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Simultaneous Analysis of 17 Organochlorine Pesticides in Natural Medicines by GC/MS with Negative Chemical Ionization

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Many methods for the determination of pesticide residues in food have been reported. Although natural medicines should be confirmed to be as safe as food, few methods for the determination of pesticide residues in natural medicines have been reported. In this study, 17 organochlorine pesticides were detected in natural medicines using GC/MS with negative chemical ionization (NCI). GC/MS with NCI can detect halogenated pesticides selectively and thus is suitable for the detection of organochlorine pesticides. This study indicates that GC/MS with NCI is useful for analyzing organochlorine pesticides in natural medicines.

Key words——natural medicine; organochlorine pesticide; negative chemical ionization; GC/MS

INTRODUCTION

Various illnesses have been treated with natural medicines. Natural medicines should be confirmed to be as safe as food because patients generally take natural medicines over the long term. In recent years, pesticides have often been detected in imported food. In Japan, a maximum residue level has been set for about 800 pesticides in food; however, most natural medicines are imported, and a maximum residue level has been set for only eight pesticides in natural medicines,[1] and only a few analytical methods for the determination of pesticide residues in natural medicines have been reported. Generally, organochlorine pesticides in natural medicines have been analyzed using GC/electron capture detector (ECD).[2–5] Various methods to analyze organochlorine pesticides in food selectively using GC/MS with negative chemical ionization (NCI) have been reported.[6,7] We also analyzed pyrethroid pesticides in natural medicines selectively using GC/MS with NCI.[8] We therefore attempted to analyze organochroline pesticides in natural medicines selectively using GC/MS with NCI. In this study, we simultaneously analyzed 17 organochlorine pesticides in natural medicines selectively using GC/MS with NCI.

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MATERIALS AND METHODS

Pesticide Standards Pesticide standards were obtained from Wako Pure Chemical, GL Sciences (Japan), and Riedel de Haën (Germany). Each compound was dissolved in acetone or hexane to make 0.1 mg/ml of standard stock solution. Spiking solutions were prepared from standard stock solutions at 5 μg/ml. Working standard solutions were diluted with extracts of pesticide-free samples to prevent a matrix effect.

Reagents Acetone, hexane, and sodium chloride were pesticide analysis grade from Wako Pure Chemical. Supelclean ENVI Florisil SPE Tubes 6 ml (1 g) (Florisil) were purchased from Supelco (USA).

Natural Medicines Rhei rhizoma (locality: Sichuan), Puerariae radix (locality: Sichuan), Phellodendri cortex (locality: China), and Aurantii nobilis pericarpium (locality: Wakayama) were used in this study as Japan imports significant amounts of these four natural medicines. The four natural medicines were obtained from Mikuni (Japan) during a recent 2-year period. We confirmed that the concentrations of pesticide residues in these four natural medicines were below the detectable level with the proposed method.

Sample Preparation Natural medicines were powdered, 10 g was weighed out, and 200–400 μl of spiking solution was added. After 30 min, 25 ml of

acetone was added. After 1 h, 50 ml of hexane was added and the mixture was homogenized for 1 min. The homogenate was filtered with filter paper (Toyo Roshi, Japan). The filtrate was washed twice with 25 ml of 5% NaCl solution after washing with hexane. The 10 ml of hexane layer obtained was loaded into Florisil and preconditioned with acetone 5 ml and hexane 20 ml. Pesticides were eluted with 25 ml of acetone-hexane (3 : 17). The eluate was evaporated and the residue was dissolved in 10 ml of acetone-hexane (3 : 17) for GC/MS analysis.

NCI Mode GC/MS A 5973MSD was connected to a GC 6890 (Agilent, USA). GC conditions: column, DB-1701 capillary column 30 m×0.25 mm×0.25 μm (J & W Scientific, USA); helium carrier gas flow, 1.7 ml/min; injection temperature, 200℃; interface temperature, 260℃; ion source temperature, 180℃; ion mode, NCI/selected ion monitoring (SIM) mode; reaction gas, methane; oven temperature program, 50℃ for 1 min, 25℃/min to 100℃, and then 5℃/min to 280℃; injection mode, splitless; injection volume, 1 μl.

