia glutinosa Libosch* sample was 85.9%–101.4% by GC, and relative standard deviation (RSD) was 1.9%–6.0%. Catalpol determination with HPLC in a *Rehmannia glutinosa Libosch* sample was 0.2486 and 0.2559 mg/mL before and after decontaminating OCPs by SFE, respectively. Those were 0.2486 and 0.2632 mg/mL before and after decontaminating HM by SFE, respectively. After a series of experiments to optimize the final SFE, the following conditions were used to determine the OCPs: pure CO_2, extraction pressure of 15 Mpa, extraction temperature of 60°C, extraction time of 30 min, flow rate at 35 kg/h, and the final SFE conditions of HM was pure CO_2, extraction pressure of 18 Mpa, extraction temperature of 50°C, extraction time of 20 min, modifier at 2.5 mL/50 g. The SFE was used to remove the 12 OCP residues and seven HM residues from *Rehmannia glutinosa Libosch* with less residue left and negligible loss of the active constituent catalpol.

Introduction

For thousands of years, the practice and use of herbal remedies and medicines have been prevalent among the Chinese. Although Chinese herbal medicine (CHM) is an important part in traditional Chinese remedy, the safety issue in herbal medicines must always be considered (1–3). The cultivation of herbal plants used for CHMs usually takes years of time and requires and the use of pesticides to reduce pest damage (4–5). Improper use of pesticides not only pollutes the cultivating soil and ground water but also leads to accumulation of pesticides in the plants. Simultaneously, alarm has been heightened internationally for the past few years to control the contents of elements, such as cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As), and toxic contaminants such as pesticide residue in CHM products (6). Cases of severe and fatal poisoning have occurred after the consumption of contaminated CHM products or adulterated ones containing toxic substituents. Therefore, it comes as no surprise that in the recommendations put forward recently in China, the control of organochlorine pesticides (OCP) residue and heavy metal (HM) in CHM products are top priority items requiring immediate action. The quality efficacy and safety of CHM products containing HM have to be critically assessed before the products can be put in clinical trials or placed on the market (7). The OCPs and HMs are the preferentially object residual contaminants because they are still used in CHM-cultivating countries. Moreover, OCPs and HMs hold a long half-life, high accumulation, potentially harmful biological effects, and deleterious impacts on the environment (8–9).

There are specific problems concerning extraction, purification, and detection of OCPs in complex matrices in the decontamination of OCPs and HMs of CHMs. Pesticides in matrices similar to CHM have been determined using various techniques to decontaminate pesticides from the matrices. The general drawbacks, such as the use of large amounts of solvents, its time-consuming and labor-intensive process, and considerable waste production, associated with these classical extraction techniques have been reduced using matrix solid-phase dispersion and solid phase extraction (SPE) (10–12). SPE has shown to be an efficient and rapid method for the isolation of pesticides from vegetables and medical plant matrices. But the samples are prone to be contaminated again as an extracting agent. The advantage in speed and cost as well as the use of small amount of solvents in traditional SPE are further increased by the integration of decontamination methods into supercritical fluid extraction (SFE). In SPE, a lot of procedures need to be executed which include extracting, enriching and cleaning, while only a step of extracting must be done in SFE. So SFE needs less time and costs less than SPE (13–17). The extracting agent is CO_2 in the method of SFE. After extraction, the CO_2 in normal pressure is a gas, so it is easily volatilized. In this way, contamination again by the extracting agent could not posed.

Rehmannia glutinosa Libosch has been widely used to nourish the kidney and increase the blood (18). *Rehmannia glutinosa Libosch* consists of iridoid glycoside and carbo. However, catalpol is a compound of iridoid glycoside, which has no substituent group at C4. It is one of the main and active components in *Rehmannia glutinosa Libosch* (19). The content of catalpol is assigned as an active component to control the quality of *Rehmannia glutinosa Libosch*. In the present study, catalpol is assigned as an active component. After *Rehmannia glutinosa Libosch* is decontaminated, its change of quality is estimated by the change of catalpol content.

The target of the present study was to establish an SFE method for receiving high decontamination rate of OCPs and HMs residuals in *Rehmannia glutinosa Libosch* and low loss of active constituents.

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Chemicals and reagents

α-, β-, γ- and –δ BHC (benzene hexachloride), pentachloronitrobenzene (PCNB), and dichlorodiphenyltrichloroethane (DDT) were supplied by National Standard Department (Beijing, China), whose purities were 97%. Heptachlor (HEPT), methyl-pentachlorophenlyl sulfide (MPCPS), and pentachloroaniline (PCA) were supplied by Accu, whose purities were 100% AccuStandard, Inc. (New Haven, CT). Methanol was HPLC-grade and others were analytical-grade and were obtained from Chemical Reagent Factory (Shenyang, China). Light petroleum (boiling point of 30–60°C) was distilled. *Rehmannia glutinosa Libosch* from China Shenyang Medicines' and Health Products Import and Export Corp was identified by Professor Qishi Sun (Shenyang Pharmaceutical University). The standard catalpol was provided by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Water was purified and deionized using a Milli-Q ion-exchange filtration system (Millipore, Bedford, MA). Nitric acid (HNO_3), perchloric acid ($HClO_4$), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) were guaranteed reagents. Potassium borohydride (KBH_4), potassium hexacyan-oferrate (K_3FeCN_6), thiourea, potassium iodide (KI), potassium dichromate ($K_2CR_2O_7$), and sodium hydroxide (NaOH) were analytical-grade. The reagents mentioned earlier are obtained from Chemical Reagent Factory (Shenyang, China). Single element metals of Pb, Cd, Cu, Fe, Zn, As, and Hg standard solutions were purchased from National Center of Standard (1000 µg/mL).

