Determination of organochlorine pesticide residues in herbs by capillary gas chromatography

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Abstract

Objective. The capillary gas chromatographic method was introduced for determining organochlorine pesticide residues (OCPs) in traditional Chinese medicines: Gastrodia Tuber, Fructus Corni and Spica Prunellae. *Methods*. The organochlorine pesticides were extracted from herbs with mixed solvents (petroleum ether), cleaned up by concentrated H_2SO_4, and separated by capillary column with electron capture detection. *Results*. The method is linear over the range 6 − 300 μg/L for four pesticides. Correlation coefficients varied between 0.9963 and 0.9992. Limits of detection (LODs) ranged from 3.91×10^{-8} to 9.05×10^{-8} μg/L. The average recoveries were within 77.13% − 102.33% and RSD was 0.94% − 2.84%. *Conclusion*. This method is accurate and reliable for OCPs determination in the three herbs. [Life Science Journal. 2007;4(1):40 − 42] (ISSN: 1097 − 8135).

Keywords: capillary gas chromatography; organochlorine pesticide; herbs

1 Introduction

Herbal medicines have been used in medical practice for thousands of years and recognized especially as a valuable and readily available resource for health care. A World Health Organization report indicated that about 70% − 80% of the world populations rely on non-conventional medicine mainly of herbal sources in their primary health care. Traditional herbs and herbal products have been considered to be mild, non-toxic and even harmless because of their natural origin. Like other crops, medicinal plants are susceptible to insects and diseases both in the field and the storage, so pesticides are widely used for their protection. In fact, contamination of crude medicinal plants as well as their products has increasingly been reported[1,2]. This has brought concerns and fears regarding practitioner's professionalism and quality, efficacy and safety of their treatment methods and products from herbal and natural sources available in market. Because of the high cost of pesticide·free cultivation, organic cultivation is only possible on a small scale and wild raw material is of insufficient quantity to meet the needs to the herbal drug market. The ever increasing consumption of medicinal plants necessitates large scale cultivation of medicinal plants which is not possible without use of pesticides. One of the characteristics of herbal medicine preparation in traditional systems is that the herbal substrates are extracted with hot water during decoction process. Thus it would be toxicological interest to determine the actual amount of pesticides that is transferred from herbal substrates to infusion during its preparation, so that how much pesticide human intake may be measured. Many research workers have studied the pesticides residues in herbal material which are mainly based on surveying and monitoring the market samples. The objective of the present work is to investigate the transfer of pesticides from a range of herbal substrates, commonly used in traditional system of medicine, to their respective decoctions. This kind of data is useful as a pattern of reference in the management of pesticide residue problems and would help to formulate regulatory guidelines and recommendation for fixing of maximum residue limits on these products for quality assurance and control in herbal preparation. Attention is usually focused on contamination by organochlorine pesticides (OCPs) due to their toxicity and persistence in environment and contamination by common pesticides[3,4]. There are extensively reported methods for monitoring pesticide residues in herb, food and feedstuff[5-7]. They are based on either liquid-liquid extraction (LLE) or solid-phase extraction (SPE), followed by gas chromatography (GC) or HPLC separations employing wide range of detectors. For GC separations, electron capture detector (ECD) are popular for detection of OCPs residues. Although the use of organochlorine pesticides has been restricted or forbidden

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by legislation, these compounds are still under investigation and they are included in the method adopted by the ChP-Chinese Pharmacopoeia. This paper describes an analytical method for the determination of organochlorine pesticide residues in three herbs. The extraction and clean-up procedures are carried out in a single step by concentrated H_2SO_4. The pesticide compounds are quantitatively eluted with petroleumether and analised by GC-ECD.

2 Materials and Methods

2.1 Reagents and apparatus

Selected pesticides and working solutions: The four stereoisomer of benzenehexachloride (α-BHC, β-BHC, γ-BHC, δ-BHC) 2, 4-dichloronitrobenzene (internal standard) were obtained from the National Center of Standard Substance Research, with purity higher than 98.5%. Organic solvents are petroleum ether, standard stock solution (3000 μg/L) of each pesticide was prepared in petroleumether and the solutions required for preparing a standard curve (6, 18, 30, 60, 120, 180, 240 μg/L) and standard addition 1ml(180 μg/L) were prepared from the standard stock solution. All the solvents and chemicals used were of analytical grade and deionized water.

Apparatus: A varian 6890N gas-liquid chromatograph equipped with a 230 m × 0.32 mm, i.d. glass column packed with SE-52, a constant current 63Ni electron capture detector. The operating conditions were as follows: Injection volume was 2 μl; injector temperature, 200 ℃; oven temperature, 160 ℃; detector temperature 210 ℃ and the flow rate of the carrier gas (nitrogen) was maintained to 4 ml/min.

2.2 preparation of the samples

Dry herbal substrate in 60 ℃ for 4 hours, sieved and weighed 2 g sample was immersed in 10ml petroleum ether for 24 hours. The ultrasonic extract was filtered into a clean screwcap glass tube and then centrifuged at 100 rpm for 5 minutes. The supernatant was decanted into a 50 ml round-bottomed flask and then concentrated to about 1 ml at 40 ℃ using vaccum rotary evaporator. The solution was cleaned by H_2SO_4. Additionally, the acid layer was discarded and ether layer was dehydrated by anhydrous sodium sulphate, transfered to 50 ml flask and concentrated to be nearly dry at 40 ℃. Last the remnant was dissolved by petroleum ether to 10 ml measuring flask and added 1 ml internal standard solution.