Table 1. Monitoring Ions Selected for SIM

Compound	Monitoring ion (m/z)	
p, p′-DDE	35	282
o, p′-DDT	35	71
p, p′-DDD	35	71
p, p′-DDT	35	71
α-BHC	71	35
γ-BHC	71	35
β-BHC	71	35
δ-BHC	71	35
Aldrin	237	35
Heptachlor epoxide	237	35
Dieldrin	237	35
Quintozene	249	265
Hexachlorobenzene	250	284
Heptachlor	266	35
Fthalide	272	228
Endrin	272	237
Tetradifon	318	243

Table 2. Linear Ranges and Correlation Coefficients of Standard Solutions Diluted with Extracts of Pesticide-Free Samples

Compound	Range (ppb)	Correlation coefficient (γ)			
		Rhei rhizoma	Puerariae radix	Phellodendri cortex	Aurantii nobilis pericarpium
p, p′-DDE	10–500	0.9982	0.9987	0.9980	0.9986
o, p′-DDT	10–500	0.9960	0.9960	0.9981	0.9953
p, p′-DDD	10–500	0.9985	0.9982	0.9981	0.9982
p, p′-DDT	10–500	0.9930	0.9938	0.9971	0.9914
α-BHC	10–500	0.9973	0.9976	0.9972	0.9988
γ-BHC	10–500	0.9970	0.9926	0.9967	0.9985
β-BHC	10–500	0.9976	0.9987	0.9980	0.9989
δ-BHC	10–500	0.9967	0.9988	0.9975	0.9986
Aldrin	10–500	0.9979	0.9979	0.9973	0.9987
Heptachlor epoxide	10–500	0.9977	0.9988	0.9982	0.9985
Dieldrin	10–500	0.9979	0.9994	0.9979	0.9979
Quintozene	10–500	0.9964	0.9974	0.9925	0.9980
Hexachlorobenzene	10–500	0.9981	0.9989	0.9979	0.9990
Heptachlor	10–500	0.9961	0.9967	0.9962	0.9984
Fthalide	10–500	0.9932	0.9965	0.9979	0.9972
Endrin	10–500	0.9949	0.9984	0.9980	0.9988
Tetradifon	10–500	0.9953	0.9967	0.9974	0.9967

GC/ECD A Hewlett Packard GC5890 series was used. GC conditions: oven temperature program, 50℃ for 1 min, 25℃/min to 100℃, and then 5℃/min to 280℃ and held for 1 min. Other conditions were same as for NCI mode GC/MS.

RESULTS AND DISCUSSION

The monitoring ions selected for SIM detection are shown in Table 1. Matrix enhancement effects were sometimes observed in GC/MS. Standard solutions were therefore diluted with extracts of pesticide-free samples to prevent a matrix effect. The correlation coefficients of linearity can be seen in Table 2, and varied from 0.9914–0.9994. Standard solution diluted with extracts of the four pesticide-free natural medicines showed good linearity. SIM chromatograms of the Phellodendri cortex extract fortified with the pesticides are shown in Fig. 1. Pesticide peaks were clearly detectable. SIM chromatograms of the Phellodendri cortex extract (blank sample) are shown in Fig. 2. There were no interfering peaks near the pesticide peaks and thus GC/MS with NCI detected or-