Instrumentation

The liquid chromatographic system was an LC-10AT (Shimadzu, Kyoto, Japan) equipped with an SPD-M10A UV-visible detector and an NC-2000 workstation. The liquid chromatographic separation was performed on an ODS column (250 mm × 4.6 mm i.d., 5 µm, Thermo Scientific, Waltham, MA). The mobile phase was a mixture of acetonitrile-water (1:100, v/v). The flow-rate was 1.0 mL/min. The detector was operated at 205 nm with a sensitivity of 0.01 a.u.f.s. The sample of 20 µL was injected into the high-performance liquid chromatography (HPLC) system.

The gas chromatographic (GC) apparatus (GC-17A, Shimadzu, Kyoto, Japan) consisted of a CLASS-GC10 workstation, a model DB-1 Quartz capillary column (30 m × 0.25 mm i.d., 0.25 µm), and an electron capture detector (ECD). Nitrogen gas was used as the carrier and make-up gas. Its purity is 99.999%. The flow of nitrogen is 50 mL/min. The injector temperature and the detector temperature were set at 250°C and 280°C, respectively. The column temperature was initially held at 150°C, then programmed from 150 to 240°C at a rate of 15°C/min and held at 240°C for 2 min. The sample of 1 µL was injected into the GC.

A HG-9602 atomic absorption spectrometer (AAS) with hydride generation and HG-9602 workstation (Shenyang Huaguang Precision Instrument Co., Shenyang, China) was employed. Hollow cathode lamps of Pb, Cd, Cu, Fe, Zn, As, and Hg were used.

The SFE system was a model DY 168-50-05 equipped with a 5 L stainless steel extraction vessel (Shenyang Dongyu Pharmaceutical Ltd, Shenyang, China).

Methods

Extraction of organochlorine pesticides

Rehmannia glutinosa Libosch samples were dried at 60°C, ground mechanically to obtain a homogenous power, and sieved through a No. 20 mesh sieve. One gram of the powdered plant material was put into a beaker and 35 mL water–acetonitrile (35:65, v/v) was added to it. After mixing 5 min at high speed and filtering with suction through Buchner, the filtrate was transferred to a 250 mL separator. After adding a 30 mL volume of petroleum ether and shaking vigorously 1–2 min, a 1 mL of saturated NaCl solution and 60 mL distilled water were added to the filtrate. The water phase was discarded following delamination. The petroleum ether phase was washed with 5 mL of distilled water twice. The two washings were combined into the filtrate. A 1 mL dense sulfuric acid (95%–98%, g/g) was added to the extract and shaken well. After delaminating, the organic phase was transferred to another 250 mL separator. A 10 mL volume of 2% Na_2SO_4 solution was poured into the separator and shaken well. The organic phases were combined, then dehydrated by using a bed of anhydrous Na_2SO_4, and concentrated by using a vacuum rotary evaporator equipped with a 30–35°C water bath. The previously mentioned was dried under a gentle steam of pure nitrogen. The residue was dissolved in exactly 1 mL of light petroleum and injected into the GC system.

Catalpol extraction

10 mL methanol was added to 2.0 g sieved powder of a *Rehmannia glutinosa Libosch* sample in a 50-mL flask, which was refluent at 70°C for 2 h and then the residues were redissolved into 5 mL volumetric flask with methanol. The solution was cooled to room temperature and was filtered through a membrane (PTFE, 0.5 µm). The initial filtrate was discarded because the membrane can absorb the active component of the sample. So the membrane was saturated with the filtrate at first. A 20-µL sample of the filtrate was gained on the second injection into the column.