2.3 Determination of response factors

The response factor of the standard pesticides relative to the internal standard, penta-chloronitrobenzene were carried out by injecting 2 μl into the GC-ECD system of a mixture consisted of the OCPs together with the I.S. at a concentration range of 6 − 300 μg/L. The response factor was calculated based on the equation below:

$$\text{Response factor} = \frac{\text{Peak area of the pesticide standard}}{\text{Peak area of the internal standard}}$$

3 Results and Discussion

The gas chromatograph of a mixture of the benzenehexachloride standards plus the dichloronitrobenzene is shown in Figure 1. All the four enantiomeric forms are well resolved and eluted within a very reasonable time of about 10 minutes under the optimized GC conditions. Table 1 showed the retention times and the response factors for the OCPs and the linear regression equation of the calibration curve of each standard pesticide. Figure 2 showed samples and Table 2 gave results: the quantification were achieved in the three samples.

Table 1. Retention times, the linear regression equation and linearity range

BHC	Regression equation	r	Linearity range
α- BHC	y=0.0106x−0.1034	0.9963	6～300μg/L
β- BHC	y=0.0032x−0.0134	0.9992	6～300μg/L
γ- BHC	y=0.0100x−0.0894	0.9967	6～300μg/L
δ- BHC	y=0.0087x−0.0762	0.9971	6～300μg/L

Table 2. The detection results of the three samples

BHC	Gastrodia tuber	Fructus corni	Spica prunellae
α- BHC(ng/g)	40.31	44.04	not detected
β- BHC(ng/g)	27.88	32.04	not detected
γ- BHC(ng/g)	38.74	44.13	not detected
δ- BHC(ng/g)	40.49	42.31	not detected

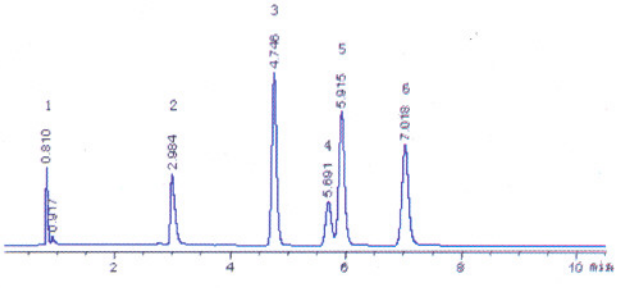

Figure 1. Gas chromatograph of OCP standards
1. solvent 2. internal standard dichloronitrobenzene 3. α-BHC 4. β-BHC 5. γ-BHC 6. δ-BHC

In order to evaluate the efficiency of the method, additional recovery analysis were carried out using petroleum ether. Table 3 showed the recovery and precision expressed as relative standarddeviation (RSD). Mean recoveries from samples fortified with α-BHC, β-BHC, γ-BHC, δ-BHC at levels from 6 to 300 μg/L, ranged from 77.13% to 102.33% with RSD values be-

tween 0.94% and 2.84%. These data have demonstrated the efficiency of the proposed method.

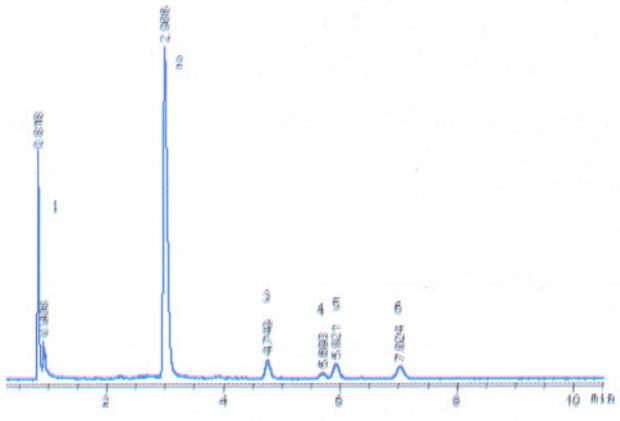

Figure 2. Gas chromatograph of the sample
1. solvent 2. internal standard dichloronitrobenzene 3. α-BHC
4. β-BHC 5. γ-BHC 6. δ-BHC

Table 3. Recovery and precision of the proposed method

Pesticides	Retention time (minute)	Mean recovery ± RSD (range) (%)
α- BHC	4.75	91 ± 8.2
β- BHC	5.69	91 ± 10.8
γ- BHC	5.92	88 ± 13.4
δ- BHC	7.02	92 ± 7.2

4 Conclusion

The apparent current explosion of interest in commercial activity in the area of herbal products should be followed by accurate quality control. This could be ascertained by imposing regulatory standards on these products that should be manufactured using good practices. The present study demonstrated transfer in decoctions of pesticides varied depending on their leaching potential. The actual amount of pesticides contributing to the dietary intake therefore may be quantified. This study showed an accurate and reliable method for OCPs determination in traditional Chinese medicines. It also showed that despite the numerous claims by both manufacturers and research groups on the usefulness of MAE for sample preparation. GC could be a more accurate alternative method for OCP determination in herbs.

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