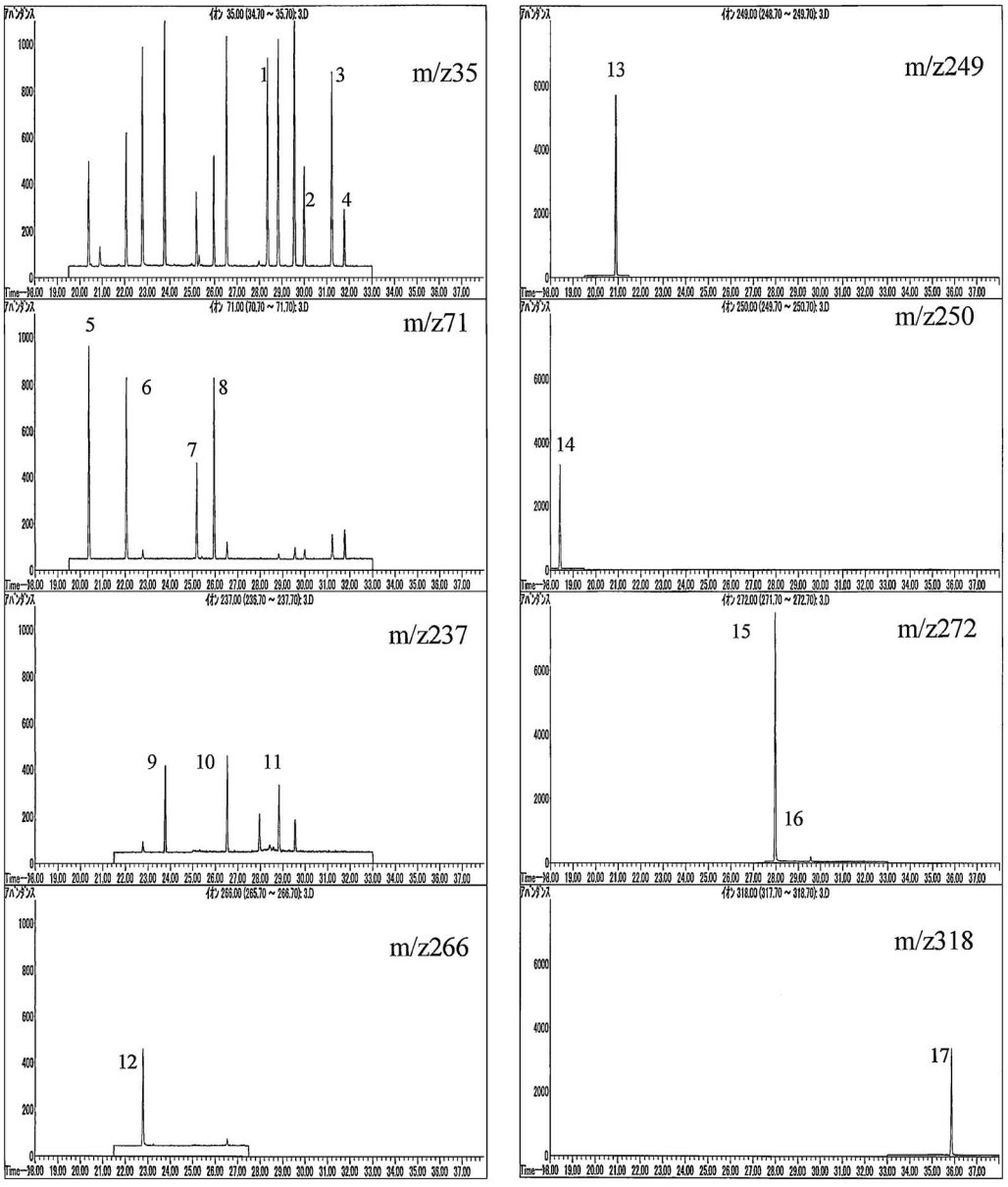

Fig. 1. SIM Chromatograms of Phellodendri Cortex Extract Fortified with Pesticides at 0.1 μg/g 1: *p*,*p*′-DDE, 2: *o*,*p*′-DDT, 3: *p*,*p*′-DDD, 4: *p*,*p*′-DDT, 5: α-BHC, 6: γ-BHC, 7: β-BHC, 8: δ-BHC, 9: aldrin, 10: heptachlor epoxide, 11: dieldrin, 12: heptachlor, 13: quintozene, 14: hexachlorobenzene, 15: fthalide, 16: endrin and 17: tetradifon