Sample preparation for determination of heavy metals

Digestion with HNO_3–$HClO_4$

5.0 g of *Rehmannia glutinosa Libosch* was levigated, weighed, kept in 30 mL mixed acid (HNO_3/$HClO_4$, 5/1) for 24 h, and heated

Table I. Recovery and RSD* of the Determination of the 12 OCPs and Catalpol in the *Rehmannia glutinosa Libosch* Sample (*n* = 3)

Pesticide	Original (ng/mL)	Added (ng/mL)	Found (ng/mL)	Mean Rec. (%)	RSD (%)
α-BHC	68.0	45.0	111.7	97.0	2.5
β-BHC	63.0	30.0	89.9	89.9	1.9
γ-BHC	76.4	45.0	115.0	85.9	3.8
δ-BHC	17.4	25.0	42.8	101	4 6.0
PCNB	45.9	25.0	68.5	90.5	2.2
PCL	12.3	20.0	29.9	87.9	4.4
HEPT	26.5	20.0	44.9	92.3	5.2
MPCPS	16.2	20.0	36.0	99.0	4.9
PP′-DDE	97.2	50.0	146.7	98.9	2.2
PP′-DDD	37.0	20.0	55.3	91.5	2.1
OP′-DDT	103.3	50.0	149.2	91.9	3.2
PP′- dDDT	57.5	30.0	86.6	96.9	2.9
Catalpol	0.2515	0.20	0.4385	93.2	3.1
			0.4275	87.7	
			0.4348	91.4	
		0.25	0.4399	75.35	1.6
			0.4422	76.28	
			0.4461	77.84	
		0.30	0.4961	81.5	2.8
			0.4914	79.90	
			0.5049	84.4	

* RSD = relative standard deviation

on hot plates until brown fumes changed to white fumes. The white fumes, which came from the mouth of the flask, were evaporated and were observed again in the flask. The solution digested should be clear or light yellow. The residue was diluted with ultrapure water of 10 mL, which was heated until it generated white fumes two times. After cooling, the previously mentioned solution was transferred into volumetric flask of 50 mL. The final volume was completed to exactly 50 mL with ultrapure water, which was used as test solution. A blank sample was run. The test solution was used directly to determine the total concentration of Pb, Cd, and Cu in medicine. The test solution diluted was determined for Fe and Zn. The test solution of 20 mL was added to 50 mL water in the 100-mL volumetric flask, which was followed by 10% KI and 5% thiourea of 7.5 mL. The final volume was completed to 100 mL exactly with ultrapure water, which was examined for As.

Digestion with H_2O_2–HNO_3

Approximately 1 g sieved powder of *Rehmannia glutinosa Libosch* sample was weighed into a 25-mL tube. Pre-digestion was performed by first adding 5 mL of HNO_3 to the sample and secondly adding 2 mL of H_2O_2. The mixture was allowed to stand overnight. On the next day, the pre-digested samples were further digested in a 95ºC water bath for 2 h. After cooling to ambient temperature, the solution was transferred into a 50-mL volumetric flask with 20 mL ultrapure water, which was added to 4–5 drops of 2% $K_2CR_2O_7$. The volume was made up to the mark with ultrapure water, which was used as a test solution of Hg. In terms of the pervious method, a blank sample was run.

SFE conditions of Rehmannia glutinosa Libosch

Samples were ground mechanically and sieved through a No. 20 mesh sieve to yield a homogenous powder. Fifty grams of the powdered plant material was taken. Extraction conditions of OCPs were as follows: pure CO_2, extraction pressure of 15 Mpa, extraction temperature of 60ºC, extraction time of 30 min, and a flow rate at 35 kg/h; the final SFE conditions of HM was pure CO_2, extraction pressure of 18 Mpa, extraction temperature of 50ºC, extraction time of 20 min, and a modifier at 2.5 mL/50 g (20–23).

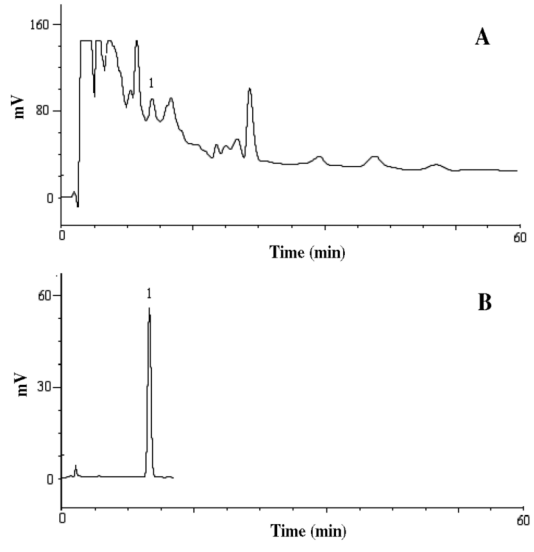

Figure 1. HPLC of a Rehmannia glutinosa Libosch sample extract (A) in comparison with that of its assigned effective constituent (catalpol) standard (B).

Results and Discussion

GC, HPLC, and AAS method validations

GC

Pesticides calibration curves were prepared by dissolving stock solutions (20 µg/mL) in cyclohexane separately. Different volumes of the resulting 200 ppb standard solution were diluted with cyclohexane to yield a series of solutions of exactly 10, 20, 40, 60, 80, and 100 ppb. Calibration curves were plotted at these six concentration levels, in which the response of each concentration was determined five times. The linearity of response (peak height, mv) versus independent variable (concentration, ng/mL) was evaluated by simple linear regression analysis. The regression equations for the 12 OCPs were: $H_1 = 1.348 \times 103C_1 + 4.821$ ($r = 0.9958$), α-BHC; $H_2 = 7.481 \times 103C_2 + 4.508$ ($r = 0.9972$), β-BHC; $H_3 = 1.117 \times 103C_3 + 3.861$ ($r = 0.9967$), γ-BHC; $H_4 = 1.414 \times 103C_4 + 10.105$ ($r = 0.9965$), PCNB; $H_5 = 1.920 \times 103C_5 + 10.119$ ($r = 0.9921$), δ-BHC; $H_6 = 1.546 \times 103C_6 + 31.463$ ($r = 0.9954$), PCL; $H_7 = 9.210 \times 103C_7 + 8.628$ ($r = 0.9991$), HEPT; $H_8 = 2.323 \times 103C_8 + 20.025$ ($r = 0.9958$), MPCPS; $H_9 = 1.012 \times 103C_9 + 4.567$ ($r = 0.9953$), pp'- DDE, $H_{10} = 8.202 \times 103C_{10} + 3.318$ ($r = 0.9948$), pp'-DDD; $H_{11} = 5.440 \times 103C_{11} + 2.142$ ($r = 0.9963$), op'-DDT; and $H_{12} = 4.930 \times 103C_{12} + 0.969$ ($r = 0.9952$), pp'-DDT. The lower limit of quantification for all pesticides was 10 ppb.

The intra-day relative standard deviations (RSDs) were: 2.8%, α-BHC; 2.4%, β-BHC; 3.2%, γ-BHC; 4.2%, PCNB; 3.4%, δ-BHC; 3.8%, PCL; 5.0%, HEPT; 4.4%, MPCPS; 3.5%, pp'- DDE; 4.3%, pp'-DDD; 3.6%, op'-DDT; and 4.4%, pp'-DDT ($n = 5$) while the inter-day RSDs was 3.2%, α-BHC; 2.2%, β-BHC; 3.5%, γ-BHC; 3.5%, PCNB; 2.2%, δ-BHC; 3.4%, PCL; 4.2%, HEPT; 4.4%, MPCPS; 4.6%, pp'- DDE; 4.3%, pp'-DDD; 3.8%, op'-DDT; and 4.6%, pp'-DDT.

Recoveries of the method

One gram of the powdered *Rehmannia glutinosa Libosch* sample was added into the mixed standard solution. The process was repeated three times. In terms of the OCP extraction, the mixture was extracted, purified, and finally determined. The result is listed in Table I. Recovery of the determination of the 12 OCPs in the *Rehmannia glutinosa Libosch* sample is 85.9%–101.4%, and RSD is 1.9%–6.0%.

HPLC

A series of standard solutions 50.0, 100.0, 200.0, 400.0, 600.0, and 800.0 µg/mL of catalpol was prepared by diluting the 1.0 mg/mL stock solution with methanol. All of the solutions were filtered through a 0.45-µm microporous filter, and 20 µL of the filtered solution was injected into the HPLC in triplicates. The calibration curve was constructed by plotting the peak-area (y) of catalpol to its concentration (x). The calibration curve of the peak-area (y) versus

Table II. Factors and Levels of SFE for OCP in *Rehmannia glutinosa Libosch*

	Factors			
Levels	A Pressure (MPa)	B Temperature (ºC)	C Time (min)	D Flow Rate (kg/h)
1	10	50	10	35
2	15	60	20	45
3	20	75	30	55

the concentration (x, µg/mL) was linear: $y = 1.093 \times 10^7 x + 1.409 \times 10^5$, $r = 0.9994$. The linear range for the determination of catalpol was 0.05–1.0 mg/mL.

The repeatability of catalpol was 0.40% ($n = 5$), and its reproducibility was 2.1%. The content was evaluated by carrying out the determination in triplicates. 20 µL of the filtrate was injected into the column. The result is 0.2486 ($n = 3$), and the RSD is 1.5%. The HPLC chromatogram is shown as Figure 1.

Table III. Orthogonal Array L9(34) and Decontamination of OCP Residue from *Rehmannia glutinosa Libosch*

Experiment No.	Factor and level				DR* of OCP (%)
	Pressure	Temperature	Time	Flow Rate	
1	1	1	1	1	44.38
2	1	2	2	2	66.30
3	1	3	3	3	31.35
4	2	1	2	3	76.89
5	2	2	3	1	83.5
6	2	3	1	2	77.08
7	3	1	3	2	81.4
8	3	2	1	3	91.6
9	3	3	2	1	81.1
I	142.0	202.7	213.1	209.0	
II	237.5	241.4	224.3	224.8	Total =
III	254.1	189.5	196.3	199.8	633.6
R	112.1	51.9	28	25	

* DR = decontamination rate.

Table IV. Orthogonal Array L9(34) and Determination of Catalpol

Experiment No.	Factor and level				RR* of Catalpol (%)
	Pressure	Temperature	Time	Flow Rate	
1	1	1	1	1	26.79
2	1	2	2	2	80.0
3	1	3	3	3	31.73
4	2	1	2	3	71.33
5	2	2	3	1	104.2
6	2	3	1	2	47.93
7	3	1	3	2	18.52
8	3	2	1	3	61.30
9	3	3	2	1	55.26
I	138.5	116.6	136.0	186.3	
II	223.5	245.5	206.6	146.5	Total =
III	135.1	134.9	154.5	164.4	497.06
R	88.4	128.9	70.6	39.8	

* RR = retaining rate.