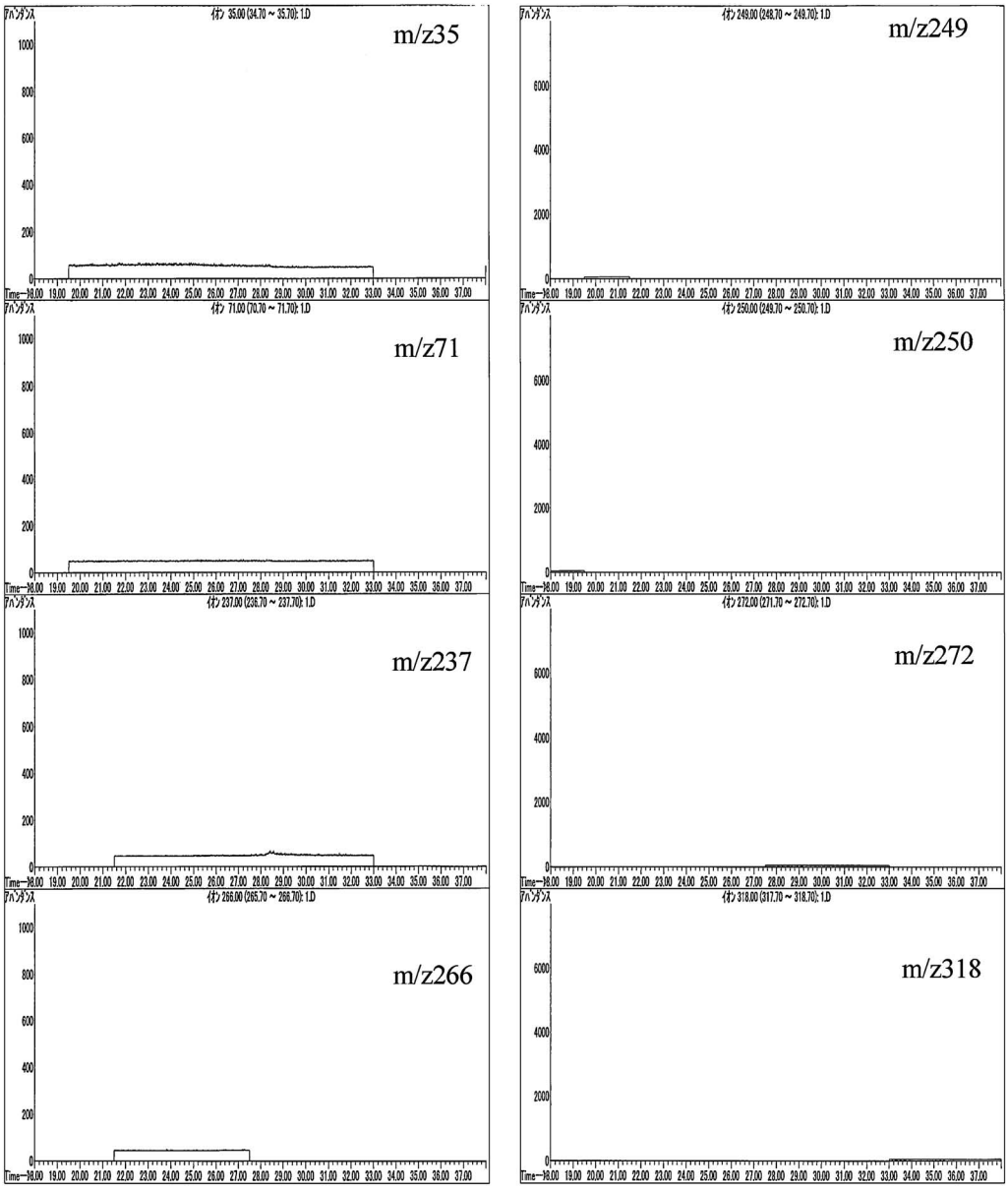

Fig. 2. SIM Chromatograms of the Phellodendri Cortex (Blank Sample)

ganochlorine pesticides selectively. GC/ECD chromatograms of the Phellodendri cortex (blank sample) and standard solution diluted with hexane (20 ppb) are shown in Fig. 3. Some interfering peaks were detected in GC/ECD. Figures 1 to 3 show that GC/MS with NCI can detect organochlorine pesticides more selectively than GC/ECD. The recovery tests were conducted 5 times for the four natural medicines at 0.1 and 0.2 ppm (data not shown). The recovery rates of 17 pesticides were between 83% and 118%, and most relative standard deviations (RSD) were less than 10% at each spiked level. The recovery rates and RSD were satisfactory. The detection limits of the 17 pesticides were less than 5 ppb.

This study indicates that GC/MS with NCI is useful for analyzing organochlorine pesticides in natural medicines. This is the first report of an analytical method for the determination of organochlorine pesticide residues in natural medicines using GC/MS with NCI.

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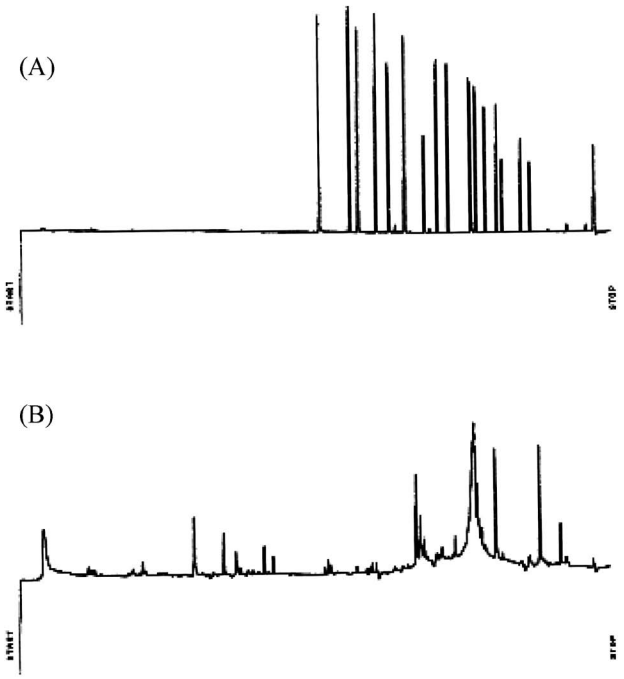

Fig. 3. GC/ECD Chromatograms of (A), Standard Solution Diluted with Hexane (20 ppb); (B), Phellodendri Cortex (Blank Sample)

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