Recoveries of the method

Nine *Rehmannia glutinosa Libosch* samples were taken with each one being 2 grams in which the content of catalpol was added according to the standard. The previous solution was extracted by means of catalpol extraction. By determining catalpol content, recoveries were calculated ($n = 3$). The results are shown in Table I.

AAS

A series of standard solutions was prepared by diluting the single element stock solution of Pb, Cd, Cu, Fe, and Zn with water, which was determined by a flame-atomic absorption spectrometry with air-acetylene flame. The previous method was applied to a blank sample. 0.50 mL, 1.00 mL, and 2.00 mL standard solutions of As were added to a 100-mL volumetric flask, respectively, followed by 50 mL of HCl (1.67 mol/L), 7.5 mL of 10% KI, and 7.5 mL of 5% thiourea. The volume was made up to the mark with water, which was determined by hydride generation AAS. 0.50 mL, 1.00 mL, and 2.00 mL standard solutions of As were added to a 100-mL volumetric flask, respectively, followed by 50 mL of HCl (0.07 mol/L) and 8–10 drops of 2% K_2CrO_7. The volume was made up to the mark with 50 mL of HCl (0.07 mol/L). The calibration curve was constructed by plotting the absorption volumes (y) of each element to its concentration (x). The calibration curve of the absorption volumes (y) versus the concentration (x, µg/mL) was linear: y(Pb) = $3.632 \times 10^{-2}x + 2.960 \times 10^{-3}$ ($r = 0.9994$), y(Cd) = $1.043 \times 10^{-1}x + 3.200 \times 10^{-4}$ ($r = 0.9998$), y(As) = $2.857 \times 10^{-2}x - 7.800 \times 10^{-3}$ ($r = 0.9996$), y(Hg) = $7.100 \times 10^{-3}x - 2.100 \times 10^{-4}$ ($r = 0.9999$), y(Cu) = $7.445 \times 10^{-2}x + 1.430 \times 10^{-3}$ ($r = 0.9999$), y(Fe) = $1.994 \times 10^{-2}x + 4.130 \times 10^{-3}$ ($r = 0.9996$), y(Zn) = $1.896 \times 10^{-1}x + 2.500 \times 10^{-3}$ ($r = 0.9999$). The linear range for the determination of Pb, Cd, As, Hg, Cu, Fe, and Zn was 0.52–4.00 ng/mL, 0.10–1.00 ng/mL, 5.0×10^{-3} – 2.0×10^{-2} ng/mL, 5.0×10^{-3} – 2.0×10^{-2} ng/mL, 0.60–4.00 ng/mL, 2.00–20.00 ng/mL, 0.60–4.00 ng/mL, respectively.

The repeatability of Pb, Cd, As, Hg, Cu, Fe, and Zn was 0.66%, 0.91%, 0.51%, 0.56%, 0.34%, 0.41%, and 0.37%, respectively ($n = 7$) and the reproducibility was 1.9%, 3.3%, 2.0%, 1.9%, 0.98%, 0.58%, and 1.1%, respectively ($n = 5$).

SFE

Decontamination of OCP. Catalpol is one of the active ingredients of *Rehmannia glutinosa Libosch*. SFE, as an effective method of extraction, is usually affected by several factors, such as pressure, temperature, time, and flow rate. With the preliminary test, these ranges of pressure, temperature, time, and flow rate were selected. A four factor-three level orthogonal test [i.e., a L9(3^4)] (Table II) was designed to optimize the SFE conditions—removal of the residual OCPs from *Rehmannia glutinosa Libosch* yet retaining catalpol as complete as possible. The results of the orthogonal array L9(3^4) decontamination of OCP residue and the AEC catalpol from the

Table V. Results of OCP and Catalpol Determination in a *Rehmannia glutinosa Libosch* Sample Before and After SFE (n = 3)

	Pesticides Residue (ng/mL)												
	α-BHC	β-BHC	γ-BHC	δ-BHC	PCNB	PCA	HEPT	MPCPS	Pp′-DDE	Pp′-DDD	Op′-DDT	Pp′-DDT	Catalpol (mg/mL)
Before SFE	96.2	106.2	191.7	103.7	60.0	165.6	34.6	5.8	213.4	15.5	361.9	313.5	0.2486
RSD (%	4.5	3.8	4.3	2.9	4.0	5.4	4.6	4.1	2.7	3.9	4.9	5.6	1.5
After SFE	7.9	16.6	24.3	5.6	15.4	—*	—	—	36.0	—	33.7	—	0.2559
RSD (%)	1.3	1.9	2.7	3.4	0.8	—	—	—	5.1	—	5.7	—	4.6

* — = limit of detection.

CHM Rehmanniae are listed in Table III and Table IV. "I" is the sum of the values of the decontamination rate (DR) of OCP when the pressure, temperature, time, and flow rate were "1", respectively. "II" is the value when those were "2". "III" was the value when those were "3". Subtract the minimum value from the maximal value, and the result is "R". According to the value of R in the Table III, we can clearly see that the most significant factor to DR of OCP was pressure, while the retaining rate (RR) of catalpol decreased significantly with increasing pressure from 15 MPa to 20 MPa from Table IV. So the pressure of 15 MPa is to be preferred. When the temperature was 60ºC, the total value of DR of OCP is maximal in Table III ("II" = 241.4%), and the total value of RR of catalpol is maximal in Table IV ("II" = 245.5%). So the extraction temperature of 60ºC is to be preferred. Because the effect of extraction time was not significant to the percentage of catalpol retained and that of decontaminated OCP, subsequent SFE is to be carried out with 30 min extraction. The flow rate was an unimportant factor on the percentage of catalpol retained of increased decontaminated OCP. So the SFE should be carried out at 35 kg/h.

Based on these results, the optimal extraction condition is to be reached at 15 MPa pressure, 60ºC temperature, with 30 min extraction time, and 35 kg/h flow rate. The SFE was used to remove the 12 OCP residues from *Rehmannia glutinosa Libosch* with less residue left and negligible loss of the active constituent catalpol as compared with other methods. Three typical GC chromatograms of the 12 pesticides standard, and CHM samples, one before and one after the SFE, are shown in Figure 2. The contents of residual OCPs in a sample of *Rehmannia glutinosa Libosch* determined are listed in Table V. We can see that PCA, HEPT, MPCPS, *pp*'-DDD, and *pp*'-DDT were completely decontaminated, and the decontamination of the other OCP residues was significant. The catalpol determination was not changed before and after SFE

Decontamination of HM. A four factor-three level orthogonal test [i.e., a $L_9(3^4)$] (Table VI) was designed to optimize the SFE conditions—removal of the residual HM from *Rehmannia glutinosa Libosch* while retaining catalpol as much as possible. As shown from the range analysis (R) in Table VII and Table VIII, pressure is the most significant influencing factor both to the decontamination rate of HMs and to the retaining rate of catalpol. When the pressure increases from 14 MPa to 18 MPa, the decontamination rate of HMs increases greatly; when the pressure increases from 18 MPa to 22 MPa, the decontamination rate becomes stable. And with the increase of pressure, the retaining rate of AEC catalpol decreased. When the pressure increases from 18 MPa to 22 MPa, the retaining rate of catalpol decreases greatly. So the pressure of 18 MPa is to be preferred.

Temperature was the second most important factor to the decontamination rate of HMs, and it was the least important factor to the retaining rate of catalpol. In Table VII, the difference between the value of "II" and the value of "III" was very small. So 50ºC is the

Table VI. Factors and Levels of SFE for HM in *Rehmannia glutinosa Libosch*

	Factors			
Levels	A Pressure (MPa)	B Temperature (ºC)	C Time (min)	D Modifier (mL/50 g)
1	14	40	10	0
2	18	50	20	2.5
3	22	60	30	5

Table VII. Orthogonal Array L9(34) and Decontamination of HM Residue from *Rehmannia glutinosa Libosch*

Experiment No.	Factor and level				DR of HM(%)
	A	B	C	D	
1	1	1	1	1	67.25
2	1	2	2	2	85.7
3	1	3	3	3	79.09
4	2	1	2	3	81.8
5	2	2	3	1	91.1
6	2	3	1	2	93.7
7	3	1	3	2	93.6
8	3	2	1	3	88.6
9	3	3	2	1	93.2
I	232.0	242.7	249.6	251.6	
II	266.6	265.4	260.7	273.0	Total =
III	275.4	266.0	263.8	249.5	774.0
R	43.4	23.3	14.2	23.5	

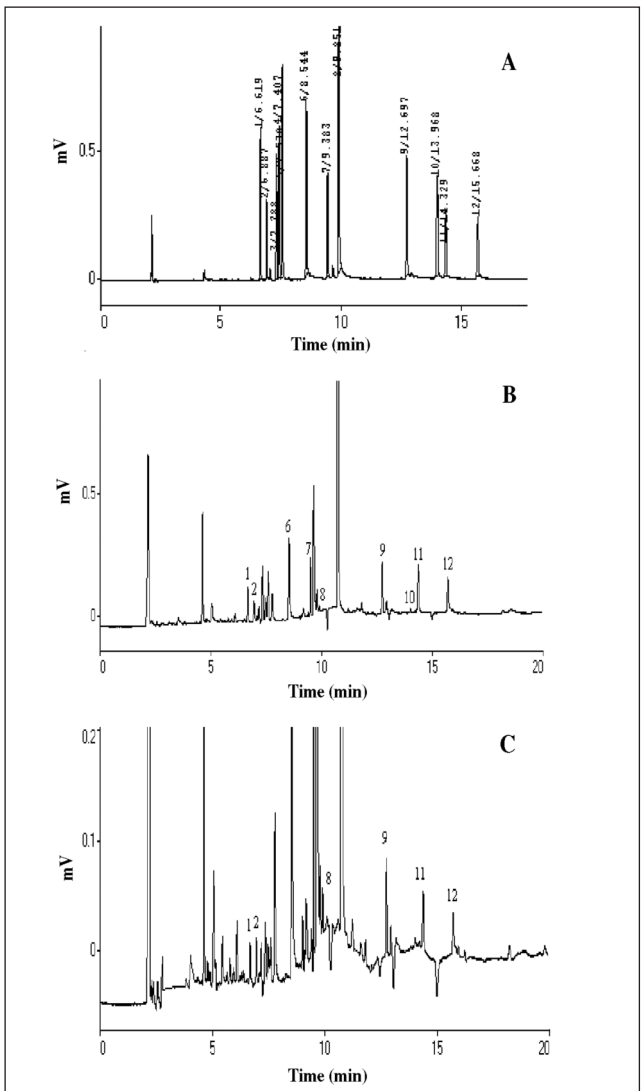

Figure 2. A gas chromatogram of the 12 pesticides, A; A gas chromatogram of 12 pesticide residue in *Rehmannia glutinosa* Libosch sample before SFE, B; A gas chromatogram of 12 pesticide residue in Rehmannia glutinosa Libosch sample after SFE, C; α-BHC, 1; β-BHC, 2; γ-BHC, 3; PCNB, 4; δ-BHC, 5; PCA, 6; HEPT, 7; MPCPS, 8; pp′-DDE, 9; pp′-DDD, 10; op′-DDT, 11; pp′-DDT, 12.

appropriate temperature with regard to the results of the two indexes, energy consumption, and the stability in keeping the thermo-sensitive ingredients of medicine materials.

Extraction time was the least important factor to the decontamination rate of HMs, and it was the second most important factor to the retaining rate of catalpol. Carried dose was not an important factor both to the decontamination rate of HMs and to the retaining rate of catalpol. The retaining rate of catalpol was the maximum value when the time was 20 min.

By direct-analysis mentioned, verification test was executed at 18 MPa pressure, 50°C temperature, 20 min extraction time, and 2.5 mL/50 mg. The decontamination rate of HM and the retaining rate of AEC catalpol were higher than in Table VII and Table VIII. So the optimal extraction condition is to be reached at 18 MPa pressure, 50°C temperature, 20 min extraction time, and 2.5 mL/50 mg modifier.

The SFE was used to remove the HM residues from *Rehmannia glutinosa Libosch* with less residue left and negligible loss of the active constituent catalpol. Table IX showed the result of HM and catalpol determination in a *Rehmannia glutinosa Libosch* sample before and after SFE. Corresponding to our aim, Cd, Hg, and As were completely decontaminated. The decontamination of Pb was pronounced.

Conclusion

The SFE method designed in this study removed the residual OCPs and HM efficiently from *Rehmannia glutinosa Libosch,* and the active constituent, catalpol, was retained unaffectedly. Furthermore, the SFE had advantages, such as high speed, smaller samples used, and less solvent consumed. So the method designed can be easily used to develop an efficient process of decontamination for CHMs.

Table VIII. Orthogonal Array L9(34) and Determination of Catapol

Experiment No.	Factor and level				RR of Catapol(%)
	A	B	C	D	
1	1	1	1	1	100.2
2	1	2	2	2	99.6
3	1	3	3	3	100.4
4	2	1	2	3	100.1
5	2	2	3	1	99.9
6	2	3	1	2	94.9
7	3	1	3	2	85.8
8	3	2	1	3	83.2
9	3	3	2	1	86.8
I	300.2	286.1	278.3	286.9	
II	294.9	282.7	286.5	280.3	Total =
III	255.8	282.1	286.1	283.7	850.9
R	44.4	4	8.2	6.6	

Table IX. Results of HM and Catalpol Determination in a *Rehmannia glutinosa Libosch* Sample Before and After SFE

	HM Residue (ng/mL)							Catalpol (mg/mL)
	Pb	Cd	Hg	As	Cu	Fe	Zn	
Before SFE	36.95	—*	1.0500	4.5060	28.820	1006.7	99.10	0.2486
RSD(%)	3.5	—	4.3	2.6	1.7	3.4	2.9	1.9
After SFE	3.34	—	—	—	16.116	962.9	86.31	0.2632
RSD(%)	2.5	—	—	—	2.8	4.5	5.0	4.8

* — = limit of detection

Acknowledgement

This work was supported by a grant from the National Natural Science Foundation of China (39970910).

References

1. W. Xican, Z. Sudi, and S. Juanfen. Determination of organic chlorine pesticide residue in Chinese traditional patent medicine by GC. *J. China Pharm. Univ.* **27(1):** 32–35 (1996).
2. Z. Wuji. The comprehensive compilation of national maximum residue limits for pesticides, veterinary drugs in foodstuffs and feedstuffs in the world, Beijing, China. Chinese publishing house for foreign economy and trade, 1995.
3. Z. Chunjie, X. Hong, L. Minghua, et al. Determination of residual benzene hexachloride and DDT in ten Chinese herbs by gas chromatography. *J. Shenyang Pharm. Univ.* **17:** 17–20 (2000).
4. C. Yuping, G. Zifeng, Z. Chunjie, et al. Determination of residual benzene hexachloide in ginseng by gas chromatography. *J. Shenyang Pharm. Univ.* **14(4):** 283–286 (1997).
5. C. Zhao, Y. Dong, X. L, et al. A study on the quantitative determination of ginseng saponin and residual esticides in ginseng extracts. *J. Shenyang Pharm. Univ.* **17:** 40–42 (2000).
6. H. Zhang, C. Zhao, C. Jin, and Q. Pan, Hydride generation-atomic absorption spectrometry for determination of trace lead, arsenic and mercury in beneficial traditional Chinese medicines. *J. Chinese Pharma.* **39(4):** 296–97 (2004).
7. C. Bin, W. Xiaoru, and Frank S.C. Lee. Pyrolysis coupled with atomic absorption spectrometry for the determination of mercury in Chinese medicinal materials. *Anal. Chim. Acta* **447:** 161–169 (2001).
8. R. S. Sheridan and J. R. Meola. Analysis of pesticides residues in fruits, vegetables, and milk by gas chromatography/tandem mass spectrometry. *J. AOAC Int.* **82(4):** 982–990 (1999).
9. L. Sojo, A. Brocke, J. Fillion, et al. Application of activated carbon membranes for on-line cleanup of vegetable and fruit extracts in the determination of pesticides multiresidues by gas chromatography with mass selective detection. *J. Chromatogr. A* **788:** 141–154 (1997).
10. T. Chester, J. Pinkston, and D. Raynie, Supercritical fluidchromatography and extraction. *Anal. Chem.* **70:** 301–319 (1998).
11. C. Zhao, G. Hao, H. Li, et al. Supercritical fluid extraction for the depuration of organochlorine pesticides residue in Angelica sinensis. *Biomed. Chromatogr.* **16:** 441–445 (2002).
12. C. Zhao, G. Hao, H. Li, et al. Decontamination of organochlorine pesticides in Radix Codonopsis by supercritical fluid extraction and determination by gas chromatography. *Biomed. Chromatogr.* **20:** 857–863 (2006).
13. L. Barnabas, R. John, M. Steven, Selective Supercritical fluid extraction of organochlorine pesticides and herbicides form aqueous samples. *J. Chromatogr. Sci.* **32:** 547–551 (1994).
14. J. Li, F. Ge, X. Huang, Study of supercritical fluid extraction in extrating essential oils of radix codonopsis sinensis. *J. Chinese Med. Mat.* **19(4):** 187–189 (1996).
15. Y. Qiu, K. Wen, and H. Bai, Superitical fluid extraction-gas chromatographic determination of rganchlorine pesticides residues in cereals and tea. *Chinese J. Anal. Chem.* **12(12):** 1391–1394 (1997).
16. B. Chen, L. Liu, Z. Zhai, et al. Determination of major components in Psoralea Corylifolia L.by using superitical fluid extraction. *Chinese J. Chromatogr.* **18(1):** 61–63 (2000).
17. C. Nerin, R. Batlle, and J. Cacho. Determination of pesticides in high-water-content samples by off-line supercritical fluid extraction-gas chromatography-electron-capture detection. *J. Chromatogr. A* **795:** 117–124 (1998).
18. W. Liu, Z. Chen, and J. Li, Content Determination of Reducing Suger in Radix Rehmanniae Preparata by Automatic Determine Instrument of Reducing Sugar. *Li Shizhen Med. Materia. Medic. Res.* **18(2):** 420–421 (2007).
19. M. Wang, H. Liu, and L. Huang. The comparative studies on the tuberows roots of Rehmannia glutinosa in different producing areas. *Chinese Trad. Herb.* **37(3):** 444–446 (2006).
20. T. Chester, J. Pinkston, and D. Raynie, Supercritical fluid chromatography and extraction. *Anal. Chem.* **70:** 301–319 (1998).
21. A. Mehdi, M.T. Combs, and L.T. Taylor. Supercritical Fluid Extraction of Metal Ions and Metal Chelates from Different Environments. *J. Chromatogr. A* **774:** 37–49 (1997).
22. Y. Lin, N. G. Smart, and C.M. Wai. Supercritical Fluid Extraction and Chromatography of Metal Chelates and Organometallic Compounds. *Trends Anal. Chem.* **14(3):** 123–132 (1995).
23. S. Nemoto, K. Sasaki, M. Toyoda, et al. Effect of extraction conditions and modifiers on the supercritical fluid extraction of 88 pesticides. *J. Chromatogr.* **35:** 467–477 (1997).

Manuscript received May 22, 2007;
Revision received March 15, 2008;